Evaluation of children aged from 3 to 4 years with Prader-Willi Syndrome who received oxytocin between 0 and 6 months of age during the OTBB2 study and comparison with a group of untreated and age-matched children with PWS

OT2SUITE

Sponsor code: RC31/16/8407

BIOMEDICAL RESEARCH PROTOCOL

Version no. 1.6 of 22 March 2017
Favourable decision obtained from the CPP on 21/11/2016 and ANSM authorisation on 22/12/2016

ID-RCB number: **2016-A01348-43**

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HISTORY OF PROTOCOL UPDATES

VERSION	DATE	REASON FOR UPDATE
V1.1	25/07/2016	Initial version
V1.2	28/07/2016	Version reviewed by the DRI
V1.3	18/08/2016	Version submitted to the CPP and ANSM
V1.4	16/11/2016	Version response to the CPP
V1.5	16/12/2016	Version amended for the ANSM
V1.6	22/03/2017	Version following Substantial Modification no. 1

PROTOCOL SIGNATURE PAGE

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LIST OF ABBREVIATIONS

PWS: Prader-Willi Syndrome

OT: oxytocin

ANSM: Agence nationale de sécurité du médicament et des produits de santé [French national agency for the safety of medicines and health products]

EMA: European Medicines Agency

fMRI: Functional Magnetic Resonance Imaging

PHRC: Programme Hospitalier de Recherche Clinique [Hospital Clinical Research Programme]

MA: Marketing Authorisation

CONTENTS

1. SYNOPSIS OF THE STUDY

SPONSOR	CHU de Toulouse
CO-ORDINATING/PRINCIPAL INVESTIGATOR	**Pr Maïthé Tauber** Reference Centre for Prader-Willi Syndrome, Endocrinology Team / Hôpital des Enfants [Children's Hospital]/330 av de Grande Bretagne/ TSA70034/31059 Toulouse Cedex 9/Tel 05 34 55 85 51
TITLE	Evaluation of children aged from 3 to 4 years with Prader-Willi Syndrome who received OT between 0 and 6 months of age during the OTBB2 study and comparison with a group of untreated and age-matched children with PWS (OT2SUITE)
JUSTIFICATION / CONTEXT	PWS is a genetic developmental disorder affecting 1 child in 20,000 at birth, which combines neonatal hypotonia with a severe deficiency in the sucking reflex which may lead to a complete lack of weight gain. Currently diagnosis is made during the first weeks of life. In the absence of specific care and prevention, obesity then occurs in the 2nd year of life in association with other endocrine anomalies probably linked to hypothalamic dysfunction. An anomaly in the neurones secreting OT in the hypothalamic paraventricular nucleus is found both in patients and in 2 animal models (Necdin mice and MAGEL2 mice obtained after invalidation of these 2 genes situated in the PWS chromosomal region). Administration of OT during the first 5 hours of life in MAGEL2 mice allows the survival of 93% of pups as opposed to 51% without administration of OT, due to deficiency in the sucking reflex. Long-term effects are also found after early administration of OT, in particular the restoration of social behaviour in adult Magel 2 mice. In our previous OTBB2 study we carried out repeated intranasal administrations of OT for 7 days at different doses (4IU every 2 days, 4IU/day or 2x4IU/day) in babies with PWS aged less than 6 months. The tolerance of these administrations was excellent: no clinically significant anomaly in the different clinical and biological parameters monitored. Investigations have shown that out of the 18 babies included, 88% have normalised sucking/swallowing after the 7 days of treatment. Significant improvement in social skills is found, with increased eye contact, less withdrawal and intensified mother-baby interactions. Our results have also shown an increase in the levels of acylated ghrelin and changes in cerebral activity with increased connectivity of the right orbitofrontal cortex. These results will be published in the Pediatrics journal in February 2017. We have presented these very promising results to ANSM and EMA and both agencies have strongly recommended that we collect long-term data on these patients treated before the age of 6 months. We therefore hope to confirm the safety and long-term effects on the children treated before the age of 4 years. On the other hand, due to its neuromodulating action, OT could have long-term effects as reported in the Magel2 mouse model. Clinical observation of the children monitored at the centre of reference in Toulouse two years after the end of the OTBB2 study supports this hypothesis. The treated children appear to have better social skills, better psychomotor development and earlier establishment of the prerequisites for communication compared with children with PWS who were not treated with OT during the first months of life.

OBJECTIVES	**Main objective:** to compare the communication skills of children with PWS aged between 3 and 4 years who have been treated for 7 days with OT in the OTBB2 study before the 6th month of life with untreated and age-matched children with PWS. **Secondary objectives**: To compare the group of treated children with PWS with the untreated group in terms of - adaptive behaviour, daily living skills, socialisation and motor skills - global behaviour - orality and eating behaviour - global development - evolution of height and weight and clinical condition - oculometric skills - cerebral activity - circulating rates of ghrelin and other peptides and neuropeptides involved in regulation of the appetite.
OUTLINE OF THE STUDY	Cross-sectional study of children with PWS comparing at the age of 3-4 years the behaviour and development of two groups of children with PWS treated or not with OT for 7 days during the first 6 months of life.
INCLUSION CRITERIA	- Male or female child with PWS (genetically confirmed diagnosis) - Aged from 3 to 4 years - The parents (or legal representative) must have signed the consent form and the child must be registered with a social security regime. - For the treated group: The child has participated in the OTBB2 study. - For the control group: The child has never received OT, age-matched to children treated with OT by plus or minus 3 months.
EXCLUSION CRITERIA	- Administrative problems: inability to give parents (or legal representative) expert information, no coverage by a Social Security regime, refusal of parents (or legal representative) to sign the consent form.
OUTCOME MEASURES	Main criterion: Score in the "Communication" field of the Vineland II scale Secondary criteria: o Scores in the fields "Daily living skills", "Socialisation" and "Motor skills", and the composite score for adaptive behaviour on the Vineland II scale o Global behaviour (CBCL) o Global development (BSID III) o Evaluation of orality and eating behaviour (questionnaire, X-ray, clinical observation) o Oculometric tests o Evolution of height and weight and clinical parameters o Cerebral activity by functional MRI o Circulating rates of ghrelin and certain peptides and neuropeptides involved in regulation of the appetite
STUDY SIZE	36 children: 18 children with PWS included in OTBB2 18 control children with PWS aged from 3 to 4 years and age-matched to the treated children (plus or minus 3 months)

EXPECTED NUMBER OF CENTRES	Monocentric
STUDY DURATION	Duration of inclusion period: 18mois Duration of participation of each patient: 3 days Total study duration: 24 months
STATISTICAL ANALYSIS OF DATA	Comparison of the 2 groups of children with regard to the studied criteria: communication, adaptive behaviour, global behaviour, eating behaviour and orality, global development, oculometric skills, cerebral activity, circulating rates of ghrelin and other neuropeptides
EXPECTED OUTCOMES	If positive effects on communication skills, eating behaviour, development and social skills in children with PWS aged between 2 and 4 years who have received early intranasal administration of OT are demonstrated with good tolerance, we will document the long-term effect of OT administered during the first months of life to babies with PWS and its modifying action on the natural history of the disease.

2. ABSTRACT

This research has been registered in http://www.clinicaltrials.gov/ the *date* under the n° *numéro*.

Long term evaluation of infants aged from 3 to 4 years old included in the OTBB2 study (repeated administrations of oxytocin in infants with PWS aged from 0 to 6 months) and comparison with not treated and age-matched PWS infants (OT2SUITE)

Follow-up of PWS infants treated by oxytocin and comparison with not-treated infants.

*S*ponsor of this research. CHU Toulouse

Background: In our previous OTBB2 study, 18 infants with PWS aged from 0 to 6 months received intranasal administrations of oxytocin (4 UI every 2 days, 4UI every day or 2x4UI every day during 7 days). Tolerance of oxytocin was excellent. Sucking assessed by the neonatal oral motor scale (NOMAS) was abnormal in all infants at baseline and normalized in 88%. Significant improvements in Clinical Global Impression scale, social withdrawal behavior and mother-infant interactions were observed. We also documented a significant increase in acylated ghrelin and connectivity of the right superior orbitofrontal network that correlated with changes in sucking and behavior.

Purpose:

In accordance with recommendations of regulatory authorities (EMA and ANSM), we want to collect long term data of patients treated with oxytocin before the age of 6 months. Morover clinical observations of these infants support long term effects on communication skills, global development and behaviour. The objective of this study is to collect data on tolerance and effects of early treatment with oxytocin in infants with PWS aged from 3 to 4 years and to compare these infants with not treated age-matched infants with PWS.

- **Primary outcome:** Evaluation of communication skills assessed by Vineland-II scale.
- **Secondary outcomes:**

Evaluation of:

  - Adaptative behavior composite and 3 domains: “Daily living skills”, “Socialization”, “Motor skills” (Vineland-II scale).
  - Behavioral troubles (CBCL questionnaire)
  - Global development (BSID III questionnaire)
  - Orality and eating behaviour (questionnaire, videofluoroscopy of swallowing, clinical observation)Eye tracking abilitiesAuxological and clinical parameters
  - Brain activity (fMRI bold)
  - Plasma levels of ghrelin and other peptides involved in feeding behaviour or energy metabolism

- **Study design**: comparison of 2 groups of infants with PWS: infants treated with oxytoxin at the age of 0-6 months and age-matched infants not treated
- **Eligibility criteria:**
  - inclusion criteria:
    - infants with PWS (genetic diagnosis confirmed)
    - aged from 3 to 4 years
    - For treated group : infant included in the OTBB2 study
    - For not treated group: infant never treated with OT
  - exclusion criteria: none
- **Arm number or label and arm type**: 2 groups of infants with PWS: infants treated with oxytoxin at the age of 0-6 months and age-matched infants not treated
- **Interventions**: *not applicable*
- **Number of subjects**: 18 infants treated with oxytocin at the age of 0-6 months and 18 infants not treated
- **Statistical analysis**: description of variables in the oxytocin treated group and comparison between the treated and the age-matched not treated group by Mann-Witney test for continuous variables and by Fisher’s test for nominal variables.
- **Key-words:** Prader-Willi, oxytocin, communication skills, oral skills, brain activity.

3. SCIENTIFIC JUSTIFICATION AND GENERAL DESCRIPTION

3.1. CURRENT STATE OF KNOWLEDGE

3.1.1. REGARDING THE PATHOLOGY

Prader-Willi Syndrome (PWS) is a rare genetic developmental disease (1/20,000 births) linked to the lack of expression of genes of paternal origin (parental imprinting) situated on chromosome 15 in the region q11-q12. For about ten years, due to the early diagnosis (1st month of life), the natural history of the disease has been well described, in particular the various nutritional phases (Miller *et al.*, 2011).

These children all present severe neonatal hypotonia and difficulties with sucking/swallowing which can lead to a severe deficit in weight gain, justifying nutrition by naso-gastric tube in 80% of cases, followed by an improvement in food intake (Miller *et al.*, 2011). A phase of weight increase without increase in food intake appears at around 2 years of age. Secondarily, after the age of 3 years, patients undergo excessive weight gain and develop increased interest in food along with significant anxiety and behavioural problems (Goldstone *et al.*, 2012, Miller *et al.*, 2011). In the absence of treatment, severe obesity appears with hyperphagia and lack of satiety together with aggravation of behavioural problems, constituting the classic profile of the disease (Goldstone *et al.*, 2012, Miller *et al.*, 2011). The physiopathological mechanism explaining the transition between anorexia and severe obesity has not yet been explained. Hyperphagia and behavioural troubles, which are consistent in these patients, have a large impact on their daily life and that of their families and hinder their socialisation (Dykens *et al.*, 2011). Moreover, these patients present endocrine deficiencies, partly linked to dysfunction of the hypothalamus, requiring hormone supplementation. At the psychiatric level, autistic spectrum characteristics and severe anxiety are often associated with mood disorders.

Patients with Prader-Willi Syndrome present communication disorders from birth. Then during childhood and adulthood they present mild or moderate intellectual impairment, attention deficit, dysfunction of executive functions, and also communication disorders, problems with social skills, emotional and linguistic disorders. One recent study showed that children with PWS have more somatic problems, difficulties in social interactions, problems with train of thought and signs of withdrawal/depression compared to age-matched control groups (Skokauskas *et al.*, 2012). The authors also specified that cut-off scores were found for the affective and somatic domain and attention deficit/hyperactivity. This complex combination of somatic, psychiatric and behavioural problems cause severe consequences for patients, their parents and family. Due to their early lack of interaction and social engagement, children with PWS do not benefit as much from the experience of daily audiovisual exposure to communicating faces in comparison with children without PWS. This can dramatically impact their skills at integrating and interpreting the acoustic and visual signals which may contribute to their socio-cognitive immaturity later in their development.

We have demonstrated a deficit in audiovisual integration and discrimination of the human voice in PWS adults in a collaborative study with a team from the brain and cognition research centre (Salles *et al.*, 2016).

3.1.1. REGARDING REFERENCE PROCEDURES AND THOSE UNDER REVIEW

<u>OT and behaviour:</u>

Oxytocin (OT) is a nonapeptide synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and is secreted into the blood system via axonal projections in the posterior lobe of the pituitary gland. The

peripheral actions of OT are well known on the triggering of labour and the stimulation and maintenance of breast feeding. In addition, OT also acts as a neuromodulator/neurotransmitter in the central nervous system, essentially via a dendritic secretion. In addition, its anorectic effect by controlling satiety has been known for several years. The central actions of OT and the peptide of the same family, arginine vasopressin or AVP, have more recently been implicated in social recognition, social memory, the attachment process (formation of the mother/child bond and intimate relationships, distress caused by separation and other aspects of social attachment) (Kosfeld *et al.*, 2005). Finally, OT has also been implicated in the regulation of the response to stress (Lischke *et al.*, 2012), and intranasal administration of OT has allowed improvement in the quality of eye contact in children with fragile X syndrome (Hall *et al.*, 2012) and in children with autistic spectrum disorders (Guastella *et al.*, 2010) in this latter case with contradictory results depending on the studies.

OT and eating behaviour:

It has been demonstrated in adult rats that OT administered intraventricularly or intraperitoneally induces a dose-dependent inhibition of nutrition (reduction of the quantity ingested, decrease in the duration of feeding, prolongation of the period of fasting) and an inhibition of the ingestion of water. In contrast, intraventricular injection of an OT antagonist results in increased appetite and prevents the anti-dipsogenic effect of OT (Arletti *et al.*, 1990). The central oxytocinergic projections appear to play a role in the control of consumption of sugary foods (Amico *et al.*, 2005, Billings *et al.*, 2006). Moreover, central OT appears to participate in limiting the ingestion of unknown foods and limiting the ingestion of appetising, sweet foods.

OT and murine PWS model:

In collaboration with the team of F. Muscatelli, we demonstrated that in knockout mice for the gene Magel2 located in the chromosomal region of PWS, which represent a good model of PWS, 50% of new-born mice died spontaneously in the first hours of life due to lack of effective suckling. A single administration of OT in the window of the first 5 hours of life restores suckling activity and allows the survival of 100% of new-born mice (Schaller *et al.*, 2010) and in the longer term in adult mice, normalises learning problems, memory and social behaviour (Meziane *et al.*, 2015).

The effect of OT on the stimulation of suckling observed at birth in knockout MAGEL2 mice thus appears different and novel from this point of view. However, recent data implicating the collaboration between OT and endocannabinoids in the hypothalamus and more specifically the supraoptic and paraventricular nucleus (McDonald *et al.*, 2008) may explain the important role of OT in suckling. Moreover, OT is implicated in early mother-child interactions and in the process of attachment.

