Escalating dose study of repeated intranasal oxytocin in infants with PWS

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OTBB-2

BIOMEDICAL RESEARCH PROTOCOL

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LIST OF ABBREVIATIONS
PWS: Prader-Willi syndrome
OT: oxytocin
GH: growth hormone
CSF: cerebrospinal fluid
NOMAS: Neonatal Oral-Motor Assessment Scale
EFS: early feeding skills
1. SUMMARY OF RESEARCH

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<table>
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<tr>
<td>TITLE</td>
<td>Escalating dose study of repeated intranasal oxytocin in infants with PWS</td>
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<tr>
<td>JUSTIFICATION / CONTEXT</td>
<td>PWS is a genetic disorder of development affecting 1/20,000 children at birth, presenting with neonatal hypotonia and severe sucking deficiency that can cause failure to thrive. Today PWS is diagnosed in the 1st week of life. Without specific management and preventive measures, obesity and other endocrine abnormalities occur in the second year of life, probably due to hypothalamic dysfunction. An abnormality of the OT-secreting neurons in the paraventricular hypothalamic nucleus has been observed in both patients and two animal models (necdin- and Magel2-null mice). We hypothesize that the early pathophysiological failure of oxytocinergic channels causes both the sucking disorder and disturbed behavior observed in this disease. Also, results in the Magel2 mouse model show that OT administration on the first day of life (and not later) restores sucking and prevents death. We therefore hypothesize that early OT administration will improve sucking and behavior in infants with PWS. The first tolerance test was conducted in 2011 (AOL in 2010) and showed no adverse effects of a single intranasal administration of OT in five infants under 6 months old with PWS. Moreover, the data suggested a positive effect on sucking/swallowing and mother-infant interactions 48 hours after administration. This new study investigates the tolerability of repeated intranasal OT administration over 7 days in babies with PWS under 6 months old.</td>
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<tr>
<td>OBJECTIVES</td>
<td>The principal objective is to study the tolerance to repeated OT administrations over the 7 days following the first administration, according to three escalating dose steps in babies with PWS under 6</td>
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months old.

The secondary objectives are:
- to perform a preliminary study of OT effectiveness on:
  - sucking/swallowing and feeding
  - mother-infant interactions before, during and after feeding
  - weight and growth changes
- to perform a physiopathological study of OT effects on:
  - cerebral metabolism
  - circulating levels of ghrelin and peptides and neuropeptides involved in appetite regulation (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH)
- to perform a minimal pharmacokinetic study: measuring the circulating OT levels before and during treatment

<table>
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<th>RESEARCH SCHEME</th>
<th>Open label phase 1-2 escalating dose study with three steps</th>
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<tr>
<td>INCLUSION CRITERIA</td>
<td>- Newborn presenting PWS (genetic confirmation of diagnosis)</td>
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<td>- Age under 6 months old</td>
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<td>- No prolongation of the QT interval on EKG</td>
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<td>- No hypokalemia</td>
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<td>- Consent form signed by parents or legal guardians and infant registered with the social security system</td>
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<td>EXCLUSION CRITERIA</td>
<td>- Administrative problems: unable to communicate full information to parents or guardians, infant not covered by the social security system, parental refusal to sign the consent form</td>
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<td>- Hypersensitivity to product excipients</td>
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<td>- Familial antecedents of genetic pathology provoking prolonged QT interval</td>
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<td>- Concomitant treatment that might provoke a prolonged QT interval</td>
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<td>- Newborn presenting hepatic insufficiency</td>
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<td>- Newborn presenting renal insufficiency</td>
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<tr>
<td>RESEARCH TREATMENT/STRATEGIES/</td>
<td>Repeated intra-nasal administrations of OT for 7 days according to three modalities:</td>
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### PROCEDURES
- Step 1: 4 IU once a day, every other day (4 administrations)
- Step 2: 4 IU once a day, every day (7 administrations)
- Step 3: 4 IU twice a day, every day (14 administrations)

### ENDPOINTS
**Primary endpoint:** adverse systemic effects and OT implicated in any of the three steps in the 7 days following the first dose

**Secondary endpoints:**
- *Assessment of effectiveness* after 7 days of treatment on:
  - Sucking/swallowing (quantity of bottle milk taken/24 hr, feeding duration, amount of milk taken/24 hr, radioscopy)
  - Mother-infant interactions before and during feeding
  - Weight and height changes
- *Physiopathological study:*
  - BOLD MRI of brain
  - Circulating levels of ghrelin and peptides and neuropeptides involved in appetite regulation (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH)
- *Minimal pharmacokinetic study:*
  - Circulating OT levels measured before the first administration and every 48 hr

### NUMBER OF SUBJECTS INCLUDED
18 (maximum number of babies)

### NUMBER OF CENTERS
1

### RESEARCH DURATION
- Inclusion period: 18 months
- Participation of each patient: 30 days
- Total research: 24 months

### STATISTICAL ANALYSES OF DATA
- Description of side effects with each dose step
- Comparison before/after administration on sucking/swallowing, mother-infant interactions, cerebral metabolism, circulating levels of OT, ghrelin and other peptides and neuropeptides involved in appetite regulation

### EXPECTED BENEFITS
If tolerance to repeated intranasal OT administration is good and infant sucking/swallowing and behavior are improved, we propose a new
| study of OT effectiveness with the optimal dose |
2. ABSTRACT

This research was registered in [http://www.clinicaltrials.gov/](http://www.clinicaltrials.gov/) on date under the n° numéro.

**Evaluation of tolerance, sucking and food intake after repeated nasal administrations of oxytocin in PWS infants**

Acronym OTBB2

CHU de Toulouse is the sponsor of this research.

This research will be conducted with the support of AOL.

**Background:** Prader-Willi syndrome (PWS) is a rare, complex, multisystem genetic disorder due to lack of expression of paternally inherited imprinted genes on chromosome 15q11-q13. The syndrome includes severe neonatal hypotonia with impaired sucking, leading to failure to thrive in the most severe cases, subsequently followed by early-onset morbid obesity with hyperphagia and deficit of satiety, as well as other endocrine dysfunctions probably due to hypothalamic dysfunction. The physiopathological mechanisms of the two main nutritional phases of PWS are unknown. Swaab reported a deficit in the OT-producing neurons of the paraventricular nucleus in patient brains. In addition to its well-known anorexigenic effect, OT is involved in establishing and maintaining social codes. Moreover, in a PWS model with Magel2-KO mice, a single OT injection at 5 hr of life prevented early death in 50% of the newborn mice by recovering normal sucking. Interestingly, this effect is no longer observed if the OT injection takes place later than this. We hypothesize that early administration of OT in babies with PWS will improve sucking and possibly mother-infant interactions. In our recent study (manuscript in preparation), we have shown that a single intranasal administration of OT is well tolerated. This escalating dose study is designed to evaluate the tolerability of repeated intranasal OT administration in 3 steps (4 IU every other day, 4 IU daily, 4 IU twice daily) in babies under 6 months old with PWS.

**Purpose**

- **Abstract: Primary endpoint:** Occurrence of adverse events, description and quantification of their severity, imputability to repeated intranasal OT administration (4 IU every other day, 4 IU daily, 4 IU twice daily) during the 7 days following the first administration.