OT in Prader-Willi Syndrome:

In 1995, Swaab et col. reported a deficit in the number and volume of neurons in the paraventricular nuclei of the hypothalamus which secrete OT in the brain of patients with PWS (Swaab *et al.*, 1995). A functional decrease in expression of the OT receptor gene was shown by Bittel et col (Bittel *et al.*, 2007). Our hypothesis is that in PWS, the lack of OT could explain both the difficulties with suckling observed at birth and, in the

longer term, the problems linked to the lack of early establishment of the circuits for regulating the appetite and certain behavioural problems.

Thus we carried out a phase I/II proof-of-concept study in infants with PWS aged under 6 months. 18 infants from all over France were included from May 2013 to July 2014 in the paediatric endocrinology department of CHU de Toulouse. During this study they received OT intranasally for 7 days, in 3 stages: 4 IU every 2 days (N=6), 4 IU per day (N=6) or 4 IU twice per day (N=6).

We evaluated the tolerance and the effects on eating behaviour and in particular sucking/swallowing, social skills by clinical observations and videos rated blindly by independent third-parties, as well as the circulating rates of acylated and non-acylated ghrelin and measurements of cerebral connectivity evaluated by fMRI.

The results show excellent tolerance with no undesirable effects or arginine-vasopressin-like effects associated with an excess of OT. No dose-related effect was observed. Sucking/swallowing was abnormal in all infants at inclusion and was normalised in 88% of the children after the 7 days of treatment; this improvement was shown by a significant reduction from 16 to 9 ($p<0.001$) in the scores on the NOMAS scale (neonatal oral motor scale) and from 18 to 12.5 ($p<0.001$) in the videofluoroscopic swallowing scores. We demonstrated a significant improvement in the clinical global impression (CGI), of the [social] withdrawal of the child (Alarm Distress BaBy scale), and in mother-child interactions (Coding Interactive Behaviour scale). We have also documented a significant increase in acylated ghrelin and in the connectivity of the upper right orbitofrontal network which are correlated to changes in sucking and behaviour. These key results are detailed in the article recently accepted for publication in Pediatrics and will appear in February (Appendix I).

It is necessary to evaluate the long-term effects to determine whether and how OT changes the course of the disorder.

Moreover, these promising effects must be confirmed by a phase III study which will begin during 2017 and for which we have already obtained financing from PHRC in 2015.

3.2. RESEARCH HYPOTHESES AND EXPECTED RESULTS

We have presented the very promising results of the OTBB2 study to ANSM and EMA and both agencies have strongly recommended that we collect long-term data on these patients treated before the age of 6 months.

The OTBB2 study has made it possible to show the major effects of OT on infants with PWS: on the global clinical status of the patient, eating behaviour, social skills, cerebral connectivity and circulating levels of ghrelin.

These early effects of the administration of oxytocin persist for 3 weeks after the last administration and clinical observations of the children monitored at the centre of reference in Toulouse support a long-term effect. The 18 treated children appear to have better social skills, better psychomotor development and earlier establishment of the prerequisites for communication compared with the children with PWS who were not treated. These observations are consistent with the results reported in Magel2 mice which demonstrate that early administration of OT normalises learning, memory and social skills in the adult mice. Moreover, functional modifications have been observed after treatment with oxytocin in these infants.

In accordance with the request of the agencies, we hope to re-evaluate the 18 children treated in the OTBB2 protocol and compare them to a control group of children with PWS who have never been treated with OT. The comparison will focus on communication skills, adaptive behaviour, global behaviour, orality and eating behaviour, global development, evolution of height and weight and clinical parameters, oculometric skills, cerebral activity and circulating rates of peptides implicated in eating behaviour.

The control group will consist of 18 children with PWS aged between 3 and 4 years, age-matched to the experimental group, seen at the reference centre for evaluation and/or routine monitoring.

3.3. RISK/BENEFIT BALANCE

The risks linked to participation in this study are:

- The risk linked to X-rays is low because with current methods the irradiation is 20 µGray which is far lower than 1 non-digital chest X-ray.
- There is currently no known risk linked to MRI examination other than the standard contraindications. Patients with PWS routinely have cerebral imaging carried out generally after 2 years. There is no need for anaesthesia or sedation. MRI examinations have already been performed by our team on patients with PWS with no major difficulty in the vast majority of cases.

It is important to note that all these examinations are likely to be carried out as part of the care of the children with PWS at the Reference Centre.

- For the oculometric assessment, no undesirable effect is expected with the eye-tracking system, a non-ionising and non-invasive technique. The infra-red light emitted by the eye tracker (TOBI) is not harmful to the human eye.

Taking account of the minimal risks incurred by the subjects, the risk/benefit balance for the children with PWS can be considered acceptable for this study. The benefit is above all collective: if an improvement is observed in the long-term state of health of the children with PWS treated with OT during their first 6 months of life, this will document our hypothesis of a positive long-term effect and will support our request for market authorisation in the near future, so that all babies with PWS can be treated.

3.4. EXPECTED OUTCOMES

If the safety and long-term effects (between 2 and 4 years) of early and repeated intranasal administration of OT are demonstrated, we will be in a favourable position to begin our next phase III study with a view to obtaining the MA for oxytocin in Prader-Willi Syndrome.

4. OBJECTIVES OF THE STUDY

4.1. MAIN OBJECTIVE

The main objective is to compare the communication skills of children with PWS aged between 3 and 4 years who have been treated for 7 days with OT before their 6th month of life in the OTBB2 study with untreated and age-matched children with PWS.

4.2. SECONDARY OBJECTIVES

The secondary objectives are to compare the group of treated children with PWS with the group of untreated children in terms of:

- adaptive behaviour, daily living skills, socialisation and motor skills
- global behaviour
- orality and eating behaviour
- global development
- evolution of height and weight and clinical condition
- oculometric skills
- cerebral activity
- circulating rates of ghrelin and other peptides and neuropeptides involved in regulation of the appetite (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH).

5. OUTLINE OF THE STUDY

A cross-sectional biomedical study comparing at the age of 3-4 years the behaviour and development of two groups of children with PWS treated or not with OT for 7 days during the first 6 months of life. This national, mono-centric study will take place at CHU de Toulouse Purpan.

6. ELIGIBILITY CRITERIA

6.1. INCLUSION CRITERIA

The OTBB2 study finished in July 2014 and it was possible to include 18 babies who were seen regularly at the reference centre; all the children included in OTBB2 will be offered the study. This will be the treated group.

For the treated group:

- Male or female child with PWS (genetically confirmed diagnosis) who has participated in the OTBB2 study (i.e. has received intranasal administrations of OT for 7 days before the age of 6 months), aged from 3 to 4 years at the time of inclusion.

For the untreated control group:

- Male or female child with PWS (genetically confirmed diagnosis) aged from 3 to 4 years at the time of inclusion and never treated with OT, age-matched to the children treated with OT plus or minus 3 months.

The parents (or legal representative) must have signed the consent form and the child must be registered with a social security regime.

6.2. NON INCLUSION CRITERIA

- Subject participating in another study including an exclusion period and still under way at inclusion.
- Administrative problems:

- inability to give parents or legal representatives expert information,
- no coverage by a Social Security regime

- Refusal by the parents or legal representative to sign the consent form.

NB: If a patient presents a contraindication for MRI, he/she can be included in the study but the MRI will not be carried out.

6.3. RECRUITMENT METHODS

For the group of children treated with OT:

The team from the reference centre will contact the families. There should not be any patients lost to follow-up, given that the patients who participated in the OTBB2 study and treated with OT are regularly monitored as part of their normal care by the PWS Centre of Reference at CHU de Toulouse.

For the control group of untreated children:

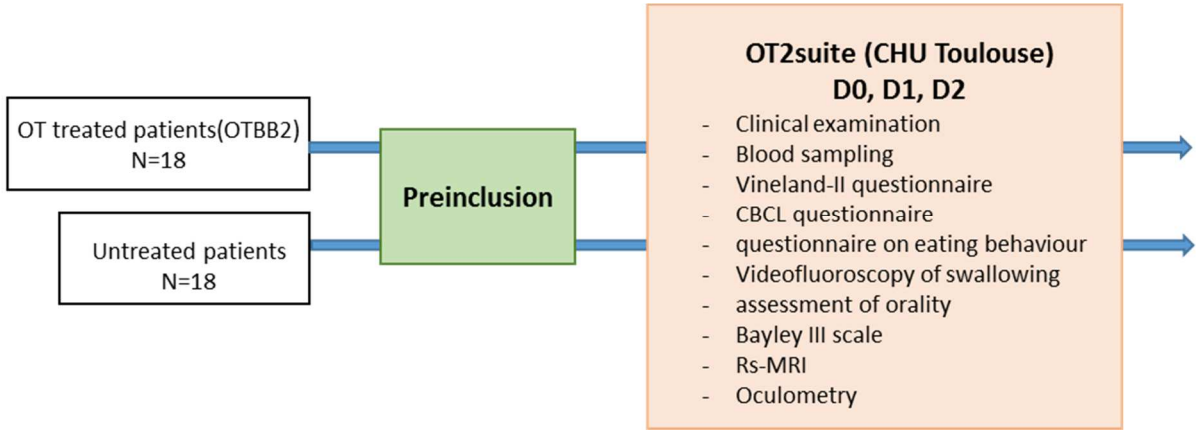
Since treatment with OT is not authorised, no child with PWS currently benefits from this treatment. For the group of control patients, the team at the reference centre will present the study by telephone to the families whose children meet the inclusion criteria ahead of a planned hospital stay at the reference centre. They will be included after the signature of the informed consent form by the parents or legal representative. Approximately 40 children with PWS are born in France every year. The inclusion period of 18 months will therefore be sufficient to include the 18 patients planned in this group.

After an adequate reflection period (between 2 and 10 days), if the parents agree to participate in this protocol they will contact one of the investigators at the reference centre who will organise the inclusion visit at CHU de Toulouse. The children will be hospitalised at CHU de Toulouse in the PWS reference centre, only one parent may sleep in the room with the child, the other may be accommodated in a suitable facility close to the Children's Hospital.

We will also explain to the family that, with some exceptions, participation in the study will not extend the duration of hospitalisation of these children for the routine assessments carried out, which is generally 3 days. The study will thus be carried out during a routine hospital stay in the reference centre.

7. STRATEGY OF THE STUDY

7.1. STRATEGY OF THE STUDY



7.2. COMPARISON STRATEGY

In the 18 babies included in the OTBB2 study we have not observed a serious adverse effect linked to OT. These children appear to have improved from the point of view of behaviour, development, communication and social skills. We wish to compare them to a control group of children with PWS not treated with OT, age-matched and monitored in a comparable manner at the reference centre in order to analyse the long-term effect of OT. This strategy will allow us to document the modifying effect of early treatment with OT on the course of the disease.

8. ASSOCIATED TREATMENTS AND PROCEDURES

Chronic treatments will be continued and noted. In general, there are few at this age. Growth hormone treatment is started during the second semester of life. This treatment has also been started according to recommendations in the children included in the OTBB2 protocol. No treatment will be forbidden due to participation in the study.

9. OUTCOME MEASURES

9.1. PRIMARY OUTCOME MEASURE

The primary outcome measure is the score for the domain of Communication obtained using the Vineland II adaptive behaviour evaluation scale (cf. appendix 2).

This tool, which has been validated in France from the age of 1 year in different populations (autism, attention deficit/hyperactivity disorder, mental retardation, etc.), allows for the multidimensional assessment of skills and disabilities in daily living.

The validity of this tool has been tested in different populations with and without intellectual deficiency, aged from 3 to 16 years. Vineland II is configured to have a mean of 100 and a standard deviation of 15 (Sparow, 2015).

This tool has several applications, including the evaluation of the effects of various treatments or clinical interventions on the level of adaptive functioning.

This questionnaire is adapted to PWS and is currently used in the care of the children monitored at the reference centre. It has been the subject of a recent publication concerning the effect of growth hormone in a population of 96 children and adolescents with PWS aged from 4 to 21 years (Dykens *et al.*, 2016).

For children aged under 7 years, Vineland II examines four domains: Communication, Daily Living Skills, Socialisation and Motor Skills.

The 4 domains are summarised in a composite score for adaptive behaviour which is defined as the execution of activities of daily living required for personal and social autonomy.

Vineland II is based on standard scores for describing the global functioning of a person and his/her level of functioning in each of the domains of adaptive behaviour. A standard score indicates the deviation between the raw score of the subject and the mean gross score, taking into account the standard deviation in the distribution of gross scores.

9.2. SECONDARY OUTCOME MEASURES

The secondary outcome measures are:

- Scores in the fields Daily living skills, Socialisation and Motor skills, and the composite score for adaptive behaviour on the Vineland II scale
- The CBCL (Child Behaviour Check List questionnaire, cf. appendix 3), often used to assess the psychopathology of patients (behavioural and emotional disorders) and in particular those with PWS (Dykens *et al.*, 2007, Skokauskas *et al.*, 2012, van Lieshout *et al.*, 1998). We will use the version aimed at children from 1.5 to 5 years of age. The CBCL is usually completed by the parents at home prior to their arrival and allows an global score to be obtained, as well as a score for the following 6 sub-scales:
    - Anxiety / Depression
    - Withdrawal
    - Emotional reactivity
    - Somatic complaints
    - Attention problems
    - Aggressive behaviour

  The results are expressed as a “total problem” score and in sub-scores. For the “total problem” scale, T scores from 50 to 59 are normal; T scores from 60 to 63 are borderline; above 63 are pathological.
- BSID-III (Bayley Scales of Infant and Toddler Development, 3rd edition, N. Bayley, 2006) is a standardised test designed to measure the global development of infants/young children aged from 1 to 42 months. This assessment battery examines all the spheres of development of the child. It is composed of 5 scales evaluating cognitive abilities, language (receptive and expressive) and motor skills (fine and gross) administered by the psycho-motor therapist of the reference centre. This test will be used regardless of the age of the child, taking account of the developmental delay observed in patients with PWS. The French translation, adaptation and validation of BSID-III form the subject of a national PHRC started in 2012 in Toulouse (principal investigator: Dr C Arnaud) and which will finish in December 2016. We have access to the final French version and are in contact with Dr C. Alberge who led this study and used this version. The French standards must be available during 2017. The Bayley development scales are those most frequently used in clinical research. We are working with the neonatal

department of CHU de Toulouse, and in particular with Dr C. Alberge who began using this test as part of the routine evaluation and care of her patients. It therefore seems relevant to use the most recent version of this scale. Dr Alberge believes that there is no problem using this test at the age of 4 years, even if the upper age limit is 42 months, given that these children have a developmental delay. In order to rate the competences of the child in detail and make qualitative observations, the application of the BSID-III scale will be filmed. The use of video media for rating this test is standard practice.

- The assessment of eating behaviour, comprising:
  - a questionnaire on eating behaviour (cf. Appendix 4) is designed and used in clinical routine by the psychologist of the PWS Reference Centre in Toulouse. Indeed, no validated scale currently takes into account the eating behaviour problems encountered specifically in children with PWS, particularly at this age. It is completed during the interview with the parents.
  - a filmed assessment of eating behaviour during a meal.
- The assessment of orality, comprising:
  - a recording of the voice
  - a videofluoroscopy of swallowing, which represents the “gold standard” for the objective assessment of swallowing problems. It will be carried out and interpreted by the speech and language pathologist of the Reference Centre who carries these out routinely. This is a video radioscopy, the recorded images of which can be reviewed and discussed after the examination, by another expert if need be. The contrast medium used for the radioscopy of the swallowing process is Micropaque®, produced by Laboratoire Guerbet (cf. SPC, appendix 5). It is an oral suspension. In the context of the trial, each child will receive 10 ml of Micropaque® diluted in 20 ml of apple juice.
- The cerebral activity, studied by BOLD effect in functional MRI (carried out at INSERM U825 in Baudot ward, Purpan Hospital under the responsibility of Prof. Pierre Payoux, the head of this unit). Two types of acquisition will be carried out: a morphological MRI and a resting-state functional MRI (rs-fMRI). No injection of contrast medium will be administered to the subjects during this examination. The examination will last approximately 20 minutes. This examination has already been carried out during the OTBB2 study on the 18 infants with PWS.
- Oculometric tests, which allow the gaze position and eye movements in response to visual or auditory stimulation to be studied. The type of eye movement, saccadic eye movements and the position on which the observer focuses are good indicators of the type of social interaction. These oculometric tests are carried out using a TOBII eye tracker, which uses pupillary and corneal refraction of infrared light to track the rotation of the eyes. Eye-tracking is innovative technology, powerful and non-invasive, which is widely used to study social cognition and the perception of emotions, particularly in young children with autism. We have acquired this device which will be used in a study on young children with PWS under the initial supervision of the PhD student (Jimmy Debladis) whose thesis is co-directed by P Barone of the Brain and Cognition Research Centre (CerCo) and M Tauber.