- **Secondary endpoints:**
  - Preliminary study of efficacy on:
• sucking and swallowing and quantity of milk intake/day
• mother-infant interactions before, during and after feeding
• weight gain and growth
• Pathophysiological study of:
  • cerebral metabolism (BOLD fMRI)
  • plasma levels of ghrelin and other peptides involved in feeding behavior or energy metabolism (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide (PP), orexin A, αMSH)
  • Minimal pharmacokinetic study: measurement of circulating OT levels before administration and every 48 hrs.
• Study design: escalating dose study with three steps
• Eligibility criteria:
  • inclusion criteria:
    o infants with PWS genetically confirmed
  • under 6 months old exclusion criteria:
    o infants presenting hepatic insufficiency
    o infants presenting renal insufficiency
    o infants with abnormal ECG
• Interventions: three steps of intranasal OT administration over 7 days
  • 4 IU of OT every other day for the six first babies
  • 4 IU of OT daily for the next six babies
  • 4 IU of OT twice daily for the last six babies
• Number of subjects: 18
• **Statistical analysis:**

  - Description of adverse events at every step
  
  - Comparison before/after administration of OT on sucking/swallowing, mother-infant interactions, weight gain, quantity of feeding intake per day
  
  - Evolution of brain metabolism by BOLD fMRI
  
  - Evolution of circulating levels of ghrelin and other neuropeptides involved in appetite regulation
  
  - Evolution of circulating OT levels
3. JUSTIFICATION AND GENERAL DESCRIPTION

3.1. CURRENT STATE OF KNOWLEDGE

PWS was first described in 1956. It is caused by the lack of expression of the paternal allele of the q11-q13 region of chromosome 15 due to microdeletion, maternal disomy, translocation, or imprinting defect [1]. Clinically, PWS is a genetic disorder of development affecting 1/20,000 children at birth, characterized by neonatal hypotonia and severe sucking deficit that may require nasogastric tube feeding to prevent failure to thrive. In the absence of adapted management and preventive measures, this phase may be followed in the second year by obesity with hyperphagia and impaired satiety. The obesity is generally associated with other endocrine abnormalities (GH deficiency, hypogonadism) and behavioral disorders and is probably related to a hypothalamic syndrome. An abnormality of the OT-secreting neurons in the paraventricular hypothalamic nucleus has been observed in both patients [2] and two animal models (necdin- and Magel2-null mice [3,4]). The OT deficit (an anorexigenic hormone) and early increase in circulating ghrelin (an orexigenic hormone) [5] found in patients could explain the two phases of the syndrome: a sucking deficit followed by obesity with hyperphagia, impaired satiety and behavioral disorders. In addition, the results obtained in the Magel2 mouse model shows that OT administration on the first day of life (and not later) restores sucking and prevents death [3].

OT has a wide range of functions, both peripheral (the oldest known concerns uterine muscle and mammary glands) and central (concerning appetite and satiety regulation and, more recently, the implementation of social functions, emotion regulation and attachment phenomena).

OT and feeding behavior

In adult rats, OT administered by intraventricular or intraperitoneal route induced a dose-dependent inhibition of nutritional intake (reduced food intake, reduced feeding duration, prolonged fasting period) and water ingestion. Conversely, intraventricular injection of an OT antagonist increased appetite and prevented the antidipsogenic effect of OT [6]. The results of other studies in rats [7] indicated the role of OT in appetite regulation. Central oxytocinergic projections appeared to play a role in controlling the consumption of sugary foods [8, 9]. Amico et al. [8] demonstrated that targeted deletion of the OT gene in mice was associated with a high intake of sugary foods. In addition, central OT seemed to limit the intake of unknown foods and sugary, appetizing foods. Recent data also suggest its role in the control of satiety.

The effect of OT on the stimulation of sucking observed in Magel2-KO mice at birth therefore seems different and new from this viewpoint. However, recent data on the collaboration between OT and endocannabinoids [10] in the hypothalamus, specifically in supraoptic and paraventricular nuclei [10],
might explain the important role of OT in sucking. In addition, OT is involved in early mother-child interactions and attachment processes. In PWS, OT deficiency could therefore explain both the sucking problems observed at birth and subsequently the long-term disorders related to a defect in early establishment of appetite regulation circuits and certain behavior disorders [6].

We will not describe the major effects of OT on social behavior and emotions here, as they are not the subject of this study.

**OT in patients with PWS**

1) PWS patients have a dysregulation of OT

In 1995, Swaab et al. [2] studied the post-mortem brains of individuals with PWS and found a significant reduction (42%, \( p = 0.016 \)) in the number and volume (54%, \( p = 0.028 \)) of neurons expressing OT in the paraventricular hypothalamic nucleus in 5 patients (aged 22-64 years) in comparison with the findings in 27 healthy subjects of the same age and sex. However, in 1998, Martin et al. [11] reported a high rate of OT in the cerebrospinal fluid (CSF) of patients with PWS. It should nevertheless be noted that this study had methodological limitations because the number of patients was low and patients and controls were not matched. Furthermore, circulating OT levels have been reported to be normal in adult patients with PWS [12]. The data on OT levels in plasma or CSF are few and contradictory. Peripheral OT levels are probably a poor reflection of the activity of oxytocinergic pathways, especially at the hypothalamic level.

2) OT administration in patients with PWS

In a double-blind pilot study of OT vs placebo promoted by the CHU de Toulouse (University Hospital of Toulouse), we showed for the first time that adult patients with PWS who had received a single intranasal dose of OT (24 IU) had fewer tantrums, arguments and feelings of sadness than those who had received placebo. [13] Moreover, appetite seemed to decrease in the OT group.

More recently, our 2011 tolerance study (AOL 2010) in 5 babies with PWS under 6 months old showed no adverse effects of a single intranasal administration of OT (2 IU and 4 IU). In addition, the data analysis indicated a positive effect on sucking/swallowing and mother-infant interactions 48 hours after administration.
3.2. RESEARCH HYPOTHESES AND EXPECTED RESULTS

Infants with PWS have poor suck and demand little, seeming to show a certain lack of interest in food, which often prompts the placement of a nasogastric tube or more rarely gastrostomy.

Today these treatments are shorter because of the parental support given from the time of diagnosis, but in most cases symptoms persist for at least the first 6 months and prolonged bottle feeding times. Also, it increasingly seems that these feeding disturbances are less closely related to hypotonia and more to a real lack of interest in food. In a second phase from the age of 18 months to 3 years, excessive weight gain and hyperphagia occur, with a deficit of satiety, strong food compulsions, incessant search for and obsession with food, and food storing, all of which result in early and severe obesity. Two teams recently reported that the weight curve begins to rise even before the onset of hyperphagia [14,15]. This sudden and early onset of obesity can probably be explained by the reduced energy requirements related to the lower basal metabolism (approximately 60% of normal for their age) and could be linked to the early rise (before obesity) in ghrelin [5]. The only abnormal appetite-regulating hormones in PWS are oxytocin (anorectic hormone involved in satiety), which seems to be deficient and certainly dysregulated, ghrelin (orexigenic hormone involved in food obsession), which is high in blood very early on and throughout life, and pancreatic polypeptide (PP, which is low).