- Circulating rates of acylated and non-acylated ghrelin and certain peptides and neuropeptides involved in regulation of the appetite (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH).
- The evolution of height and weight and clinical parameters collected on a routine basis.

10. CONDUCT OF THE STUDY

10.1. STUDY SCHEDULE

- Start of inclusions: January 2017
- Duration of inclusion period: 18 months
- Duration of participation of each patient: 3 days
- Total study duration: 24 months

10.2. SUMMARY TABLE OF FOLLOW-UP

	Pre-inclusion M-6 / M-3	**Inclusion D0**	**Visit D0-D1-D2**
Eligibility criteria	X	X	
Collection of informed consent		X	
Medical consultation and clinical examination Weight Height HC Waist/hip circumference SBP, DBP, HR			X
5 ml blood sample during the general biological test carried out for follow-up			X
Evaluations: - VINELAND-II assessment scale - CBCL questionnaire - BSID III - Evaluation of eating behaviour - Evaluation of orality - MRI - Oculometry			X
Collection of AEs and SAEs			X

10.3. PRE-INCLUSION VISIT

The pre-inclusion visit is performed by the investigator during a follow-up hospitalization or by telephone ahead of a planned hospital stay at the reference centre. The patient information sheet will be sent to the family by email or by post.

During this pre-inclusion visit, the investigating doctor will present the study to the parents (or legal representative). The presentation of the study will take place at the latest 90 days prior to the inclusion visit to explain the objective of the research, the nature of the constraints, foreseeable risks and the expected

outcomes of the research. The doctor will also outline the rights of the patient and his/her parents (or legal representative) in the framework of biomedical research and will check the eligibility criteria. The parents (or legal representative) will then have time to read the project, contact the investigator if necessary and will be entirely free to give a positive or negative response. After this information session, the parents (or legal representative) have a reflection period of 2 to 10 days.

The investigating doctor is responsible for obtaining the written informed consent from the parents (or legal representative) of the patient. The consent form will be signed by at least one parent (or by the legal representative) at the latest by D0 at the inclusion visit, before any clinical or paraclinical examination is carried out as part of the research.

The various copies of the information leaflet and consent form signed by the parent(s) will then be distributed as follows:

- a copy of the information leaflet and signed consent form is given to the patient and his/her parents.
- another copy is kept by the investigating doctor in a safe place inaccessible to third parties.

10.4. INCLUSION VISIT (D0)

The inclusion visit will be carried out under the responsibility of an investigating doctor of the study at the Hospital of Toulouse during a 3-day hospital stay planned for the normal follow-up of the patient. It will be verified that the information on the set-up and objectives of the research has been understood correctly.
During this visit, the investigator will ensure that the consent form has been signed by the parent(s) (or the legal representative) and the eligibility criteria will be checked.

10.5. FOLLOW-UP VISIT D0 - D1 - D2

The assessments planned for the study are spread over the 3 days of the hospital stay (D0, D1, D2) in the paediatric endocrinology department of Hôpital Pierre-Paul Riquet / the Children's Hospital where the child is admitted.

- A clinical examination is carried out in order to record the weight, height, head circumference and waist/hip circumference. Blood pressure and heart rate are measured.
- During the fasting blood test carried out for the usual follow-up of the patient, an additional 5 ml tube is taken to measure the circulating rates of ghrelin and certain peptides and neuropeptides involved in regulation of the appetite.
- The psychologist administers the Vineland-II questionnaire in consultation with the parents, as well as the eating behaviour questionnaire (approx. 1.5 hours). He/she will collect and if necessary complete the CBCL questionnaire with the parents.
- The speech therapist will administer the “language” scale of BSID III to the child (approx. 1 hour).
- The psycho-motor therapist will administer the “motor skills” and “cognition” scale of BSID III to the child (approx. 1 hour).
- A midday meal is filmed in order to assess eating behaviour.
- A radioscopy of the swallowing process (15 mins + installation time) and a recording of the voice are made by the speech and language pathologist. Depending on availability, these examinations will be carried out

either in the radiology department of the Children's Hospital or in the ENT department on the site of Hôpital Larrey. In the latter case, the patient and his/her parents will be transported by a hospital ambulance.

At the Brain and Cognition Research Centre, in Baudot ward on the Purpan site, the assessments will include:
- an fMRI (30 minutes + installation time)
- oculometric tests using eye-tracking (30 minutes).

10.6. STOPPING RULES FOR THE STUDY

Withdrawal from the study may take place:

- ✓ by the considered decision of the parents or legal representative,
- ✓ or by necessity, after a decision by the investigator, in the event of the occurrence of an unacceptable adverse event.

10.7. CONSTRAINTS LINKED TO THE STUDY AND POSSIBLE INDEMNIFICATION OF THE SUBJECTS/PATIENTS

The main burden for the patient is to undergo additional assessments during their routine 3-day hospital stay at CHU de Toulouse, in particular clinical tests, a videofluoroscopy, a medical imagery examination and an additional 5 ml blood sample.
It is important to state that these examinations form part of the standard examinations carried out at the reference centre on children with PWS.
The person must not be included in another interventional biomedical study for the duration of the study. There is no subsequent exclusion period.

10.8. COLLECTION OF BIOLOGICAL SAMPLES

A plasma bank will be set up in order to additionally measure hormonal levels of ghrelin and certain peptides and neuropeptides involved in regulation of the appetite (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH).
During the fasting blood test carried out for the normal follow-up of the patient, an additional EDTA tube of 5 ml will therefore be taken. The tubes will be centrifuged, the plasma collected and aliquoted then stored at -80°C before measurement. The samples will be coded using a code including the acronym of the study (OT2suite) and the identifying number of the patient specific to the research (cf. 14.3).
The plasma bank will be kept at -80°C at the Biological Resources Centre (CRB) of CHU de Toulouse under the responsibility of the principal investigator, Prof. M Tauber. At the end of the study, the collection will be kept for 10 years in order to be able at a later date to make hormonal level measurements not accessible at the time of the study.

11. MANAGEMENT OF ADVERSE EVENTS AND NEW FACTS

11.1. DEFINITIONS

Adverse event (article R. 1123-39 of the Public Health Code)

Any detrimental event occurring in a person taking part in biomedical research, whether or not this event is linked to the research or to the product being studied.

Adverse reaction (article R. 1123-39 of the Public Health Code)
Any unwanted event due to the research.

Serious adverse event or reaction (article R. 1123-39 of the Public Health Code and ICH guideline E2B)
Any unwanted event or reaction which:
results in death,
endangers the life of the person participating in the research,
requires admission to hospital or a longer stay in hospital,
causes a significant or persistent incapacity or handicap,
is a congenital anomaly or birth defect,
or any event considered to be medically serious (according to the medical judgement of the investigator),
and regarding the medicine, regardless of the dose administered.

Unexpected adverse reaction (article R.1123-39 of the Public Health Code)
For research involving non-medical products such as OT2suite, an unexpected adverse reaction is considered to be any undesirable effect the nature, severity or evolution of which does not correspond to the information relating to the actions taken and methods used during the research. The reference document for assessing the unexpected nature [of the reaction] will be the protocol, in particular paragraph 11.2.

New fact (ruling of 24 May 2006)
New safety information, liable to lead to a re-evaluation of the risk/benefit ratio of the study, or which could be sufficient to consider modifying documents relating to the study, the conduct of the study, or if applicable, to the use of the product.

11.2. DESCRIPTION OF THE EXPECTED SERIOUS ADVERSE REACTIONS

- linked to the radioscopy of the swallowing process: no reaction expected at the low level of radiation received (20µG).

- linked to the MRI: if the contraindications are respected, no serious adverse reaction is expected for the fMRI study, given that no contrast agent is injected and this method is non-invasive and does not use ionising radiation.

- linked to the oculometric tests (eye-tracking): no serious adverse reaction is expected with the eye-tracking system, a non-ionising and non-invasive technique. The infra-red light emitted by the eye tracker (TOBI) is not harmful to the human eye.

- linked to the blood samples: apart from exceptional complications (infection, inflammation, pain at the puncture site), no serious reaction is expected.

These examinations form part of the standard examinations carried out at the reference centre on children with PWS.

Any serious adverse reaction not mentioned in this paragraph will be considered as "unexpected" in this study.

11.3. ACTION TO BE TAKEN IN CASE OF AN ADVERSE EVENT

In the event of the occurrence of an adverse event, whether serious or not, the child will remain hospitalised and will not return home until clinical and biological parameters have returned to normal.
The investigator evaluates the gravity of each adverse event. He must notify the sponsor, without delay from the date on which he is made aware of it, of every serious adverse event or any new fact, if one arises:

- ✓ from the date of signature of the consent form,
- ✓ throughout the patient follow-up provided for by the study,

EVENT TYPE	METHODS OF NOTIFICATION	PERIOD FOR NOTIFYING THE SPONSOR
Non-serious adverse event	In the case report form	No immediate notification
Serious adverse event	Initial SAE declaration form + written report if necessary	**Immediate notification of sponsor**

Name of pharmacovigilance manager for the sponsor: *Dr Pascale OLIVIER-ABBAL*

CHU de TOULOUSE

Tel: +33 (0)5 61 14 59 98 or +33 (0)5 61 77 85 56
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All these events must be followed-up until complete resolution. Additional information (additional declaration form) concerning the evolution of the event, if not mentioned in the first report, will be sent to the sponsor by the investigator.

The investigator must document the event as fully as possible, giving the medical diagnosis if possible, and establish a causal link between the serious adverse event and the study procedures.
The investigator may transmit, in addition to the SAE declaration form, copies of laboratory results or records of examinations or hospitalisation mentioning the serious adverse event, including relevant negative results, without omitting to anonymize these documents.
If the SAE is not resolved following the initial declaration, the investigator must send the sponsor one or more additional declaration(s) until it is completely resolved (with or without sequelae).

11.4. DECLARATION AND RECORDING OF UNEXPECTED SAES AND NEW FACTS

The sponsor/pharmacovigilance unit will declare the unexpected SAEs and new facts occurring during the research according to the mandatory time limits:

- to the ANSM,
- to the competent Ethics Committee (CPP). The committee will ensure, if necessary, that the subjects participating in the study have been informed of the adverse reactions and that they confirm their consent.

11.5. ANNUAL SAFETY REPORT

Once a year, the sponsor will draw up a safety report comprising:

- the list of serious adverse events likely to be linked to the study, including expected and unexpected serious reactions,
- a concise and critical analysis of the safety of participants in the study.

This report will be sent to ANSM and the CPP within the regulatory time limits.

12. STATISTICAL ASPECTS

12.1. CALCULATING THE STUDY SIZE

The main objective is to compare the communication skills of children with PWS aged between 3 and 4 years who have been treated for 7 days with OT in the OTBB2 study before the 6th month of life with untreated and age-matched children with PWS.

Analysis of the primary outcome measure will be a comparison of the distribution of scores for the Communication domain of Vineland-II:

- Comparison of the means if the hypothesis of normality is verified (graphic verification + description of coefficients of skewness and kurtosis): Student's test and variance comparison test to determine whether the Student's test should take account of unequal variances between the two groups,
- Non-parametric comparison of ranks in the event of non-normal distribution of scores: Mann-Whitney test.

The test of hypotheses which will be carried out in the case of normal distribution of scores for Communication is as follows:

H0: the mean of scores for Communication is identical in the two groups (treated group/control group) ($\mu treated = \mu control$)

H1: the mean of scores for Communication differs between the two groups ($\mu treated \neq \mu control$)

If the p-value in this test is less than 5%, the null hypothesis will be rejected and it will be concluded that there is a significant difference in the Communication scores between the two groups at the threshold of 5%.

Hypotheses for calculating the number of subjects required:

- Bilateral test for comparison of means;

- Expected mean in the control group: this value will be varied: 60, 65, 70, 75, 80, 85 points (estimates of the author of the publication (Dykens et al 2016) concerning children with PWS between 4 and 21 are 79.57 (std. dev = 14.12) on average in group 1 versus 65.05 (std. dev = 17.31) in group 2);
- Standard deviation equal in the two groups and fixed at 15 points;
- Desired power: 80%;
- Significance threshold (α risk): 5%;
- Difference to be shown (clinically relevant): 15 points (i.e. 1 standard deviation);
- Ratio between the size of the two groups (Control Group / Treated Group): this value will be varied: 1 (number of subjects equal in the two groups), 2 (two times more patients in the Control group), 3 (three times more patients in the Control group).

Estimate:

With the STATA 14 SE software, we use the command *"power twomeans"* in order to estimate the number of subjects required. Estimates are based on the formulae presented by Armitage, Berry and Matthews (2002); Chow, Shao and Wang (2008); and Dixon and Massey (1983)[1].

Moreover, we use the tables proposed by Julious (2004)[2] as a basis in the case of a study in parallel groups, for a comparison of means (bilateral test), with a power of 90% and an α risk of 5%.

Results:

To ensure a statistical power of 80% and to show a difference of standard deviation between the scores of the Control group and the Treated group, assuming a mean varying from 60 to 85 in the control group, with an α threshold fixed at 5%, the number of subjects that need to be included would be: 17 patients per group if we include as many patients in the Control group as in the Treated group.

This study will be carried out with the 18 patients included in the preceding OTBB2 study. The number of patients to include in the Control group will be 18 children of the same age.

12.2. STATISTICAL METHODS USED

A statistical analysis plan (SAP) will be defined before the database is frozen. All the statistical analyses detailed in the SAP will be carried out by a statistician from the Methodological Support Unit for Research of CHU de Toulouse using Stata® or SAS® software.

All the study variables will be presented by exposure group (children having received early OT versus children never treated with OT) using the descriptive statistics appropriate to each type of variable.

- Continuous variables: number of non-missing observations, median, 1st and 3rd quartile, minimum and maximum, average and standard deviation.

[1] Armitage, P., G. Berry, and J. N. S. Matthews. 2002. Statistical Methods in Medical Research. 4th ed. Oxford: Blackwell.
Chow, S.-C., J. Shao, and H. Wang. 2008. Sample Size Calculations in Clinical Research. 2nd ed. New York: Dekker.
Dixon, W. J., and F. J. Massey, Jr. 1983. Introduction to Statistical Analysis. 4th ed. New York: McGraw–Hill.
[2] Julious, S. A. 2004. Tutorial in biostatistics : Sample sizes for clinical trials with Normal data. Statistics in Medicine; 23:1921-1986.

- Categorical variables: number of non-missing observations, number and percentage of each modality (percentages will be calculated on the basis of the non-missing observations).

Taking into account the limited staff complement, the analyses will primarily be exploratory. For the primary and secondary outcome measures, the total scores and scores per dimension on the different scales (Vineland-II, CBCL, BSIDIII) will be calculated according to the recommendations of the authors. Missing data may be imputed according to the recommendations. The differences in scores obtained in the group of children exposed to treatment before the age of 6 months and the group of control children who have never been treated with oxytocin will be calculated, as well as their confidence intervals at 95%. Comparisons between the exposure groups will be made using a Student's test or a Mann-Whitney test for the scores for the different scales and continuous variables and with a Fisher's exact test for categorical variables. No correction will be applied for the multiplicity of the tests.
For the MRI, the analyses will be made by the MRI team using the same method as that used in the OTBB2 study (article under publication).
All the statistical comparisons on the principal and secondary criteria will be carried out with an alpha risk of 5%.

13. MONITORING OF THE STUDY

This follow-up study does not need an independent surveillance committee, however a scientific council will be set up comprising the study investigators, project leaders and CRA study coordinators, parties involved in the study, a person from the Methodological Support Unit for Research and a member of the DRCI, and will meet once a year or more frequently if necessary.

14. RIGHTS OF ACCESS TO DATA AND SOURCE DOCUMENTS

14.1. ACCESS TO DATA

The sponsor is tasked with obtaining the agreement of all the parties involved in the study in order to guarantee direct access to all study sites, source data, source documents and reports for the purposes of quality control and auditing by the sponsor.
The investigators will make available the individual documents and data strictly necessary for the follow-up, quality control and auditing of the biomedical research to persons having access to these documents in accordance with current laws and regulations (articles L.1121-3 and R.5121-13 of the Public Health Code).

14.2. SOURCE DATA

Any original document or object (medical record, result of a biological examination, report of an imaging test, video recordings) making it possible to prove the existence or accuracy of data or facts recorded in the course of the research is defined as a source document.

14.3. DATA CONFIDENTIALITY

In line with current legislation (articles L.1121-3 and R.5121-13 of the Public Health Code), persons having direct access to source data will take all necessary precautions to ensure the confidentiality of information relating to research, participating persons, particularly as regards their identity, and the results obtained. These persons, as well as the investigators themselves, shall be subject to professional secrecy.

The subjects participating in the study will be coded using an identifier. For the treated group, the identifiers will correspond to those of the OTBB2 protocol, i.e. the number of the order of inclusion in OTBB2 (from 1 to 18) followed by the 1st letter of the surname and 1st letter of the first name of the patient. For the control group, the identifiers will correspond to the number of the order of inclusion in OT2suite (starting from 19) followed by the 1st letter of the surname and 1st letter of the first name of the patient.

The investigator will ensure that the anonymity of each person participating in the study is guaranteed. No information allowing the identification of persons will be communicated to third parties other than those authorised by regulatory requirements to hold this information (and who are bound by the obligation of professional secrecy).

During or at the end of the biomedical research, the data collected concerning the participating persons and transmitted to the sponsor by the investigators (or any other specialised associates) shall be anonymised. They must not in any case allow the names or addresses of the persons concerned to appear in uncoded form.

The sponsor will ensure that the legal representative of each person participating in the research has given his/her consent in writing for access to the individual data about the participant that is strictly necessary for quality control of the study.

15. QUALITY CONTROL AND ASSURANCE

15.1. INSTRUCTIONS FOR COLLECTING DATA

All information required by the protocol must be entered into the case report forms and an explanation must be given for any missing information. The data must be collected as it is obtained and transcribed into these case report forms neatly and legibly.

Incorrect data removed from the case report forms will be clearly struck out and the new data will be copied next to the struck out information, together with the initials, date and possibly an explanation by the investigator or authorised person who has made the correction.

15.2. STUDY FOLLOW-UP

The follow-up of the study will be carried out by a clinical research associate. He/she will be tasked by the coordinating investigator with:

- the logistics and monitoring of the study,
- drawing up reports concerning its progress,
- checking the updating of the case report forms (request for additional information, corrections, etc.),
- sending samples,
- transmission of SAEs to the sponsor.

He/she will work in accordance with the standardised operating procedures, in collaboration with the clinical research associate delegated by the sponsor.

15.3. QUALITY CONTROL

A clinical research associate authorised by the sponsor will regularly visit the investigation centre, during the set-up of the research, once or more during the study according to the rate of inclusions, and at the end of the study. During these visits the following elements will be reviewed:

- informed consent,
- compliance with the study protocol and the procedures defined therein,
- quality of the data collected in the case report form: accuracy, missing data, coherence of data with source documents (medical files, appointment calendars, originals of laboratory results, etc.),

Every visit will be the subject of a written monitoring report.

15.4. DATA MANAGEMENT

A list identifying the subjects participating in the study will be kept in the investigator's file.

The information will be collected for each subject in a standardised case report form completed by the investigator or CRA. The case report form will include the identification of the subject (coded), clinical data and study data.

The source document (existing medical file) will be kept for each subject participating in the study; observation and medical follow-up concerning the study must be recorded in the source document.

All the data from the study will be transcribed in the case report form and kept by the investigating doctors of CHU de Toulouse under the responsibility of Pr M Tauber. The case report forms will be completed legibly and indelibly using a blue or black ball point pen. In case of error, the incorrect data will be struck through with a single line, so that the original information remains legible, and the correct information will be written next to it. Each correction will be explained and authenticated (dated and signed or initialled by the investigator). The principal investigator will sign each case report form to certify his/her agreement with the data included therein. The study data is then entered into a file created using Excel software by simple data entry with rereading by the study CRAs.

The data is saved automatically after the entry of new data and is kept for 15 years after the end of the study.

The data is validated in accordance with the data management plan defined jointly between the coordinating investigator and the Centre of Methodology and Data Management (methodologist, data manager and statistician).

The process of freezing/unfreezing the data is carried out in accordance with the procedure set up in the Centre of Methodology and Data Management (freezing of raw data in XML format and in the form of an SAS table).

15.5. AUDIT AND INSPECTION

An audit may be carried out at any time by persons authorised by the sponsor and independent of the study managers. The aim of the audit is to ensure the quality of the research, the validity of its results and compliance with the law and current regulations.

The investigators undertake to conform with the sponsor's requirements and with the competent authority with regard to an audit or inspection of the study.

The audit may apply to all stages of the research, from development of the protocol to the publication of results and classification of the data used or produced in the context of the research.

16. ETHICAL AND REGULATORY CONSIDERATIONS

The sponsor and the investigator(s) undertake that this research shall be carried out in accordance with law number 2004-806 of 9 August 2004, and in accordance with Good Clinical Practice (I.C.H. version 4 of 1 May 1996 and decision of 24 November 2006) and the Helsinki Declaration (cf. full version http://www.wma.net).

The study is conducted in accordance with this protocol. Except in urgent situations requiring the implementation of precise therapeutic actions, the investigator(s) undertake(s) to respect all points of the protocol, in particular regarding the collection of consent and notification and the follow-up of serious adverse events.

This study received the favourable decision of the Comité de Protection des Personnes (CPP) Sud-Ouest et Outre-Mer I [Ethics Committee for South-West France and Overseas] on 21/11/2016 and the authorisation of ANSM on 22/12/2016.

CHU de Toulouse, the sponsor for this study, has taken out an civil liability insurance policy with the company GERLING France in accordance with the provisions of article L1121-10 of the Public Health Code.

The data recorded in this study will be processed electronically at CHU de Toulouse subject to the law no. 78-17 of 6 January 1978 relating to information technology, files and civil liberties as amended by law 2004-801 of 6 August 2004.

This study falls within the framework of "Reference Methodology" (MR-001) under the provisions of article 54 paragraph 5 of the amended law of 6 January 1978 relating to information, files and liberties. This change has been approved by the decision of 5 January 2006. CHU de Toulouse has signed a commitment to comply with this "Reference Methodology".

- This study is registered in the European RCB database under registration number 2016-A01348-43 in accordance with article L1121.15 of the Public Health Code.
- This study is registered on the website http://clinicaltrials.gov/.

- The collection of biological samples carried out in the context of this study was declared to ANSM at the same time as the request for authorisation of the study. After the study, the conservation of the collection of biological samples will be declared to the minister in charge of research and the Director of the Regional Health Authority (and submitted to the CPP for an opinion if there is a change in the purpose of the study).

PROTOCOL AMENDMENT

Any substantial amendment, i.e. any amendment likely to have a significant impact on the protection of persons, on the conditions of validity and on the results of the research, on the interpretation of the scientific documents supporting the implementation of the study or on the methods of conducting the study, shall be the subject of a written amendment which is submitted to the sponsor; the latter must, prior to its implementation, obtain a favourable decision from the CPP and authorisation from ANSM.
Non-substantial amendments, i.e. those which do not have a significant impact on any aspect of the research, are communicated to the CPP for information.
All amendments are validated by the sponsor, and by all the parties involved in the study concerned by the amendment, prior to submission to the CPP and ANSM. This validation may require a meeting of the CS and/or CIS.
All amendments to the protocol must be brought to the notice of all the investigators participating in the study. The investigators undertake to respect the content of these.
Any amendment which alters the care of patients or the benefits, risks and burdens of the study shall be the subject of a new information note and a new consent form, the collection of which follows the same procedure as that described above.

17. RETENTION OF DOCUMENTS AND DATA RELATING TO THE STUDY

The following documents relating to this study are archived in accordance with Good Clinical Practice:

- By the investigating doctors:

- for a period of 15 years following the end of the study

• The protocol and any amendments to the protocol
• The case report forms (copies)
• The source files of the participants who have signed a consent form
• All other documents and correspondence relating to the study
• The original informed consent forms signed by participants

All these documents are under the responsibility of the investigator for the regulation archiving period.

- By the sponsor:

- for a period of 15 years following the end of the study

• The protocol and any amendments to the protocol
• The original case report forms
• All other documents and correspondence relating to the study
• A copy of the informed consent forms signed by participants
• The documents relating to serious adverse events

All these documents are under the responsibility of the sponsor for the regulation archiving period.
They may not be moved or destroyed without the permission of the sponsor. At the end of the regulation archiving period, the sponsor will be consulted for their destruction. All the data, documents and reports may be audited or inspected.

18. RULES CONCERNING PUBLICATION

18.1. SCIENTIFIC COMMUNICATIONS

Analysis of the data is performed by the study investigators in conjunction with Dr C. Arnaud, University Department of Epidemiology, Health Economics and Public Health, Faculty of Medicine, Toulouse. This analysis results in a written report which is submitted to the sponsor, who will transmit it to the CPP and to the competent authority.

Any written or verbal communication of the results of the research must receive the prior agreement of the coordinating investigator and, if necessary, any committee created for the study.

Publication of the principal results shall mention the name of the sponsor, all investigators who included or followed-up patients in the study, the project leader and CRA coordinators, the methodologists, biostatisticians and data managers who participated in the study, members of the committee(s) created for the study and the source of financing. Account shall be taken of the international rules for writing and publication (*The Uniform Requirements for Manuscripts* of the ICMJE, April 2010).

18.2. COMMUNICATION OF RESULTS TO PATIENTS

In accordance with law no. 2002-303 of 4 March 2002, patients are informed, if they so request, of the global results of the study.

18.3. TRANSFER OF DATA

The collection and management of data is performed by CHU de Toulouse. The conditions of transfer of all or part of the study database are decided by the sponsor of the study and shall be the subject of a written contract.

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20. APPENDICES

APPENDIX 1: CONFIDENTIAL / ARTICLES UNDER PUBLICATION

DO NOT DISTRIBUTE

Oxytocin in infants, a promising treatment for poor oral feeding and social skills: first results in Prader-Willi syndrome.

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Abstract

Background: As supported by pre-clinical data, early treatment with oxytocin (OXT) could restore sucking after birth. Patients with Prader-Willi syndrome (PWS) display poor feeding and social skills as infants and a decreased number of hypothalamic OXT-producing neurons was documented in adults.
Methods: We conducted a proof-of-concept phase I/II escalating dose study of a short course (7 days) of intranasal OXT administration with three steps. We enrolled 18 infants with PWS under 6 months old (6 infants in each step) who received 4 IU of OXT either every other day, daily or twice daily. We investigated the tolerance and the effects on feeding and social skills clinically and on blinded videos, as well as circulating ghrelin and brain connectivity by fMRI.
Results: Neither adverse events nor AVP-like effects were reported. No dose effect was observed. Sucking assessed by the neonatal oral motor scale (NOMAS) was abnormal in all infants at baseline and normalized in 88% with a significant decrease in both NOMAS and videofluoroscopy of swallowing scores from 16 to 9 ($p<0.001$) and from 18 to 12.5 ($p<0.001$), respectively. Significant improvements in Clinical Global Impression scale, social withdrawal behavior (Alarm Distress BaBy scale) and mother-infant interactions (Coding Interactive Behavior scale) were observed. We also documented a significant increase in acylated ghrelin and connectivity of the right superior orbitofrontal network that correlated with changes in sucking and behavior.
Conclusions: Intranasal OXT is well tolerated in infants with PWS, improves oral feeding and social skills, increases circulating acylated ghrelin and modifies brain connectivity.

Introduction

Oxytocin (OXT) is a neuropeptide that plays an important role in modulating social interactions and mother-infant bonding (Alves et al., 2015, Carson et al., 2013, Carter, 2014, Insel, 2010, Numan and Young, 2015). Quantitative neuroanatomical studies of post-mortem human hypothalamic tissue from patients with Prader-Willi syndrome (PWS) have demonstrated a reduced number and volume of OXT neurons in the paraventricular nucleus in comparison with controls (Swaab et al., 1995). Similarly, an alteration in the OXT system was described in PWS mice models (Schaller et al., 2010). Interestingly a single OXT injection before the first 5 hours of life, rescued 100% of the newborn Magel2 KO mice from early death by restoring normal sucking activity (Schaller et al., 2010). Magel2 KO mouse is now considered as a mouse model for PWS and autism spectrum disorder (ASD) as truncated mutations in Magel2 gene have been reported in some patients with ASD (Schaaf et al., 2013) Restricted production of mature OXT despite normal prohormone production was detected specifically in the hypothalamus of the Magel2 KO pups. Altogether these data suggest that OXT is involved in the pathophysiology of PWS and ASD12.

PWS is a rare complex genetic neurodevelopmental disease comprising several nutritional phases (Goldstone et al., 2008, Miller et al., 2011). From birth to 9 months infants with PWS display severe hypotonia, poor interactions and anorexic behavior with poor suck that may cause life-threatening complications like aspiration. Nasogastric tube feeding (NGT) is started at birth in more than 80% of the infants to ensure normal weight gain (Bachere et al., 2008).

Genetic diagnosis which is now made in the first months of life (Goldstone et al., 2008) offers a unique opportunity for early treatment with OXT. We report here the results of a proof-of-concept phase I/II

study of a short course of intranasal OXT (7 days) in 18 infants with PWS under 6 months old on safety, feeding and social skills, ghrelin levels and brain connectivity.

Methods

Patients: Eighteen infants with a genetic diagnosis of PWS were recruited and hospitalized in our French reference center for PWS with the presence of at least one parent. A detailed description of the population is shown in Table 1.

Study protocol: This proof-of-concept monocentric phase I/II escalating dose study was divided into three steps of 7 days of OXT treatment (Syntocinon®), each step recruiting six consecutive infants under 6 months old. Infants received 4 IU intranasal OXT every other day (4 administrations) in the first step, 4 IU intranasal OXT daily (7 administrations) in the second step, or 4 IU intranasal OXT twice daily (14 administrations) in the third step. The study protocol (available at NEJM.org; ClinicalTrials.gov number, NCT 02205034) was approved by the research ethics committee of the Hospital of Toulouse. Written informed consent was provided by the two parents.

Evaluation of tolerance: Each infant was examined daily by the team pediatrician, and blood pressure and heart rate were monitored three times per day and for 2 hours after each OXT administration. An electrocardiogram was performed before and 5 min after each OXT administration. Biological parameters, glucose, potassium, sodium and osmolality were assessed every 2 days. Diuresis and urinary density were monitored daily. After discharge, tolerance was evaluated by the parents with a standardized daily case report form, and the local pediatrician via links with the study team, until the end of the study at day 30.