The main evidence supporting early intervention with OT comes from a recent study [4] in mice. In a Magel2-KO mouse model, the administration of a single injection of OT on the first day of life completely prevented mouse death by restoring the sucking reflex, although death usually occurs in 50% of the cases in both sexes. It now needs to be demonstrated that OT secretion or maturation is defective in these animals due to the inactivation of the Magel2 gene and that exogenous OT administration in this biological window corrects the sucking defect and prevents death. This was the first demonstrated of the early effect of OT on sucking [4].

Early OT administration could have the same effects in infants with PWS and would thus significantly improve their early feeding disturbances and weight gain.

This disease currently has no treatment and the hypothesis that early treatment within a restricted biological window (first months of life) has positive effects justifies this early-life study. It is also possible that early correction of the OT deficit will prevent the second-phase abnormal eating behavior and obesity and considerably transform the quality of life of these patients. In situations other than the PWS, early feeding disorders with poor weight gain followed by excessive weight catch-up are more frequently associated with obesity [16]. We filed an international patent in 2010 for oxytocin and its analogs known
in PWS, through an INSERM transfer with a partnership between INSERM and the CHU de Toulouse, and all seems on track for the patent to be granted.

We hypothesize that early OT administration will improve sucking and behavior (mother-child interactions) in infants with PWS. The first tolerance study in 2011 (AOL 2010) showed no adverse effects of a single intranasal OT administration in 5 infants with PWS under 6 months old. In addition, data analysis indicated a positive effect on sucking/swallowing and mother-infant interactions 48 hours after the dose. This new study aims to investigate the tolerance to repeated intranasal OT administrations for 7 days in infants with PWS under 6 months old, with escalating doses in three steps.

3.3. JUSTIFICATION OF METHODOLOGICAL CHOICES

The narrow biological window demonstrated in Magel2-KO mice and the current state of knowledge on the development of appetite-regulating centers prompts us to intervene as early as possible. In humans, the establishment of appetite regulating centers probably occurs in the first 6 months of life. This period is also the biological time of attachment and may therefore match the early action phase of OT on neuronal plasticity [17, 18].

We have little data on OT effects in neonatal humans. The studies on the effects of nasal OT administration on behavior have been conducted in healthy and autistic adults.

A first safety study in 2011 (AOL 2010) showed no adverse effects of a single intranasal OT administration (at doses of 2 IU and 4 IU) in 5 infants under 6 months old with PWS. In addition, data analysis indicated a positive effect on sucking/swallowing and mother-infant interactions 48 hours after dosing.

This new study will investigate the tolerability of repeated intranasal OT administration for 7 days according to three dosing schedules (4 IU every 2 days, 4 IU daily in a single administration and 4 IU twice a day) in infants with PWS under 6 months old. As in our first study, 2 IU was administered to three infants 3 and 4 IU were administered to two infants 2 were used without any side effects, we chose the dose of 4 IU to study the tolerance of repeated OT administrations over the 7 days following the first dose.

Like the first study, this study will be conducted with infants in our inpatient unit of the PWS Reference Center at the Children's Hospital of Toulouse. The Center is near the ICU, whose staff is aware of the study and is informed of the arrival of each infant. The infant will be monitored for pulse frequency and

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respiratory rate for the 2 hours following each administration of OT and the monitoring parameters will be the same as in our first study (clinical and laboratory).

**Drug administration**

There are no data on whether OT administration should be repeated in the newborn. Our first study suggested that the effect on sucking and mother-child interactions appeared after 48 hours and persisted.

Nevertheless, although sucking seemed to be improved in these babies, we suspect that repeated administrations would achieve greater efficiency. Given the lack of data and the particular care required with newborns, we propose an escalating dose study of OT in three successive steps and a preliminary study of OT efficacy and pathophysiological effects. In adults, the usual dose for single administrations is 24 IU and in studies with repeated OT doses, twice daily administration is most often reported to have good tolerance [19,20]. In babies, OT is secreted during feeding and circulating levels are higher than in adults. In this empirical study, we propose to start with a first step of 4 IU every 2 days with 6 babies; once this step is completed, a report on the results of the OT doses and safety will be communicated to ANSM, which will decide on study continuation. If ANSM approves, 6 other babies will be included in the second step of 4 IU daily, and we will again wait for approval before including last 6 babies in the third step of 4 IU twice a day.

On empirical grounds, we have decided to monitor the infants for 7 days following the first dose to check for the onset of a healthy sucking reflex (effect obtained in Magel2-KO mice) [4] and an early effect on brain plasticity [17-18].

Six babies per step is above the usual recommendation of 3 subjects [21] but is justified by the age of the subjects of this research.

We also intend to determine the effects of OT in order to have preliminary data on efficacy regarding:

- sucking
  - qualitatively: We will use the Neonatal Oral-Motor Assessment Scale (NOMAS; Palmer, 1990 [22]) and certain items from the Early Feeding Skills (EFS; Thoyre, 2005 [23]) scale that seem suitable for babies with PWS. Both scales were developed for premature babies. The scoring will be done by a team physician who is specialized in oral problems in infants. videofluoroscopy of swallowing is the gold standard for objective evaluation of disorders of swallowing and feeding (24); with this technique, we will be able to review and discuss the images after the exam. The
same physician will conduct the study and interpret the findings. The contrast product will be Micropaque® and it will used in very small quantity.

- quantitatively: We will also evaluate the quantity of food intake at each feeding session for every 24 hours and the duration of each feeding.

- weight gain for the 7 days following the first OT administration and weight and growth changes over one month.

- the infant’s overall behavior and mother-infant interactions before, during and after bottle feeding as evaluated with the checklist from the University of Bobigny, the PMI team of Seine-Saint-Denis and INSERM Unit 292, ). In addition, video recordings will be made three times (before, 48 hours after and 7 days after the first OT administration) to evaluate the interactions.

- Brain metabolic activity at rest by fMRI. We have chosen the resting state for our study because recent works have shown that intense metabolic brain activity in resting humans is organized as an intrinsically recruited neuronal network (i.e., default mode network: DMN). The DMN can be modified by pharmacological interventions.

- The observed modifications will help to specify the impact of treatment on neuronal pathways. Morphological MRI will be coupled with rs-fMRI before the first administration to look for any abnormalities that might interfere with the interpretation of functional abnormalities:

- on circulating hormone levels:

  - ghrelin: Circulating levels are high very early in life in PWS and we hypothesize that they will drop under OT treatment.

  - Other hormones will be measured because they are known to be abnormal or possibly implicated in PWS, such as PP, which is lower in PWS patients, orexin A, which is lower in the Magel2 mouse model, leptin, cortisol, and αMSH, which are implicated in appetite regulation.

  These hormones can be measured as in the first study on microplaques (Multiplex assay, Millipore® kit) using a very small quantity of plasma.

- We plan to perform a minimal pharmacokinetic study in these babies. We will measure circulating OT before and every 48 hours after the first administration up to day 7.
3.4. BENEFIT/RISK RATIO

Today children with PWS are diagnosed in the neonatal period because of obvious hypotonia that prompts genetic testing. The result is usually given within the first month of life (median of diagnosis in the French database in 2011).