Evaluation of oral feeding skills

Sucking and swallowing were evaluated before the first and after the last OXT administration by a speech and language pathologist (SLP) with expertise in PWS. We used the Neonatal Oral-Motor Assessment Scale (NOMAS; see Table S1)(Palmer et al., 1993) which is a composite scale comprising 28 items with three sucking pattern categories (normal, disorganized or dysfunctional) assessing jaw and tongue aspects and functions and the rhythm and synchronization between sucking and swallowing and between sucking/swallowing and respiration. Higher scores indicate greater impairment. We also calculated the percentage of infants with a score ≤ 10 versus a score >10. A score ≤ 10 corresponds to a normal or near normal sucking pattern without severe or life threatening respiratory complications. The same day, a dynamic videofluoroscopy of swallowing was performed by the same SPL and scored with a grid of nine relevant items that she uses in routine practice (see Table S2). Videofluoroscopy is normal if the score is 11, and the maximum abnormal score is 29. The SLP evaluated behavior before and during feeding using a clinical global impression (CGI) scale (see Table S 3).

Blind evaluation of social skills: In addition, we assessed social withdrawal behavior and mother-infant interactions using the Alarm Distress Baby scale (ADBB) and the validated Coding Interactive Behavior (CIB) scale respectively (Feldman, 2007, Guedeney et al., 2013, Viaux-Savelon, 2014). The ADBB and CIB were scored in a blinded manner on videos of feeding taken before and after OXT administration by two experts who did not participate in either designing the protocol or conducting the trial. The ADBB scale is a composite scale comprising eight items with a normal score under five (Guedeney et al., 2013). For the CIB scale each of the 42 items was rated from 1 (a little) to 5 (a lot) and grouped into

five composites. In addition, according to the infant's age, 26 specific infant items based on Brazelton's Neonatal Behavioral Assessment Scale (Viaux-Savelon, 2014) were rated and grouped into two additional composites (See Table S4). Psychoanalytic infant observation through emotional welcome followed by thinking (Bick, 2007) was performed with the parents' consent, by a psychologist trained to E Bick's method, before the first and after the last OXT administration at the hospital.

Sampling and hormone assays: Blood sampling was performed every 2 days after a minimum of 3 hours of fasting. The samples were drawn into EDTA tubes with anti-protease 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) (Sigma-Aldrich Chemicals) at a concentration of 2 mg/ml for the ghrelin and OXT measurements. With these procedures, it has been shown that acidification was not necessary to prevent ghrelin degradation (Blatnik and Soderstrom, 2011). Samples were stored at -20°C prior to measurements and were assayed not longer than 6 months after collection. Measurement of acylated (AG) and unacylated ghrelin (UAG) was performed as described by Delhanty et al. (Delhanty et al., 2015). OXT was measured in blood samples as described by Pequeux et al. (Pequeux et al., 2001).

Brain connectivity study during resting state using fMRI: The brain connectivity is defined as the temporal correlation of neuronal activity as evidenced by BOLD signal of anatomically separated brain regions. With resting-state functional MRI (RS-fMRI), it is possible to investigate whole-brain (van den Heuvel and Hulshoff Pol, 2010). Regions are said functionally connected if they demonstrate synchronous BOLD fluctuations at rest and then form a network. There are multiple resting-state networks that pertain to different brain functions that can be detected from the time-series scans Independent Component Analysis (ICA) -based methods(Beckmann et al., 2005) are the most commonly used and display high level of consistency (Damoiseaux et al., 2006). To investigate the effect of OXT on brain connectivity, we scanned 17 of 18 infants at rest without sedation before and after OXT administration. We applied ICA (Group ICA fMRI Toolbox; GIFT; (http://mialab.mrn.org/software/gift/, Version 4.0a) (Damoiseaux et al., 2006) to find a set of statistically independent spatial components. This group analysis allowed us to identify important network and then provide the associated map for each subject and each condition where the value in each voxel is a Z-score measure of the pixel connectivity to the network. (see Methods S4).

Statistical analysis:

Data are presented for the whole population and for each dose step. The continuous variables were expressed as medians and ranges and the categorical data as numbers and percentages. Continuous variables before and after treatment were compared using Wilcoxon signed rank tests in the whole population and in each dose step. The score changes were compared between dose steps using Kruskall-Wallis ranking tests when significant evolution was observed in the whole study group. Changes in connectivity Z-score before and after OXT were measured with a paired t test Correlations between connectivity Z-score changes in fMRI and changes in the oral feeding scales (NOMAS, videofluoroscopy) and behavior changes (CGI, ADBB, CIB) were estimated for the whole sample by the Kendall Tau-b coefficient. Data analysis was performed with Stata v11.2 software. P-values ≤ 0.05 were considered as statistically significant.

Results

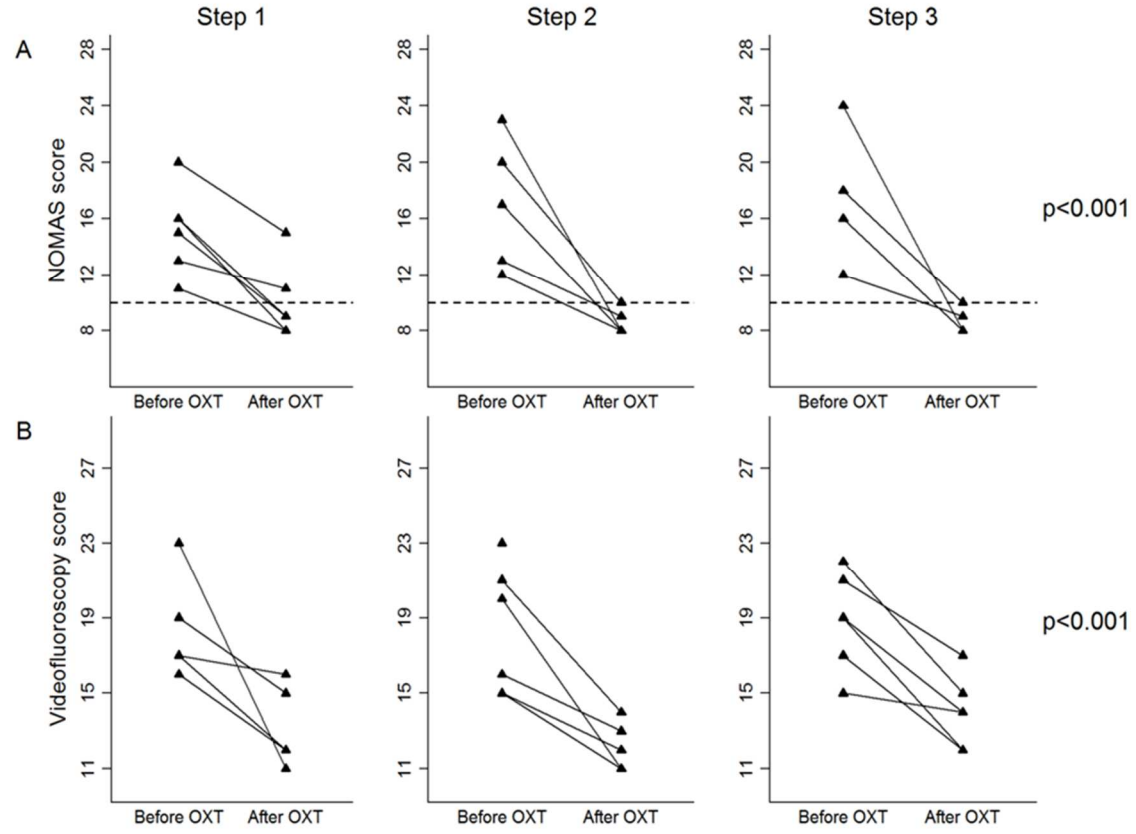
Tolerance

We observed no adverse event in relation to OXT for any of the parameters specifically surveyed and no other event occurred during the 7 days of OXT administration and up to day 30. Overall, the tolerance to OXT was excellent, with no cardiovascular or antidiuretic AVP-like effects. Plasma OXT levels were highly variable before and after OXT administration and did not significantly change in each step (data not shown).

Effect of OXT on oral skills

The NOMAS score significantly improved after treatment in the whole group, with a change in median score from 16 to 9 (p-value<0.001). At baseline, the score varied widely from 11 to 24 with no infant having a normal score. After OXT treatment, eight infants (47%) reached a score of 8, which is strictly normal and 15 (88%) had a score ≤10, meaning that they displayed a near normal sucking pattern (Figure 1A). Only two patients included in step 1 did not normalize their score after treatment. Overall, the changes in NOMAS scores did not differ between the three dose steps (p-value = 0.504).
The scores of the videofluoroscopy of swallowing significantly improved after treatment in the whole group from a median value of 18 to 12.5 (p-value <0.001), and in each dose step (see Figure 1B).
No significant difference according to dose step (p-value=0.588) was found. Improvement is obvious as shown in the videofluoroscopy of swallowing before and after OXT of 1 infant (see video 1 and video 2 available at NEJM.org).

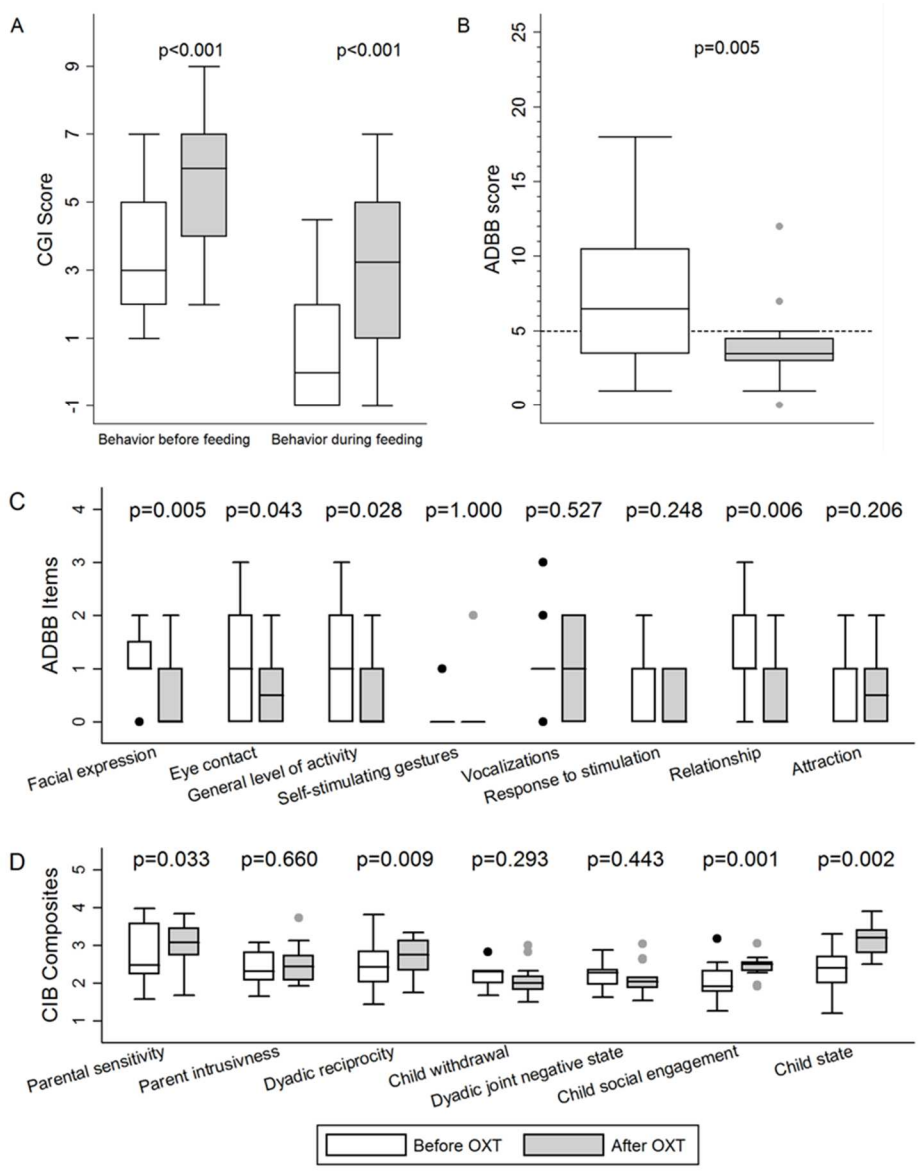
Figure 1: Evaluation of sucking/swallowing before and after Oxytocin



Effect on behavior and social skills: As shown in Figure 2A , the CGI score significantly improved after treatment from a median score of 3 to 6 before feeding (p-value=0.001) and 0 to 3.5 during feeding (p-value=0.001) in the whole group.

At baseline, the median ADBB score was 6.5, with 62% of the infants with an ADBB score ≥5.The score significantly improved in the whole group from 6.5 to 3.5 (p=0.005) with a normal score in 81% after OXT (figure 2B). We observed significant improvements on facial expression, from a median score of 1.0 to 0.0 (p-value=0.005); eye contact, from 1.0 to 0.5 (p-value=0.043); general level of activity, from 1.0 to 0.0 (p-value=0.028); and relationship, from 1.0 to 0.0 (p-value=0.006) (Figure 2C). For the total ADBB score as well as for these four items, the observed changes did not significantly differ between the three steps. Figure 2D shows the changes in composites of the CIB scale in the whole group. We observed significant improvements after treatment on parental sensitivity, from a median score of 2.47 to 3.08 (p-value=0.033); dyadic reciprocity, 2.43 vs. 2.75 (p-value=0.009); child social engagement, 1.91 vs. 2.50 (p-value=0.001); and child state, 2.40 vs.3.20 (p-value=0.002). These significant changes did not differ between the three steps.

Figure 2: Evaluation of behaviour and social skills before and after Oxytocin

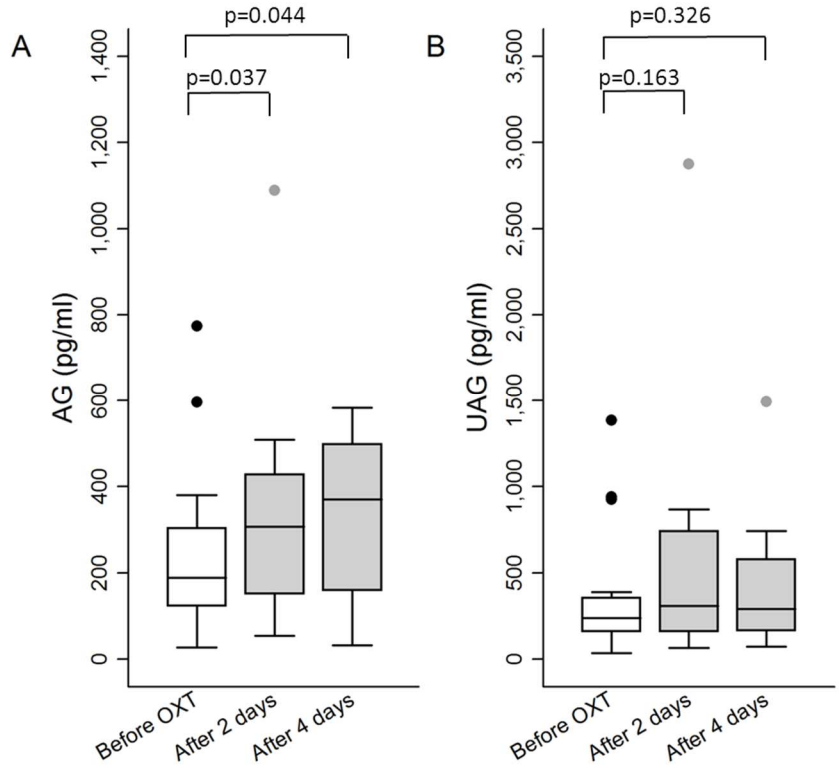


Infants' observation revealed an improvement in the parents' interrelations with the baby in gaze, holding and handling. This observation seemed to be a support for the emergence of staggering and depressed feelings in the parents caused by the stress generated by the diagnosis of PWS.