The infants are usually hospitalized and some have a feeding tube placed to complement bottle feeding when the weight gain is insufficient. In the French database of 298 children, 92% were hospitalized at birth for a mean stay of 38 days and 85% had the tube placed for a mean of 30 days (from 1 to 180 days).

It is difficult to evaluate the benefit/risk ratio since the data on OT in newborns are scarce.

Nevertheless, our first study indicated a positive benefit/risk ratio in favor of good tolerance (no adverse effects after a single OT administration at either 2 IU or 4 IU) and a positive effect on sucking/swallowing and behavior.

The risk from videofluoroscopy is low because with the current methods the radiation dose is 20 μgray, which is much less than a single non-digitized pulmonary X-ray. We will carry out the usual X-ray study of sucking/swallowing during the first visit to our Reference Center. The infants included in the study will also undergo videofluoroscopy 7 days after the first OT administration.

There is no known risk associated with MRI. Patients with PWS have routine brain imaging, although generally later (after 2 years).

The time to examine these babies is very brief, as they move very little due to their hypotonia. There is no need for anesthesia or sedation.

The risk from blood sampling is extremely low. These infants will have blood drawn once on arrival at the Center and then there will be four other samples drawn for the study. The amounts collected to monitor for adverse effects are low and we will use the microplate technique for the hormone assays, which requires only 200 μL. The team nurses are experienced at drawing blood from the infants.

3.5. EXPECTED BENEFITS

If the tolerance to repeated intranasal OT administration is good and the effects on sucking/swallowing and/or the mother-infant interactions are good as well, we will propose another OT tolerance study using an optimal dose (effective dose with no side effects).

3.6. RESEARCH OBJECTIVES

3.6.1. PRINCIPAL OBJECTIVE
The principal objective is to study the tolerance to repeated intranasal OT administrations of OT for 7 days according to three dosing schedules (4 IU every 2 days, 4 IU daily in a single administration and 4 IU twice a day) in infants with PWS under 6 months old.

3.6.2. SECONDARY OBJECTIVES

The secondary objectives are the following:

- Perform a preliminary efficacy study on:
  - sucking/swallowing and food intake
  - mother-infant interactions before, during and after feeding
  - gains in weight and height

- perform a physiopathological study on OT effects:
  - on brain metabolism
  - on circulating levels of ghrelin and certain peptides and neuropeptides involved in appetite regulation (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH):
    - perform a minimal pharmacokinetic study: to measure the circulating levels of OT before and under treatment.

4. RESEARCH DESIGN

This study is a prospective, open escalating dose study, with three successive steps. It will be performed in a single center (CHU de Toulouse in the Prader-Willi Syndrome Reference Center). However, patients will be recruited in all Competence Centers, as in the first study.

5. ELIGIBILITY CRITERIA

5.1. INCLUSION CRITERIA

Male and female infants with PWS:
- genetically confirmed diagnosis
- under 6 months old
- hospitalized in the endocrinology unit of the Children’s Hospital Reference Center for PWS of the CHU de Toulouse)
- no prolonged QT interval on EKG
- no hypokalemia
- the parents or legal guardians have signed the consent form and the infant is enrolled with the national health service

5.2. NON-INCLUSION CRITERIA

- administrative problems: unable to give full information to parents or legal guardians, infant not covered by the social security health system, refusal of parents or legal guardians to sign the consent form
- hypersensitivity to product excipients
- familial antecedents of genetic pathology provoking a prolonged QT interval
- concomitant treatment that might provoke prolonged QT interval
- infant nourished exclusively by nasogastric tube
- infant with cardiac rhythm abnormalities on EKG
- infant with hepatic insufficiency
- infant with renal insufficiency

5.3. RECRUITMENT MODALITIES

Infants with genetically confirmed PWS will be recruited directly by the Reference Center or through the Competence Centers, which will receive all information and documents for this study, as for the first study, with 5 babies included between July and October 2011.

Study information will also be put up on the websites of the Prader-Willi France Association and the Reference Center of the CHU de Toulouse.

If we assume a prevalence of 1/20,000 births, 40 infants are born with PWS every year in France. We will therefore stay in close contact with the Competence Centers to be kept up to date. As in our first study, the Competence Centers will give verbal and written information on the study to parents and will suggest a telephone conversation with one of the investigating physicians.

After a time for reflection (between 2 and 10 days), parents who accept to participate will contact one of the Reference Center investigators (Pr Tauber, Dr Diene, Dr Çabal-Berthoumieu), who will organize visit 1 (V1) for study inclusion and hospitalization at the CHU de Toulouse. The study will be conducted at the Children’s Hospital of the CHU within the framework of the PWS Reference Center. When the infants are hospitalized, only one parent will be able to sleep in the room with their infant, and the other will be put up in housing close to the Children’s Hospital.
We will also clearly explain to the families that the hospital stay of 9 days, which is the average hospital stay for infants of this age, should not be exceeded except in rare cases. While hospitalized, the infants will undergo the same exams routinely made in the Reference Center in the first 3 months of life.

We do not expect recruitment problems. Many parents and health professionals contacted us at the end of our first study asking to participate in the new OT protocol.

6. TREATMENT IN THIS RESEARCH

The treatment is intranasal oxytocin, which is not currently available in France—only the injectable form has marketing authorization (AMM). However, several European countries (Switzerland, Germany) have an AMM for the intranasal form (marketed as Syntocinon®/Spray, vials of 5 ml to 40 IU/ml). The specifications for Syntocinon are provided in. We will request authorization to import intranasal OT in the context of a clinical trial from ANSM.

Different administration modalities will be studied to try to assess the best dose.

For the first 6 patients, the dose will be 4 IU every 2 days for 7 days, or 4 administrations (Figure 1); if the treatment is well tolerated, the following 6 patients will receive 4 IU daily for 7 days, or 7 administrations (Figure 2); the last 6 patients will receive a dose of 4 IU 2 times a day for 7 days, or 14 administrations (Diagram 3).

OT will be administered with Rhinyle®, a small flexible graduated tube used for Minirin® (see Minirin® instructions), as in the first study.

Our procedure is the following:

- hold the two ends of the Rhinyle® up. The needed amount of OT (100 µl corresponding to 4 IU) will be drawn from the Syntocinon®/Spray vial using a calibrated micropipette (0 to 200 µl) and a sterile cone (0 to 200 µl) and introduced into the Rhinyle®.

- grasp the Rhinyle® between thumb and index finger about 1 cm from the end and insert it into the nostril until the fingertip touches the nostril.

- adjust the other end of the Rhinyle® to a syringe and push quickly into the Rhynile® so the solution reaches the nasal cavity, without going into the pharynx.
The oxytocin should be stored at 2-8°C if longer than one month, otherwise at room temperature (15-25°C).

There is no known contraindication for intranasal OT. Our first study of a single OT dose in the same population showed no adverse effects. The patients will first be under the care of the team physician who is there in the daytime, Monday through Friday. In the evening and on weekends the investigating physicians will hire an on-duty physician. The intensive care unit and the medical surveillance department will continue to be informed of the study and each infant’s hospitalization. Throughout the trial, there will be emergency medical equipment in the pediatrics department for the caretaking team, who will be trained in its use. In the case of a serious adverse event (SAE), transfer to the ICU will be rapid as it is part of the CHU de Toulouse and the ICU staff is aware of the study and of each infant in it, as in the first study.

7. ASSOCIATED TREATMENT

Chronic treatment will be continued and carefully followed. There are few treatments at this age (usually anti-reflux). No treatment will be grounds for ineligibility except those that might provoke prolonged QT interval.

8. ENDPOINTS

8.1. PRIMARY ENDPOINT

We will use the same criteria as in the first study: monitoring for systemic adverse effects and evaluation of OT implication by:

Expected side effects:
- Heart rhythm disorders
- Abnormalities in blood pressure
- Respiratory diseases
- hyponatremia
- hypoglycemia

These effects, if they occur, will be graded according to their intensity: not clinically significant, moderate, or severe based on standards for age (Huault & Labrune, 1993 Pédriatre d’urgence 4ème édition, Médecine-Sciences Flammarion).

They will be detected by examination and biological exams:

- heart rate monitoring, O2 saturation in the 2 hours following each administration
- ECG performed 5 to 10 minutes after each administration
- blood pressure and heart rate readings 3x/day for 7 days after the first administration
- urine output over 24 hours and urinary densitometry (2x/day) for 7 days of administration
- regular blood glucose assays (alternating dextro and blood sampling)
- serum sodium and potassium, blood osmolarity every 2 days following administration

8.2. SECONDARY ENDPOINTS

- Sucking/swallowing:
  - using NOMAS scale and a grid (S1 of the article) chosen by our multidisciplinary team among various validated scales
  - number of bottles taken in 24 hours, amount of milk taken at each feeding, feeding duration, efficiency of two feedings/24 hours (amount of milk taken in the first 5 minutes of feeding)
  - Videofluoroscopy of swallowing before and 7 days after the first administration.
- Mother-infant interactions before, during and after feeding, according to the evaluation grid for CGI before and 3 days and 7 days after the first administration and on videos of feeding
- Height and weight: daily weight, size at baseline and at study exit
- Brain metabolism: resting state functional MRI (rs-fMRI) before and 7 days after the first administration
- Blood levels of OT, ghrelin and certain peptides and neuropeptides involved in appetite regulation (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH) before and every 48 hours after the first administration

9. RESEARCH SCHEDULE

9.1. CALENDAR
- Start of inclusions: May 2013
- Duration of the inclusion period: 18 months
- Duration of each patient’s participation: 30 days
- Total research duration: 24 months

9.2. PWS INFANT MONITORING

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The visits at 30 days (in gray) will be made in the Competence Centers near the infant’s home. The other visits will be part of hospital stay at the CHU de Toulouse. Between V9 and V30, the parents will record the number of bottles taken every 24 hours and the quantity taken at each feeding, in a dedicated notebook.

**9.3. PRE-INCLUSION VISIT (V0)**
The investigating physician will conduct the pre-inclusion visit after contacting the physician that usually follows the infant. This visit is by telephone: information about the study is given to the parents or legal guardians in the 10 days before inclusion (D1) and the physician answers any questions about the objective, the constraints and predictable risks, and the expected benefits of the research.

9.4. INCLUSION VISIT

The inclusion visit is on D1, and the investigating physician of the endocrinology department team of the Toulouse Children’s Hospital is in charge.

CONSENT FORM COLLECTION

Before any exam related to the research, the investigator will collect written consent from the parents or legal guardians, after assuring that they have been fully informed. The investigator will also reiterate patients' rights in the context of biomedical research and verify the eligibility criteria. The investigating physician gives the parents or legal guardians the information brochure, the form authorizing use of video data and the consent form. If agreement to participate is given, both parties sign and date the consent form.

The following documents are then distributed:

- copies of the brochure, the video authorization form and the signed consent are given to the parents.

- the original copy is kept by the investigating physician (even if the family moves away during the study) in a place inaccessible to third parties.

- at the end of the inclusions or at the end of the study at the very latest, copies of the consent forms are sent to the promoter or its representative on the terms set out for the investigators.

All infants in the study will have a first evaluation of weight and height, with an EKG performed and interpreted by a pediatric cardiologist from the pediatrics department of the CHU de Toulouse. If an EKG was performed for medical reasons before V1, it can be used with the interpretation recorded in the medical chart.

An EKG is routinely performed after each OT administration to monitor for prolongation of the QT interval. Blood pressure and pulse rate will be assessed 3x/day. The following will be quantitatively evaluated: number of feedings, amount of milk taken at each feeding, feeding duration, and the amount of milk taken in the first 5 minutes of feeding for two feedings/24 hours. A blood sample of 0.6 ml will be collected into heparinized microtubes to monitor sodium, potassium and glucose levels. A 1-ml sample
will be collected in a tube containing aprotinin to assay the peptides involved in appetite regulation (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH). A 1-ml blood sample in an EDTA tube will be taken in the middle of the interval between two feedings (2 hours after feeding for a 3-month-old baby with feedings every 4 hours). Blood samples will be taken in the morning 1 hour after feeding. A local anesthetic cream like Emla® will be used for all blood sampling in the study.

BOLD imaging for fMRI will be performed before the administration of OT at INSERM U825 (Dir. P. Celsis) at the Pavilion Baudot, Purpan Hospital, under the supervision of Prof. Pierre Payoux. A member of the project team will accompany the infant with one of the parents if they wish and will remain for the duration of the exam (30 minutes: 15 minutes set-up and 15 minutes for the exam).

All medical data in the medical record will also be collected in the case report.

9.5. FOLLOWING VISITS

9.5.1. Visit 2: V2

The second visit will take place on day 2, just after V1. videofluoroscopy of swallowing will be performed and then a nurse from the PWS Reference Center of the CHU de Toulouse will administer an intranasal nebulized dose of OT (1 nebulization = 1 dose of 4 IU).

One of the investigating physicians will be present for all administrations from V2 to V5 and will be reachable by phone on the weekend for V6 and V7. A physician from the ICU of the Children’s Hospital will be informed of the study and of each inclusion.

After OT administration, pulse and blood pressure will be continuously monitored for 2 hours (thereafter, 3x/day), urine output will be monitored for 24 hours (diaper weighing), and urine density by a dipstick 2x/day.

A glucose test will be performed prior to OT administration and bottle feeding to establish a reference value of blood glucose. To avoid daily blood sampling, the glucose test will be carried out on days when there is no blood sampling. We assume that it will not be necessary to monitor daily serum sodium and potassium and blood osmolarity because we will monitor daily urine output over 24 hours and urine density 2x/day by dipstick for antidiuresis caused by a vasopressin-like effect related to OT.

Moreover, food intake will be quantitatively and qualitatively assessed and weight gain will be monitored. Mother-infant interactions will also be assessed with the evaluation grid (Annex 4) and video recordings.
Twice daily, the infant will be assessed for any systemic adverse effects by clinical examination.

The electrodes connecting the infant to the cardiac monitor will be kept in place until V8 in order to resume continuous monitoring if necessary.

9.5.2. Visit 3

V3 will take place the day after V2.

Depending on the dose step, the visit will comprise one or two OT administrations and monitoring as detailed above (see visit V2). A glucose test will be performed.

The quantity of food intake and weight gain will be assessed.