Effect of OXT on circulating ghrelin

As shown in Figure 3, AG levels significantly increased between baseline and, respectively, 2 days (from 188.5 to 306.5 pg/ml, p-value=0.037) and 4 days (from 188.5 to 370 pg/ml, p-value=0.044) after the first OXT administration. No significant changes were observed in UAG levels. Changes between baseline and 4 days after the first OXT administration did not significantly differ between the three dose steps (p-value=0.889).

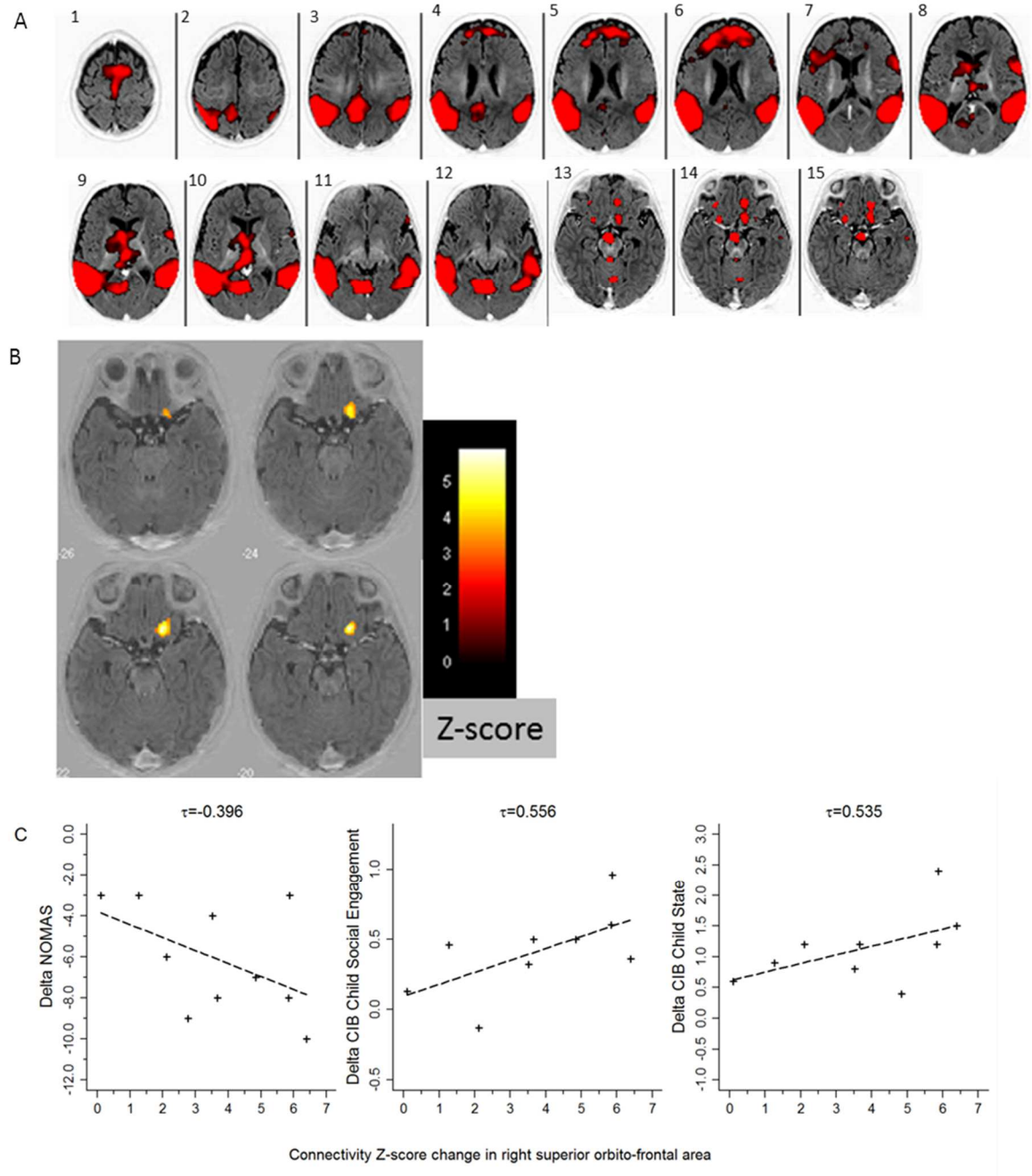
Figure 3: Circulating acylated and unacylated ghrelin before and after Oxytocin



Effects of OXT on brain connectivity study during resting state using fMRI:

In the brain connectivity analysis, we focused on the network encompassing many areas in the orbitofrontal cortex (OFC), in the striatum and the anterior cingulate known as key regions in the hunger and drive to eat. Interestingly this network involves also the Superior Temporal Gyrus which is involved in social cognition the supramarginal gyrus and the temporoparietal junction which are important area in social ability , the inferior frontal opercular and the Supplementary Motor Area (fig. 4A). As shown in Figure 4B, inside this network, the connectivity of the right superior orbitofrontal cortex increased after OXT treatment (paired t-test, p-value<0.05; familywise error corrected). In addition, the mean change calculated over all the voxels in this area for each subject was correlated with the changes in NOMAS (tau-b=-0.396), the CIB child social engagement composite (tau-b= 0.555) and the CIB child state composite (tau-b=0.535) (figure 4C)

Figure 4: Brain connectivity before and after Oxytocin.



Discussion

This proof-of-concept study demonstrates that a short course of repeated intranasal OXT administration is well tolerated and improves oral feeding and social skills in infants with PWS. Given the preclinical data with OXT, the early diagnosis of PWS and the possible role of OXT in the pathophysiology of the disease, PWS is a good model to study the effects of early OXT administration. The effects on oral feeding and social skills in human infants are reported here for the first time. In our study an abnormal sucking pattern was present at baseline in all infants and was normalized in 88% of them after OXT. Such improvement is highly clinically relevant as there was a complete rescue of the life-threatening respiratory complications of poor feeding skills in this population. It is likely that OXT improves oral

motor function of the face and head including the larynx and pharynx. Interestingly, these structures and the nerves that innervate them were described as part of a system (polyvagal theory) (Porges, 2011) that permits social engagement and communication (Carter, 2014).

In addition, after OXT treatment the infants displayed an increased interest in food that may have been related to the observed increase in circulating AG, called “the hunger hormone” as it is the only known orexigenic hormone(Cabral et al., 2015). Indeed we have data showing that AG is lower in infants with PWS compared with controls and OXT treatment seems to normalize it (data not shown). Importantly, we document the positive effects of OXT treatment on behavior and social engagement and on mother-infant interactions. After OXT, the infants were more alert, less fatigable, more expressive, and have less social withdrawal. They initiated mutual activities and, were more engaged in relationship through gaze, behavior and vocalizations. These modifications helped the parents to be more sensitive. The dyad was less restricted and there was a better reciprocal exchange, thus engaging the dyad in a positive transactional spiral as well as optimizing feeding. This improvement was also reported by infant observation in gaze, holding and handling. In humans, “mutual gaze” is the most fundamental manifestation of social attachment (Carter, 2014). Indeed we are aware that these changes may have been due to OXT and/or to the support and guidance provided by the expert team to the parents. Interestingly, similar improvement in parent-infant interactions was reported when OXT was given to the parent (Weisman et al., 2013).

Importantly, these early effects of OXT persisted 3 weeks after the last OXT administration and we have now preliminary data supporting the long-lasting effects one year after the end of the study on feeding, behavior and social skills in 16 infants. This is in line with the results reported in the Magel2 deficient mice showing that a short course of early treatment with OXT in pups rescued feeding at birth and had late effects normalizing learning, memory and social cues in adult males (Meziane et al., 2015). In addition, anatomical and functional modifications of the brain OXT-system were partly restored by OXT treatment (Meziane et al., 2015).

We also document the increasing connectivity of the right superior orbitofrontal network after infants treatment with OXT. The OFC contains major cortical representations of taste and food texture, olfactory and somatosensory inputs, from both the mouth and other parts of the body, as well as information about real faces, gestures and movement (Rolls, 2004). Overall, the OFC is involved in motivational behavior such as feeding and drinking, emotional decision-making and social behavior by implementing learning mechanisms through decoding the reward and punishment values of stimuli (O'Doherty et al., 2000). We found it highly relevant that changes in right superior OFC connectivity were correlated with changes in infant oral motor skills and CIB composite scores. Of note, a link between oral feeding in premature babies and cognitive development at 2 years was recently documented (Wolthuis-Stigter et al., 2015). Taken together, these findings suggest the hypothesis that the observed increased right superior OFC connectivity after OXT preserves OFC function, which may be important for long-term effects.

The preclinical data supporting the protective action of maternal OXT during delivery by preventing the deleterious effects of enhanced activity of GABA signaling(Tyzio et al., 2014), in addition to the current evidence of changes in brain connectivity after OXT administration in infants under than 6 months old, suggest a continuum in the role of OXT from birth to early post-natal life.

In conclusion, these results reveal that a short course of intranasal OXT administration rescues oral feeding and social skills in infants with PWS. These promising effects need to be confirmed by a phase 3 trial. In addition it will be important to evaluate the long term effects to determine if and how OXT changes the course of the disease. We anticipate that these results will also have a broad impact for infants with severely impaired sucking and infants with an early diagnosis of ASD.

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Legends of figures

Figure 1: Evaluation of sucking/swallowing before and after Oxytocin. All evaluations were performed by the same team speech and language pathologist. Individual data in each step are plotted. Step 1: 4 IU intranasal OXT every other day (4 administrations); step 2: 4 IU daily intranasal OXT (7 administrations); step 3: 4 IU twice daily intranasal OXT (14 administrations). **1A/** Clinical evaluation of sucking/swallowing was scored using the NOMAS. For this study as recommended for quantification, we scored each present item 0 or 1 when listed in the normal sucking pattern and 2 or 3 points when listed in the disorganized and dysfunctional sucking pattern category. One item of the disorganized category may be scored up to 4 when it documents the occurrence of severe complications (increased stridor, aspiration) driven by the impaired synchronization between sucking/swallowing and breathing (score can vary between 8 and 28, score $\leq$10 defining a near normal sucking pattern represented as a dotted line). **1B/** Videofluoroscopy of swallowing was scored using a grid with 9 items (score can vary between 11 and 29, the lower value defining a normal swallowing pattern).

Figure 2: Evaluation of behaviour and social skills before and after Oxytocin. **2A/** Scores of Clinical global impression (CGI) before (white box) and after the last OXT administration (grey box) before and during feeding. Evaluations were performed in 17 infants by the team speech and language pathologist concomitantly with the clinical scoring of sucking/swallowing. **2B/** Total Alarm Distress BaBy (ADBB) score of the 15 infants scored twice, before (white box) and after OXT administrations (grey box). The dotted line represent the cut-off value of normal score (<5). **2C/** Composites of the ADBB scale scored before (white box) and after OXT administrations (grey box). **2D/** Composites of the Coding Interactive Behaviour (CIB) scale scored before (white box) and after OXT administrations (grey box). Scoring was performed (Grading was obtained) in 15 infants except for the parental intrusiveness (N=14) using blinded videos taken before and during feeding by the same child psychiatrist.

In the boxplot the median is represented by a line subdividing the box. The length of the box thus represents the interquartile range (IQR). Whiskers are drawn to span all data points within 1.5 IQR of the nearer quartile. Any data points beyond the whiskers are shown individually. In the absence of any point beyond the whisker, the whisker identifies the extreme observed values (minimum or maximum). The scores of CGI, ADBB and CIB scales observed before and after treatment were compared using Wilcoxon signed rank tests for paired data.

Figure 3: Circulating acylated and unacylated ghrelin before and after Oxytocin**. 3A/**Evolution of plasmatic acylated ghrelin (AG) and **3B/**unacylated ghrelin (UAG) before the 1st OXT administration, 2 days and 4 days after the 1st OXT administration in 15 infants. Morning sampling were performed at

least 4hours after last feeding. The level of AG and UAG observed before and after treatment were compared using Wilcoxon signed rank tests for paired data.

Figure 4: Brain connectivity before and after Oxytocin. **4A/** Among the 17 infants who were evaluated twice, 7 infants were discarded either because the head motion exceeded our limits or because the images showed a significant static magnetic field (B0) inhomogeneity. Ten infants (4 in step 1, 3 in step 2 and 3 in step3) were kept for further analysis. Component obtained after ICA showing the regions with high connectivity (Z-score>3.1) (red regions) forming the network we selected for further analysis. The regions are superimposed onto spatially normalized anatomical image on one subject. The network is composed of the following cerebral areas: Supplementary Motor Area (1), Precuneus/Inferior Parietal (2), Supramarginal/Angular/Posterior Cingulate/Precuneus (3), Frontal/Temporal/Anterior Cingulate (4-6) ,inferior frontal opercular/Right Rolandic Operculum (7-8), Left calcarine/Lingual/Thalamus/Caudate (8-12), orbitofrontal/Rectus/Vermis (13-15). **4B/** Voxels showing greater connectivity Z-score in Post-OXT than Pre-OXT in 10 infants. The voxels are superimposed on anatomical T1 images of one subject. Changes in connectivity Z-score before and after OXT were measured with a paired t test Only the right superior orbito-frontal area, survived threshold at $p<0.05$ Family Wise Error corrected (small volume correction) and spatial extent >30. The small volume was defined as a 10 mm radius sphere centered on the max Z-score voxel. **4C/** Correlations between connectivity Z-score changes in right superior orbito-frontal area and NOMAS changes, CIB social engagement changes and CIB infant state changes were estimated for the whole sample by the Kendall Tau-b coefficient.

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ANNEXE 2 : RESULTATS DE L'ECHELLE D'EVALUATION DU COMPORTEMENT SOCIO ADAPTATIF DE VINELAND-II

Sujet : _____ Date : _____ Age : _____ Forme destinée au parent / à l'intervenant

SYNTHESE DES RESULTATS VINELAND-II

Notes de domaines et de sous-domaines

Sous-domaine / Domaine	Note brute	Note d'échelle-v Table B.1	Note standard de domaine Table B.2	___% Intervalle de confiance Table C.1/C.2	Rang percentile Table C.3	Niveau d'adaptation Table C.4	Age Equivalent Table C.5	Stanine Table C.3	Points Forts et Points faibles : Note moins Médiane*	Points Forts et Points faibles : F (Fort) f (Faible)
Réceptif				±						
Expressif				±						
Ecrit				±						
Communication	Somme :			±						
Personnel				±						
Domestique				±						
Communauté				±						
Vie quotidienne	Somme :			±						
Relations interpersonnelles				±						
Jeu et temps libre				±						
Adaptation				±						
Socialisation	Somme :			±						
Globale				±						
Fine				±						
Motricité	Somme :			±						

Somme des Notes standard de domaine = ____

	Note standard Table B.2	___% Intervalle de confiance Table C.2	Rang percentile Table C.3	Niveau d'adaptation Table C.4		Stanine Table C.3
Note composite de comportement adaptatif		±				

* *Pour le détail du calcul de la note médiane, voir Chapitre 3 du manuel.*

Forces/Faiblesses :
F = Note standard - Médiane ≥ 10
f = Note standard - Médiane ≤ -10

Forces/Faiblesses :
F = Note d'échelle-v - Médiane ≥ 2
f = Note d'échelle-v - Médiane ≤ -2

Indice de comportements problématiques	Note brute
Internalisation	
Externalisation	

Items critiques de comportements problématiques
Items (entourer les items notés 2 ou 1, indiquer la sévérité)
1 S M 2 S M 3 S M 4 S M 5 S M 6 S M 7 S M 8 S M 9 S M 10 S M 11 S M 12 S M 13 S M 14 S M

ANNEXE 3 : FEUILLE DE PASSATION DU QUESTIONNAIRE CBCL

INVENTAIRE DE COMPORTEMENTS POUR LES ENFANTS ÂGÉS DE 1 1/2-5 ANS

Merci de répondre en écrivant en lettres capitales.