A clinical examination will be performed twice a day to check for any systemic adverse effects.

9.5.3. Visit 4

V4 will be on day 4, the day after V3.

Depending on the dose step, the visit will comprise one or two OT administrations and monitoring as detailed above (see visit V2).

The quantity of food intake and weight gain will be assessed.

A 0.6-ml blood sample will be drawn into a heparin microtube to monitor serum sodium, serum potassium and glucose. A 1-ml sample will be drawn into an aprotinin tube to measure the peptides involved in appetite regulation (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH). A blood sample will be drawn into a 1-ml EDTA tube for OT measure. Local anesthesia with an Emla®-type cream will be used for all blood sampling.

A clinical examination will be performed twice a day to check for any systemic adverse effects.

9.5.4. Visit 5

V5 will be on day 5, the day after V4.

Depending on the dose step, the visit will comprise one or two OT administrations and monitoring as detailed above (see visit V2).

The quantity of food intake and weight gain will be assessed, and a glucose test will be performed.
Mother-infant interactions will be assessed using the evaluation grid and video recordings.

A clinical examination will be performed twice a day to check for any systemic adverse effects.

### 9.5.5. Visit 6

V6 will be on day 6, the day after V5.

Depending on the dose step, the visit will comprise one or two OT administrations and monitoring as detailed above (see visit V2).

The quantity of food intake and weight gain will be assessed.

A 0.6-ml blood sample will be drawn into a heparin microtube to monitor serum sodium, serum potassium and glucose. A 1-ml sample will be drawn into an aprotinin tube to measure the peptides involved in appetite regulation (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH). A blood sample will be drawn into a 1-ml EDTA tube for OT measure. Local anesthesia with an Emla®-type cream will be used for all blood sampling.

A clinical examination will be performed twice a day to check for any systemic adverse effects.

### 9.5.6. Visit 7

V7 will take place the day after V6.

Depending on the dose step, the visit will comprise one or two OT administrations and monitoring as detailed above (see visit V2). A glucose test will be performed.

The quantity of food intake and weight gain will be assessed.

A clinical examination will be performed twice a day to check for any systemic adverse effects.

### 9.5.7. Visit 8

V8 will be on day 8, the day after V7.

Depending on the dose step, the visit will comprise one or two OT administrations and monitoring as detailed above (see visit V2).

The quantity of food intake and weight gain will be assessed.
A 0.6-ml blood sample will be drawn into a heparin microtube to monitor serum sodium, serum potassium and glucose. A 1-ml sample will be drawn into an aprotinin tube to measure the peptides involved in appetite regulation (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH). A blood sample will be drawn into a 1-ml EDTA tube for OT measure. Local anesthesia with an Emla®-type cream will be used for all blood sampling.

A clinical examination will be performed twice a day to check for any systemic adverse effects.

**9.5.8. Visit 9**

Pulse and blood pressure will be monitored 3x/day.

The quantity and quality (evaluation grid) of food intake and weight gain will be assessed. The infant’s height will be measured. A glucose test will be performed.

Mother-infant interactions will be assessed using an evaluation grid and video recordings.

Videofluoroscopy of swallowing and brain fMRI will be performed.

The infant will be discharged at the end of all exams.

**9.5.9. Follow-up visits (V10-V29)**

From the 10th to the 29th day, the parents will record in a dedicated notebook the number of feedings per 24 hr and for each feeding, the duration and quantity of milk ingested.

**9.6. END OF RESEARCH VISIT: V30**

V30 will be conducted on D30±2 days by a physician from the Competence Center closest to the infant’s home. The evaluation will comprise a clinical examination, a quantitative evaluation of food intake (number of bottles per 24 hr, quantity of milk per 24 hr), and weight and height measurement. Pulse and blood pressure will be checked.

A 1-ml blood sample will be drawn into an aprotinin tube to measure the peptides involved in appetite regulation (leptin, cortisol, insulin, GLP-1, PYY, pancreatic polypeptide, orexin A, αMSH). A blood sample will be drawn into a 1-ml EDTA tube for OT measure. Local anesthesia with an Emla®-type cream will be used for the blood sampling. Samples will be stored at -20°C and sent to the Toulouse CHU for measurements.

A recapitulation of the study is presented below.
*The modality of OT administration will vary with the step that is open (4 IU every 2 days, 4 IU once a day, or 2x 4 IU every day).

**9.7. STOPPING THE STUDY**

The study may be ended for the following reasons:

- a decision made by the parents or the legal guardians
- necessity, after consideration by the investigator in the following cases:
  - study conditions not respected (poor compliance with treatment)
  - adverse effects (whether serious or not)

**9.8. RESEARCH CONSTRAINTS AND COMPENSATION**

Infants included in this study cannot be enrolled in other studies, as this might compromise the findings.
These participating infants will not be listed in the Ministry of Health’s national register of persons who consent to participate in biomedical research.

**9.9. COLLECTION OF BIOLOGICAL SAMPLES**

For each patient, 0.6-ml blood samples in heparin microtubes, 1-ml samples into aprotinin tubes and 1-ml into EDTA tubes will be collected on V1, V4, V6, V8 and V30. The tubes will be centrifuged, the plasma/serum collected and aliquoted and stored at -20°C until assay. The samples will be retained only for the study duration on the premises of the Pediatric Investigation Unit of the Children's Hospital under the supervision of the lead investigator, Prof. M Tauber.

**10. MANAGING ADVERSE EVENTS AND NEW DEVELOPMENTS**

**10.1. DEFINITIONS**

**Adverse event:** Any harmful effect that occurs in a person who is participating in biomedical research, whether or not this effect is related to the research or the product under study.

**Serious adverse event:** Any adverse effect that:

- results in death
- endangers the life of the person participating in research
- requires hospitalization or prolongation of hospitalization
- causes a disability or a significant or lasting disability
- is reflected by a congenital abnormality or malformation
- or any effect considered to be medically serious

and is related to the drug, regardless of the dose administered.

**Unexpected adverse event:** Any adverse effect of the drug whose nature, severity or evolution is not consistent with the information given in the request-for-study-approval submitted to the CCP and the authorization request submitted to competent authority.

**New development:** New information on safety that might lead to a reassessment of the benefits and risks of research, or that might be sufficient to lead to the amendment of the research documents, the conduct of the research and, where appropriate, the use of the product.
10.2. DESCRIPTION OF EXPECTED SERIOUS ADVERSE EVENTS

-linked to the drug experiment:

- heart rhythm disorders
- blood pressure abnormalities
- respiratory diseases
- hyponatremia
- hypoglycemia

Given the possible arrhythmogenic effect described with the intravenous form of OT, an EKG will be performed prior to administration of the nasal spray and 5-10 minutes after each administration.

Blood pressure and pulse will be monitored for 2 hours after each administration. If any values are abnormal, monitoring will continue until normalization is confirmed by medical examination.

The literature indicates no adverse events in patients who received intranasal OT or in our first study in infants with PWS who received 2 IU and 4 IU of OT.

Because of a possible anti-diuretic effect of OT by a vasopressin-like effect, we will monitor daily urine output over 24 hours and urine concentration with the dipstick test 2x/day.