NOM DE L'ENFANT Nom Prénoms

SEXE DE L'ENFANT ☐ Masculin ☐ Féminin

ÂGE DE L'ENFANT

DATE DE RÉPONSES Jour ____ Mois ____ Année ____

DATE DE NAISSANCE Jour ____ Mois ____ Année ____

Répondez à cet inventaire en vous fondant sur votre propre avis au sujet du comportement de votre enfant, même si cet avis n'est pas partagé par d'autres personnes. N'hésitez pas à écrire vos commentaires à côté de chaque question ou dans l'espace réservé pour cela en bas de la page 2. ***Vérifiez que vous avez répondu à toutes les questions.***

ACTIVITÉ PROFESSIONNELLE HABITUELLE DES PARENTS, même si cette profession n'est pas exercée actuellement.
Soyez aussi précis que possible - par exemple spécifiez : mécanicien auto, professeur de collège, ouvrier, tourneur, femme au foyer, vendeur de chaussures, sergent dans l'armée

PROFESSION DU PÈRE : _____

PROFESSION DE LA MÈRE : _____

CET INVENTAIRE EST REMPLI PAR (écrivez votre nom et vos prénoms) : _____

Votre sexe: ☐ Masculin ☐ Féminin

Votre type de parenté avec l'enfant :
☐ Parent biologique ☐ Beau-parent ☐ Grands-parents
☐ Parent adoptif ☐ Parent de famille d'accueil ☐ Autres (spécifiez)

Vous trouverez, ci-dessous, une liste de comportements qui décrivent les enfants. Répondez en vous fondant sur le comportement ***actuel*** de votre enfant - ou observé ***au cours des deux derniers mois.*** Pour chaque comportement, entourez la réponse qui correspond le mieux à votre enfant. Si le comportement décrit est ***très vrai ou souvent vrai,*** entourez le ②. Entourez le ①, si le comportement est ***parfois ou un peu vrai.*** Si le comportement décrit n'est ***pas vrai*** pour votre enfant, entourez le ⓪. Merci de donner une réponse pour tous les comportements, du mieux que vous pourrez, même si certains ne semblent pas s'appliquer à votre enfant.

0 = Pas Vrai (à votre connaissance) 1 = Parfois ou un Peu Vrai 2 = Très ou Souvent Vrai

0	1	2	1.	A des douleurs diverses (sans cause médicale connue - ***sauf*** des maux de ventre ou de tête)
0	1	2	2.	A des comportements trop jeunes pour son âge
0	1	2	3.	A peur d'essayer de nouvelles choses
0	1	2	4.	Evite de regarder les autres dans les yeux
0	1	2	5.	Ne peut pas se concentrer ou maintenir son attention longtemps
0	1	2	6.	Ne peut pas rester assis(e) tranquille, est agité(e) ou hyperactif(ve)
0	1	2	7.	Ne supporte pas que les choses ne soient pas à leur place
0	1	2	8.	Ne supporte pas d'attendre; veut tout tout de suite
0	1	2	9.	Mâchonne des choses qui ne sont pas à manger
0	1	2	10.	Se cramponne aux adultes ou est trop dépendant(e)
0	1	2	11.	Demande constamment de l'aide
0	1	2	12.	Constipé(e), ne va pas à la selle (quand il/elle n'est pas malade)
0	1	2	13.	Pleure souvent
0	1	2	14.	Cruel(le) avec les animaux
0	1	2	15.	Provoquant(e)
0	1	2	16.	Ses exigences doivent être satisfaites immédiatement
0	1	2	17.	Détruit ses affaires personnelles
0	1	2	18.	Détruit des affaires appartenant à sa famille ou à d'autres enfants
0	1	2	19.	A de la diarrhée ou des selles liquides (en l'absence de maladie)
0	1	2	20.	Désobéissant(e)
0	1	2	21.	Perturbé(e) par tout changement de routine
0	1	2	22.	Ne veut pas dormir seul(e)
0	1	2	23.	Ne répond pas quand on lui parle
0	1	2	24.	Ne mange pas bien (décrivez) : _____
0	1	2	25.	Ne s'entend pas bien avec les autres enfants
0	1	2	26.	Ne sait pas s'amuser, se conduit en petit adulte
0	1	2	27.	Ne semble pas se sentir coupable après s'être mal conduit(e)
0	1	2	28.	Ne veut pas sortir de la maison
0	1	2	29.	Facilement frustré(e)
0	1	2	30.	Facilement jaloux(se)
0	1	2	31.	Mange ou boit des choses qui ne sont pas des aliments - ***sauf*** les bonbons (décrivez) : _____
0	1	2	32.	A peur de certains animaux, situations ou lieux (décrivez) : _____
0	1	2	33.	Se sent facilement peiné(e)
0	1	2	34.	Se blesse souvent, a tendance à avoir des accidents
0	1	2	35.	Se bagarre souvent
0	1	2	36.	Se mêle de tout
0	1	2	37.	Trop perturbé(e) quand il/elle est séparé(e) de ses parents
0	1	2	38.	A du mal à s'endormir
0	1	2	39.	A des maux de tête (sans cause médicale connue)
0	1	2	40.	Tape les autres
0	1	2	41.	Retient sa respiration
0	1	2	42.	Fait mal aux animaux ou aux gens sans en avoir l'intention
0	1	2	43.	A l'air malheureux(se) sans raison évidente
0	1	2	44.	De mauvaise humeur, désagréable, hargneux

Assurez-vous d'avoir répondu à toutes les questions.
Allez ensuite au verso.

Version 2005-1-5
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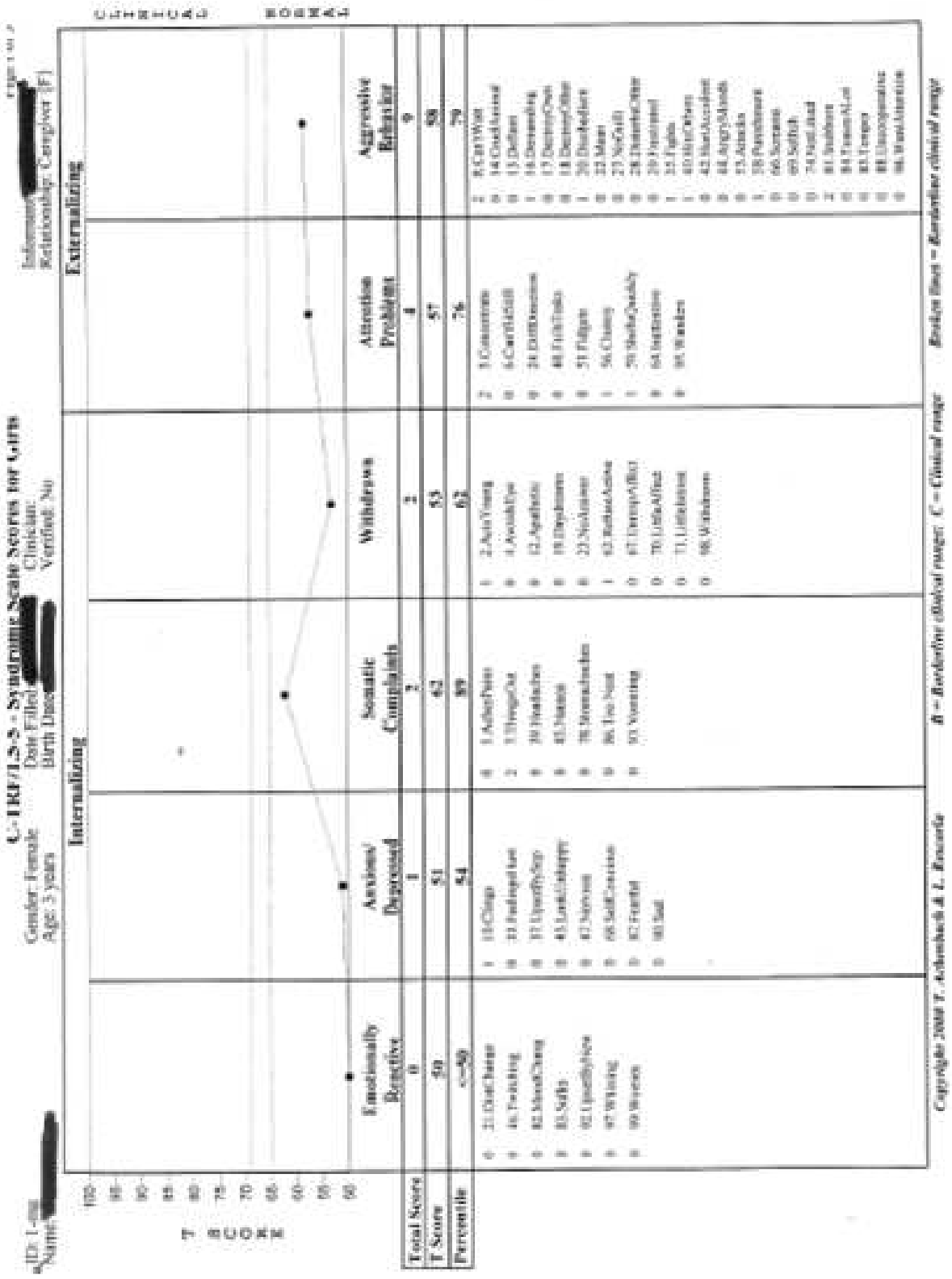
Page 1


C-TRF/1.5-5 - Syndrome Scale Scores for Girls
Gender: Female
Age: 3 years
Clinician:
Verified: No
Internalizing
Externalizing
Emotionally Reactive
Anxious/ Depressed
Somatic Complaints
Withdrawn
Attention Problems
Aggressive Behavior
Total Score
T Score
Percentile
T SCORE
BORDER
CLINICAL
Copyright 2000 T. Achenbach & L. Rescorla
B = Borderline clinical range; C = Clinical range
Broken lines = Borderline clinical range

ANNEXE 4 : ECHELLE D'EVALUATION DU COMPORTEMENT ALIMENTAIRE

- Enfant : ……..
- Date : …… Age : ……

- Evaluation faite par la mère le père

	Pas du tout d'accord	Plutôt pas d'accord	Plutôt d'accord	Tout à fait d'accord
1. Votre enfant mange de façon excessive (vu son âge) ⇒ depuis quand son appétit est-il augmenté ? ……				
2. Il est excessivement préoccupé par la nourriture				
3. Il réclame des quantités importantes lorsqu'on le sert				
4. Il met trop de nourriture à la bouche à la fois				
5. Il compare le contenu de son assiette à celui des autres				
6. Il finit son assiette intégralement en systématique				
7. Il a tendance à vouloir finir l'assiette des autres				
8. Il a faim à peine sorti de table				
9. Il mange entre les repas				
10. Il mange en cachette				
11. Il a tendance à se faire des réserves de nourriture				
12. Il mange des choses inhabituelles ou aliments impropres à la consommation. Description : ……				
13. On doit contrôler son accès à la nourriture. Méthodes : ne pas laisser traîner à vue fermer le frigo à clef enfermer dans les placards autre ……				

- 14. Moment de la journée où il a le plus faim :
  matin midi goûter soir
  pas de différence observée

- 15. Description ouverte du comportement de l'enfant en situation alimentaire

……
……
……

ANNEXE 5 : RCP DU MICROPAQUE

Fiche info | Résumé des caractéristiques du produit | Notice

SOMMAIRE

MICROPAQUE, suspension buvable ou rectale - Résumé des caractéristiques du produit

ANSM - Mis à jour le : 14/12/2015

1. DENOMINATION DU MEDICAMENT

MICROPAQUE, suspension buvable ou rectale

2. COMPOSITION QUALITATIVE ET QUANTITATIVE

Sulfate de baryum	100,00 g
Excipients à effet notoire :	
Sorbate de potassium (E202)	0,13 g
Citrate de sodium dihydraté	0,50 g
Saccharine sodique (E954)	0,02 g
Carmellose sodique (E466)	2,00 g
Parahydroxybenzoate de méthyle sodé (E219)	0,09 g
Parahydroxybenzoate de propyle sodé (E217)	0,05 g
Arôme vanille-caramel	0,05 g

Pour 100 mL de suspension buvable ou rectale.
Pour la liste complète des excipients, voir rubrique 6.1

3. FORME PHARMACEUTIQUE

Suspension buvable ou rectale.

4. DONNEES CLINIQUES

4.1. Indications thérapeutiques

Ce médicament est à usage diagnostique uniquement.
Produit de contraste destiné à être utilisé pour :

- Exploration radiologique conventionnelle de l'œsophage, estomac, duodénum et côlon
- en double contraste ou en réplétion.
- Transit du grêle.

4.2. Posologie et mode d'administration

Posologie

Estomac (réplétion) :
Diluer 150 mL de produit avec 300 mL d'eau (concentration p/v = 33 %)
Intestin grêle :
Diluer 500 mL de produit avec 750 mL d'eau (concentration p/v = 40 %)
Lavement baryté :
en double contraste : 500 mL de produit pur (concentration p/v = 100 %)
en réplétion :

- 500 mL de produit dilué avec 1 litre d'eau (concentration p/v = 33 %)

OU

- 500 mL de produit dilué avec 1,5 litre d'eau (concentration p/v = 25 %)

Sujets âgés

Aucune adaptation posologique n'est nécessaire pour cette population de patients. Toutefois, une attention particulière doit être portée aux contre-indications et mises en garde listées ci-dessous.

Population pédiatrique

Pour les nourrissons et les enfants, le médecin adaptera la dose en fonction de l'âge et du poids de l'enfant et en tenant compte des particularités du diagnostic radiologique pédiatrique.

Mode d'administration

Agiter le flacon avant emploi puis laisser reposer jusqu'à disparition des bulles d'air.
L'examen sera réalisé :

- à jeun en cas d'exploration digestive haute,
- après lavement évacuateur administré la veille et régime sans résidu 3 jours avant l'examen en cas d'exploration digestive basse.

4.3. Contre-indications

Ce médicament est contre-indiqué en cas de :

- Hypersensibilité à la substance active ou à l'un des excipients
- Perforation ou occlusion intestinale avérée ou suspectée, y compris sténose du pylore.
- Péritonite ou fistule suspectée dans le tube digestif
- Plaies récentes ou érosions de l'oesophage ou du tube digestif ou hémorragie gastro-intestinale
- Ischémie de la paroi intestinale
- Entérocolite nécrosante
- Mégacôlon ou mégacôlon toxique
- Lâchage de suture postopératoire. Le sulfate de baryum ne doit pas être administré immédiatement avant, et jusqu'à sept jours après une intervention chirurgicale gastro-intestinale, y compris en cas de résection endoscopique, de polypectomie à l'anse, ou de biopsie à chaud du côlon. Le médicament ne doit pas être utilisé si des fuites postopératoires sont prévisibles. Ne pas utiliser le produit en cours de radiothérapie du cou, du thorax (si utilisation orale) ou de l'abdomen et jusqu'à quatre semaines après la radiothérapie.

4.4. Mises en garde spéciales et précautions d'emploi

Mises en garde spéciales

Ne pas injecter par voie parentérale.
Des effets indésirables graves associés à l'administration de produits à base de sulfate de baryum ont été rapportés : inhalation, intravasation, perforation, anaphylaxie pouvant mettre en jeu le pronostic vital ou conduire à une issue fatale. Ces effets indésirables sont souvent liés à la technique d'administration du produit, à la pathologie sous-jacente et/ou à l'hypersensibilité des patients.
Le produit doit être administré sous surveillance médicale. Les effets indésirables potentiellement graves doivent être détectés, évalués et diagnostiqués rapidement afin de permettre la mise en place d'un traitement efficace. Le personnel des services d'imagerie doit être formé et à même de gérer le diagnostic et le traitement des réactions d'hypersensibilité. Des mesures médicales spécifiques d'urgence doivent être prises en cas d'inhalation, d'intravasation ou de perforation (soins intensifs, chirurgie).
Pour prévenir les effets indésirables graves, il convient d'empêcher le passage du sulfate de baryum dans les régions parentérales telles que les tissus, l'espace intravasculaire ou les cavités corporelles, ainsi que les voies respiratoires.

Population pédiatrique

L'indication doit être soigneusement pesée chez les jeunes enfants souffrant de défaillance multiviscérale, particulièrement au niveau du système cardio-vasculaire, car l'examen et la préparation peuvent être stressants.

Sujets âgés

L'indication doit être soigneusement pesée chez les patients âgés souffrant de défaillance multiviscérale, particulièrement au niveau du système cardio-vasculaire, car l'examen et la préparation peuvent être stressants.
L'examen du côlon en double contraste est parfois difficilement supporté chez les sujets âgés ou pléthoriques difficilement mobilisables.

Précautions d'emploi

Perforation

Compte tenu du risque accru de perforation, le rapport bénéfice/risque doit être soigneusement évalué chez les patients souffrant d'atrésie de l'oesophage ou de sténose sévère (spécifiquement en aval de l'estomac), ainsi que chez les patients souffrant d'affections et de pathologies impliquant un risque accru de perforation tels qu'un carcinome intestinal, une maladie inflammatoire de l'intestin, une diverticulose, une diverticulite et une parasitose.
En cas d'atrésie de l'oesophage, l'opacification de la poche doit être évitée autant que possible. Si elle est pratiquée, la plus petite quantité possible de produit de contraste doit être administrée puis réaspirée.
Des fuites de baryum dans le rétropéritoine ou dans le médiastin peuvent se manifester par quelques symptômes immédiats mais un choc endotoxique retardé, qui se révèle fréquemment fatal, peut survenir 12 heures plus tard.
En cas de perforation intestinale, le passage du sulfate de baryum dans la cavité abdominale peut être à l'origine d'abcès, d'inflammations, de péritonites, de granulomes et d'adhérences dont l'évolution peut être fatale et qui nécessitent un recours à la chirurgie.

Inhalation

Si le produit est utilisé oralement, il convient de surveiller particulièrement les patients souffrant de troubles respiratoires et de troubles de la déglutition tels que la dysphagie, ainsi que de troubles de la vigilance, car il existe un risque d'inhalation.
Les vomissements après l'administration orale de sulfate de baryum peuvent provoquer une pneumonie d'inhalation.
Le baryum ne doit pas être administré à des patients ayant des antécédents de fausse route alimentaire. Si l'utilisation du baryum est absolument nécessaire chez ces patients, une prudence particulière s'impose. Dans le cas de passage dans le larynx, l'administration du produit doit être immédiatement interrompue.

Population pédiatrique

Chez les patients hautement prédisposés à l'inhalation (nouveaux-nés) la procédure doit débuter par une faible dose orale.
L'inhalation dans l'arbre trachéobronchique peut survenir lors de l'administration de suspension buvable de sulfate de baryum en bouteille à des nourrissons et lors de l'administration de quantités importantes par cathéter. Chez les nourrissons, l'inhalation peut entraîner un arrêt cardio-respiratoire avec issue fatale. L'inhalation de petites quantités peut occasionner une inflammation des voies respiratoires et une pneumopathie.

Sujets âgés

Chez les patients hautement prédisposés à l'inhalation (sujets âgés et ayant subi un AVC), la procédure doit débuter par une faible dose orale.

Surcharge liquidienne

Une surcharge liquidienne due à l'absorption de l'eau contenue dans les suspensions de sulfate de baryum a été rapportée. Les enfants et les patients insuffisants rénaux ainsi que les enfants atteints de la maladie de Hirschsprung sont plus exposés à une intoxication par l'eau.

Population pédiatrique

Chez les enfants atteints de la maladie de Hirschsprung, il est préférable de ne pas remplir entièrement le côlon, mais d'utiliser uniquement le volume de liquide requis pour le diagnostic.

Intravasation

Le sulfate de baryum peut traverser la paroi intestinale et pénétrer dans le drainage veineux du côlon, permettant ainsi à une embolie barytée d'atteindre le système circulatoire. L'introduction et le positionnement du matériel servant à l'administration du produit de contraste doivent se faire de façon soigneuse et correcte, en limitant l'usage du cathéter à ballonnet, afin d'éviter des lésions de la paroi.

L'intravasation est très rare mais peut avoir des complications fatales : embolie systémique et pulmonaire, coagulation intravasculaire disséminée, septicémie et hypotension artérielle sévère persistante. Cette complication est plus fréquente chez les patients âgés dont les parois rectale et vaginale sont plus fines ainsi que chez les patients souffrant d'affections colorectales, chez qui la pression intraluminale est plus forte que la résistance de la paroi du côlon en cas de colite, de diverticulite et d'occlusion intestinale. Cette complication a également été observée en raison du positionnement accidentel d'un cathéter rectal dans le vagin. Il convient donc de vérifier le positionnement correct du cathéter rectal avant le début du lavement.

Ce diagnostic est à prendre en compte pour tous les patients qui s'évanouissent pendant ou après l'administration de baryum et pour les patients souffrant de malaises dans les premières heures suivant l'examen. Il peut être confirmé par un examen radiologique sans produit de contraste. Un scanner peut permettre de détecter la dissémination du sulfate de baryum.

Fécalome baryté

Une prudence d'utilisation s'impose en cas de constipation préexistante, de troubles de la vidange gastrique, sténose colique, diverticulose et atonie du côlon, en raison du risque de fécalome baryté, notamment chez les personnes âgées.

Le sulfate de baryum peut être retenu dans des diverticules coliques où il peut entretenir ou intensifier des infections.

Les fécalomes barytés se développent à partir de baryum compacté dans les fèces. Ils sont souvent asymptomatiques mais peuvent provoquer douleurs abdominales, appendicite, occlusion ou perforation intestinale.

Le sulfate de baryum peut provoquer ou aggraver une constipation préexistante.

Pour prévenir une constipation sévère et la formation de fécalome baryté, on s'assurera du bon état d'hydratation préalable et on favorisera la mobilisation après l'examen. L'utilisation de laxatifs est à envisager (particulièrement en cas de constipation). Les patients présentant une constipation avant l'examen doivent faire l'objet d'un contrôle particulier, en raison du risque de fécalome.

Sujets âgés

Le risque de développement de fécalomes barytés est plus élevé chez les patients âgés atteints de troubles de la motricité intestinale, d'ileus, de déséquilibre électrolytique, de déshydratation ou dont l'alimentation est carencée en fibres. Pour éviter que la paroi intestinale soit endommagée, il convient d'extraire les fécalomes barytés.

Autres complications possibles

L'administration du sulfate de baryum peut provoquer des réactions vaso-vagales, des épisodes de syncope, de l'arythmie et d'autres complications cardiovasculaires. Ces réactions sont en général imprévisibles et sont mieux prises en charge si le patient reste en observation 10 à 30 minutes après l'examen.

Excipients

Ce médicament contient du glucose et du saccharose dans l'arôme vanille-caramel. Son utilisation est déconseillée chez les patients présentant une intolérance au fructose, un syndrome de malabsorption du glucose et du galactose ou un déficit en sucrase/isomaltase.

Ce médicament contient 1,76 g de sodium pour 500 ml de suspension. A prendre en compte chez les patients suivant un régime hyposodé strict.

Ce médicament contient 170 mg de potassium pour 500 ml de suspension. A prendre en compte chez les patients insuffisants rénaux ou chez les patients suivant un régime hypokaliémant.

Ce médicament contient du « Parahydroxybenzoate » et peut provoquer des réactions allergiques (éventuellement retardées).

4.5. Interactions avec d'autres médicaments et autres formes d'interactions

Le sulfate de baryum étant utilisé seul en tant qu'agent de contraste et n'étant pas absorbé, aucune interaction médicamenteuse n'est à prévoir. Cependant, l'administration simultanée d'autres médicaments n'est pas recommandée, car l'adsorption par le sulfate de baryum pourrait en réduire les effets. La prudence s'impose lorsque le sulfate de baryum est administré de manière concomitante avec d'autres médicaments à index thérapeutique étroit.

L'élimination lente de la baryte amène la présence de résidus opaques qui peuvent être gênants pour d'autres radiographies comme une urographie ou un examen tomodensitométrique dans les jours qui suivent.

4.6. Grossesse et allaitement

Grossesse

Il n'existe pas de données sur l'utilisation du sulfate de baryum chez la femme enceinte. Par ailleurs, en règle générale, toute exploration radiologique digestive doit être évitée chez la femme enceinte.

Allaitement

Aucune raison théorique ne fait suspecter le passage dans le lait. Micropaque peut être utilisé pendant l'allaitement.

4.7. Effets sur l'aptitude à conduire des véhicules et à utiliser des machines

Les effets sur l'aptitude à conduire des véhicules et à utiliser des machines n'ont pas été étudiés. Cependant, les propriétés pharmacodynamiques et pharmacocinétiques du sulfate de baryum ne sont pas de nature à avoir un effet sur l'aptitude à conduire des véhicules et à utiliser des machines.

4.8. Effets indésirables

Les effets indésirables liés à l'utilisation de Micropaque sont présentés dans le tableau ci-dessous par Système Organe Classe et par fréquence en utilisant les catégories suivantes : très fréquent (≥ 1/10), fréquent (≥1/100 à 1<1/10), peu fréquent (≥1/1 000 à 1<1/100), rare (≥1/10 000 à <1/1 000), très rare (<1/10 000), fréquence indéterminée (ne peut être estimée sur la base des données disponibles).

Système Organe Classe	Fréquence : effet indésirable

Affections hématologiques et du système lymphatique	Fréquence indéterminée : En cas d'intravasation : coagulation intravasculaire disséminée
Affections du système immunitaire	Fréquence indéterminée : réaction anaphylactique, réaction anaphylactoïde, hypersensibilité
Affections gastro-intestinales	Fréquence indéterminée : occlusion intestinale, vomissement, diarrhée, douleurs abdominales, fécalome, distension abdominale, constipation, nausée, flatulence ; en cas de perforation intestinale : péritonite
Affections respiratoires, thoraciques et médiastinales	Fréquence indéterminée : pneumopathie d'inhalation, fibrose pulmonaire
Affections du système nerveux	Fréquence indéterminée : présyncope
Affections de la peau et du tissu sous-cutané	Fréquence indéterminée : urticaire, rash généralisé

Le parahydroxybenzoate de méthyle sodé et le parahydroxybenzoate de propyle sodé peuvent provoquer des réactions d'hypersensibilité immédiates et retardées.

Les produits de contraste contenant du sulfate de baryum peuvent provoquer des réactions d'hypersensibilité d'intensité variable. Très rares, ces réactions peuvent toutefois apparaître dès la première administration. L'administration du produit doit alors être interrompue immédiatement et un traitement spécifique doit être mis en place.

Dans de très rares cas, l'administration de sulfate de baryum peut provoquer la formation de fécalomes barytés.

En cas de perforation intestinale, le passage du sulfate de baryum dans la cavité abdominale est à l'origine d'abcès, d'inflammations, de péritonite, granulomes et adhérences dont l'évolution peut être fatale et qui nécessitent un recours à la chirurgie.

En cas de passage extra luminal du sulfate de baryum, d'exceptionnels cas d'intravasation s'accompagnant d'une diffusion systémique de sulfate de baryum et potentiellement fatals peuvent survenir. L'intravasation du sulfate de baryum peut être responsable d'embolies veineuses profondes, choc, détresse respiratoire et coagulation intra vasculaire disséminée.

Déclaration des effets indésirables suspectés

La déclaration des effets indésirables suspectés après autorisation du médicament est importante. Elle permet une surveillance continue du rapport bénéfice/risque du médicament. Les professionnels de santé déclarent tout effet indésirable suspecté via le système national de déclaration : Agence nationale de sécurité du médicament et des produits de santé (Ansm) et réseau des Centres Régionaux de Pharmacovigilance - Site internet : www.ansm.sante.fr

4.9. Surdosage

De rares cas de surdosage ont été rapportés lors de l'ingestion du sulfate de baryum. Les cas sont en général asymptomatiques. Cependant quelques cas de douleur abdominale ont été rapportés.

Le surdosage par voie orale implique un risque de constipation avec, dans certains cas extrêmes, la formation de fécalomes barytés. Le traitement est constitué de lavements évacuateurs et/ou de laxatifs salins.

5. PROPRIETES PHARMACOLOGIQUES

5.1. Propriétés pharmacodynamiques

Classe pharmacothérapeutique : Produit de contraste baryté ; code ATC : V 08 BA 01

Le sulfate de baryum est utilisé comme opacifiant radiologique du tube digestif. Les contrastes naturels sont suffisants pour explorer radiologiquement le squelette ou les poumons, les examens des autres organes doivent mettre en jeu des contrastes artificiels obtenus par l'utilisation de solution ou suspension d'éléments de masse atomique élevée comme le baryum, il s'agit alors de contraste positif ou par des gaz : contraste négatif. Les deux pouvant être associés dans la technique du double contraste, par exemple.

Le baryum de masse atomique 137 a la propriété d'accroître fortement l'absorption des rayons X. Utilisé indifféremment par voie orale ou rectale, il tapisse le tube digestif dont il va permettre la localisation précise sur les images radiologiques.

5.2. Propriétés pharmacocinétiques

Le sulfate de baryum, pratiquement insoluble dans l'eau et les solvants organiques, n'est pas absorbé au niveau du tractus digestif.

5.3. Données de sécurité préclinique

Les données non cliniques issues des études conventionnelles de toxicologie en administration unique ou répétée n'ont pas révélé de risque particulier pour l'homme.

6. DONNEES PHARMACEUTIQUES

6.1. Liste des excipients

Sorbate de potassium (E202), citrate de sodium dihydraté, saccharine sodique (E954), gomme xanthane (E415), carmellose sodique (E466), parahydroxybenzoate de méthyle sodé (E219), parahydroxybenzoate de propyle sodé (E217), arôme vanille caramel*, acide sulfurique, eau purifiée.

* Arôme vanille caramel : vanilline, éthyl vanilline, mélilotine, piperonal, diacétyle, butyrate d'éthyle, acide butyrique, maltol, huile essentielle de citron, caprylate d'éthyle, caramel, propylène glycol, eau purifiée.

6.2. Incompatibilités

En l'absence d'études d'incompatibilité, ce médicament ne doit pas être mélangé avec d'autres médicaments.

6.3. Durée de conservation

2 ans

6.4. Précautions particulières de conservation

A conserver à l'abri du gel.

6.5. Nature et contenu de l'emballage extérieur

Flacon (polyéthylène opaque) fermé par un bouchon à vis (polypropylène) contenant 150 ml, 500 ml ou 2 l de suspension.

Toutes les présentations peuvent ne pas être commercialisées.

6.6. Précautions particulières d'élimination et de manipulation

Agiter le flacon avant emploi.
Tout médicament non utilisé ou déchet doit être éliminé conformément à la réglementation en vigueur.

7. TITULAIRE DE L'AUTORISATION DE MISE SUR LE MARCHE

GUERBET
BP 57400
95943 ROISSY CHARLES DE GAULLE CEDEX

8. NUMERO(S) D'AUTORISATION DE MISE SUR LE MARCHE

- 34009 306 749 9 4 : flacon (polyéthylène) de 150 mL.
- 34009 306 750 7 6 : flacon (polyéthylène) de 500 mL.
- 34009 550 021 0 9 : flacon (polyéthylène) de 2 L.

9. DATE DE PREMIERE AUTORISATION/DE RENOUVELLEMENT DE L'AUTORISATION

[à compléter par le titulaire]

10. DATE DE MISE A JOUR DU TEXTE

[à compléter par le titulaire]

11. DOSIMETRIE

Sans objet.

12. INSTRUCTIONS POUR LA PREPARATION DES RADIOPHARMACEUTIQUES

Sans objet.

CONDITIONS DE PRESCRIPTION ET DE DELIVRANCE

Médicament non soumis à prescription médicale.

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