The SPC of Syntocinon®-spray lists a number of adverse events:

- linked to the underlying pathology (PWS): possible aggravation or complication
- linked to brain MRI: no negative effect expected
- linked to blood sampling: except for exceptional complications (infection, inflammation, pain at the sampling point), no serious effect is expected
- linked to videofluoroscopy of swallowing: no effect is expected with the low dose of radiation (20µG). However, the side effects of the contrast product (Micropaque®) are listed in the RCP of this product

It should be noted that blood sampling, brain MRI and videofluoroscopy of swallowing are routine exams in the Reference Center for PWS.

Any adverse events not mentioned in this paragraphs are considered “unexpected.”
10.3. RESPONSE TO AN ADVERSE EVENT OR NEW DEVELOPMENT

In the event of an adverse effect, whether serious or not, the infant will remain hospitalized and will be discharged to home only once the clinical and laboratory parameters are normalized. Following the return to home, consultation with the doctor who knows the child will be made 2 days and 1 week after discharge. After this last visit, if no problem is detected, close monitoring will be stopped. If a problem persists, appropriate treatment will be managed by the local doctor in concert with our team.

The investigator must notify the promoter of any serious adverse event or new development without delay, as soon as he or she learns of it, if it occurs:

- after the consent form was signed
- during the entire patient monitoring period provided for in the research protocol,
- during the 10 days after the end of the patient monitoring period provided for in the research protocol, when the effect may be due to the research.

<table>
<thead>
<tr>
<th>TYPE OF EFFECT</th>
<th>MODALITIES OF NOTIFICATION</th>
<th>DELAY FOR NOTIFYING THE PROMOTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE: not serious</td>
<td>Recorded in dedicated notebook</td>
<td>No immediate notification</td>
</tr>
<tr>
<td>AE: SERIOUS</td>
<td>Declaration of an SAE + written report if necessary</td>
<td>No immediate notification to promoter</td>
</tr>
<tr>
<td>New development</td>
<td>Declaration + written report if necessary</td>
<td>Immediate notification to promoter</td>
</tr>
</tbody>
</table>

Name of promoter: CHU de Toulouse
Phone: +33 (05) 61 77 87 71 / Fax: +33 (0)5 61 77 84 11
Email: olivier@cict.fr

All these events will be followed to their complete resolution. The lead investigator will send complementary information (complementary declaration form) on the evolution of the event, if it is not mentioned in the initial report, to the promoter.

10.4. DECLARATION AND DOCUMENTATION OF UNEXPECTED SAE AND NEW DEVELOPMENTS
The promoter will immediately declare the unexpected SAEs and new developments in the course of the research:

- to ANSM

- to the relevant Committee on the Protection of Persons. The Committee will ensure, if necessary, that the subjects participating in the research were informed of adverse events and that they confirm their consent.

For all drug research, the promoter will enter any unexpected SAEs in the EudraVigilance database.

11. STATISTICAL ANALYSIS

11.1. CALCULATION OF SAMPLE SIZE

The convention in dose-escalation studies is to consider a small number of subjects (usually 3) in each step and to include 3 new subjects for the next step if no adverse events occurred in the prior step or to add 3 new patients the same dose step in the case of an adverse event [21]. In our study, given the patient ages (under 6 months) and the rarity of the disease, we chose to include 6 subjects per step to obtain more detailed safety data and to apply, as recommended, the probabilistic rules for moving to the next dose step (described in paragraph 2.3). The choice of 6 babies per step is also justified by the need for preliminary data on efficacy, which in conjunction with the safety findings, will be used to select the optimal dose for further study. The maximum dose without serious adverse events attributable to OT will not necessarily be the dose selected for further study if efficacy is satisfactory with a lower dose.

11.2. STATISTICAL METHODS

Tolerance and efficacy will be evaluated in the treated population, defined as all babies exposed to at least one dose of OT. These data will be summarized using descriptive statistics for each dose step.

Primary endpoint

The types, frequency, severity, and any relationships among adverse events emerging during treatment will be analyzed.

The primary endpoint is defined as the occurrence of at least one systemic adverse effect attributable to OT administration and will be considered a binary qualitative variable. The occurrences of adverse events will be descriptively analyzed. The number and percentage of subjects with at least one systemic adverse effect will be described globally and then in detail by the type of effect. The distribution of adverse events
according to their intensity (not clinically significant, moderate, severe intensity) will be described in terms of numbers and frequencies, and represented by a pie chart.

Secondary endpoints

Preliminary criteria of efficacy will be analyzed for each step by quantifying the evolution of the parameters over 7 days (comparison between data before and after repeated OT administration).

Preliminary efficacy study

- Study of the effect on sucking/swallowing:

  o qualitatively: comparing scores on rating scales between V2 and V9. videofluoroscopy of swallowing performed at V2 and V9 will be interpreted by the physician from the Reference Center specialized in orality disorders.

  o quantitatively: comparison of the amounts of food intake over 24 hours and the average duration of feedings from V1 to V30, the milk transfer rate (Quantity of milk intake/duration of intake), efficiency (quantity of milk taken in the first 5 minutes of feeding) for two feedings/24 hr from V1 to V9

- Weight gain between V1 and V30

- Evolution of overall behavior evaluated by the grid of mother-infant interactions at V2, V5 and V9

Pathophysiological study: in similar fashion, the following will be described:

- changes in functional brain activity in default mode rs-fMRI between V1 and V9

- changes in circulating hormone levels (OT, ghrelin and other hormones involved in appetite regulation) between V1, V4, V6, V8 and V30

Minimal pharmacokinetic study: the evolution in OT levels before and every 48 hours for 7 days (4 doses).

12. SURVEILLANCE

A monitoring committee composed of physician-investigators Professor JL Montastruc (Clinical Pharmacology, Faculty of Medicine of Toulouse), Pascale Olivier (in charge of pharmacovigilance), and a representative of the Directorate of Research will meet every 6 months for the duration of the study. It
will analyze the data, notably the safety data, and plan for actions to take in the case of repeated serious adverse events not clearly linked to OT (see section 3.3).

13. RIGHT OF ACCESS TO DATA AND SOURCE DOCUMENTS

13.1 ACCESS TO DATA

The promoter will obtain the agreement of all parties involved in the research to guarantee direct access to all research locations, source data, source documents and reports so that the promoter can control quality and carry out audits.

The investigators will make available the documents and individual data that are strictly necessary for biomedical research monitoring, quality control and auditing to those individuals who have the right to access in accordance with the laws and regulations in force.

13.2. SOURCE DATA

Any original document or object (medical, biological examination result, video recording) that proves the existence or accuracy of an item of data or recorded information during the research is defined as a source document.

13.3. DATA CONFIDENTIALITY

In accordance with the existing legislation (Articles L.1121-3 and R.5121-13 the Code of Public Health), all individuals with direct access to source data will take all necessary precautions to ensure the confidentiality of all information relating to the experimental drug, the research, and the research subjects particularly regarding their identity and the results. These individuals, along with the investigators themselves, are bound by professional secrecy.

During and after the biomedical research, the data collected on subjects and transmitted to the promoter by the investigator (or other specialists involved) will be made anonymous. In no case will names and addresses appear.

The subjects participating in the study will be coded; they will be identified by the first letter of their last name, the first letter of their first name, followed by their year of birth.

The promoter will ensure that each subject has given written authorization to access the personal data that is strictly necessary for quality control.

14. MONITORING AND QUALITY CONTROL
Guidelines for data collection

All information required by the protocol must be recorded on paper case report forms and an explanation must be provided for each missing item. Data should be collected as and when they are obtained and recorded neatly and legibly in these case reports.

Erroneous data must be clearly crossed out and new data recorded next to the crossed-out information, initialed and dated, and possibly a justification by the investigator or authorized person who made the correction.

14.1. STUDY MONITORING

The research will be followed by a clinical research assistant (CRA; Mme C Molinas Cazals), who will be charged by the coordinating investigator with:

- the logistics and surveillance of the research
- progress reports
- verifying updated report forms (requests for additional information, corrections, etc.)
- sending out samples
- transmitting SAEs to the promoter

She will work in accordance with standard operating procedures and in collaboration with the managing clinical research associate delegated by the promoter.

14.2. QUALITY CONTROL

A clinical research associate appointed by the promoter will regularly visit each investigator center, during the implementation of the research, one or more times during the research process according to the rhythm of the inclusions, and at the end of research. During these visits, the following will be reviewed:

- informed consent
- compliance with the established research protocol and procedures
- quality of the data collected in the case reports: accuracy, missing data, data consistent with the source documents (medical records, appointment books, original lab results, etc.)
- treatment management
All visits will be written up as monitoring reports.

14.3. DATA MANAGEMENT

All subject identities will be coded; subjects will be identified by the first letter of their last name, the first letter of their first name, followed by their year of birth. A list of subject identities will be kept in the investigator’s files.

The investigator will ensure that the anonymity of each subject is guaranteed. No identifying information will be given to third parties other than those statutorily entitled to that information (and are bound by professional secrecy).

The information on each subject will be collected on a standardized report form completed by the investigator or the CRA. This notebook will include the identification of the subject, clinical data and the study data.

The source document (the medical record) will be required for each subject; observation and medical monitoring for the study will be included in the source document.

All study data will be transcribed in the report forms and retained by the investigating physicians of CHU de Toulouse under the supervision of Prof. M Tauber. The report forms will be completed legibly and indelibly in blue or black ballpoint pen. In the case of error, the incorrect information will crossed out with a single stroke, with the initial data remaining visible, and the correct information will be written to the side. Each correction will be explained and authenticated (dated and signed or initialed by the investigator). The principal investigator will sign each report form attesting to her agreement with the data contained therein.

14.4. AUDITS AND INSPECTIONS

An audit may be conducted at any time by persons independent of those in charge of the research commissioned by the promoter. The audit is to ensure the quality of the research, the validity of its results and compliance with laws and regulations.

The investigators agree to comply with the requirements of the promoter and the competent authority regarding an audit or inspection of the research.

The audit can cover any stage of the research, from protocol development to the publication of results and classification of data used or produced in the framework of the research.
15. ETHICAL AND REGULATORY CONSIDERATIONS

The promoter and investigators agree that all research will be conducted in accordance with Law No. 2004-806 of August 9, 2004, and in accordance with Good Clinical Practices (Decision of November 24, 2006) and the Declaration of Helsinki (ethical principles for medical research involving human subjects, Tokyo 2004).

The research will be conducted in accordance with the present protocol. Except in emergency situations requiring specific therapeutic procedures, the investigators will respect the protocol on all points, especially with regard to obtaining consent and the notification and monitoring of serious adverse events.

This research has received the approval of the Committee for the Protection of Persons (CPP): Southwest and Overseas I on March 18, 2013.

The CHU de Toulouse, promoter of this research, subscribed to a civil liability insurance policy with Gerling Insurance Company (10066482010013).

The data recorded during this research will undergo computerized processing at the CHU de Toulouse in accordance with Law No. 78-17 of January 6, 1978, on Data Processing, Data Files and Individual Liberties, modified by Law No. 2004-801 of August 6, 2004.

CHU de Toulouse has declared the research to the National Commission for Computing and Liberties (CNIL) under the methodology of the MR-001 reference.

- This research is registered in the European database EudraCT as No. 2012-005325-67.

- The collection of biological samples carried out as part of this research was declared to ANSM at the same time as the application for authorization for the research. After the research, the conservation of the biological samples will be reported to the Minister of Research and the Director of the Regional Health Agency (and submitted to the CPP for review if change of purpose in the research).

AMENDMENT TO THE PROTOCOL

Any substantial change, i.e., any change likely to have a significant impact on the protection of persons, on the conditions of validity and the results of research, on the quality and safety of products tested, and on the interpretation of scientific papers that support the conduct of research or its modalities, will be written up and submitted to the promoter. Prior to its implementation, the promoter must obtain the approval of the CPP and authorization from Afssaps.
Non-substantial modifications, i.e., those that have no significant impact on any aspect of research, will be communicated to the CPP as information.

All amendments to the protocol must be made known to all investigators involved in the research. The investigators will undertake to respect the contents.

Any amendment modifying the care of patients or the benefits, risks and limitations of the research must be made clear in a new information note and a new consent form, which will be collected following the same procedure as above.

16. CONSERVATION OF RESEARCH DOCUMENTS AND DATA

The following research documents will be archived in accordance with Good Clinical Practices:

by the investigating physicians:

- for a period of 15 years following the end of the study:
  • the protocol and any amendments to the protocol
  • the report forms
  • The source documents for all participants who signed a consent form
  • all other documents and correspondence related to the research
  • the original copy of the informed consent forms signed by participants

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

by the promoter:

- for a period of 15 years following the end of the study:
  • the protocol and any amendments to the protocol
  • all other documents and correspondence related to the research
  • documents related to serious adverse events

All these documents are the responsibility of the promoter for the prescribed archiving period.
No removal or destruction can be made without the agreement of the promoter. At the end of the required archival period, the promoter will be consulted about destruction. All data and all documents and reports may be subject to audit or inspection.

17. PUBLICATION GUIDELINES

17.1. SCIENTIFIC COMMUNICATIONS

The analysis of data from the clinical sites will be carried out by the principal investigator, Prof. M Tauber. This will be written up and sent to the promoter, who will forward it to the CPP and the competent authority.

Any written or oral communication of the research results must receive the prior approval of the coordinating investigator and, if any, the committees established for the research.

The publication of the main results will indicate the name of the promoter, all investigators that included or monitored patients, the methodologists and biostatisticians who participated in the research, and the members of any committees established for the research. Account will be taken of the international rules for writing and publication (Vancouver Convention, February 2006).

17.2. COMMUNICATING THE RESULTS TO PATIENTS

According to Law No. 2002-303 of March 4, 2002, patients will be informed of the overall research results on their written request.

17.3. DATA DISPOSAL

Data collection and management will be assured by the principal investigator, Prof. M Tauber. The conditions for the disposal of all or part of the research database will be decided by the promoter of the research and will be subject to a written contract.

18. REFERENCES


**20. APPENDICES**

**APPENDIX 1: LIST OF INVESTIGATORS**

<table>
<thead>
<tr>
<th>NO</th>
<th>CENTER</th>
<th>COMPLETE NAME AND ADDRESS</th>
<th>LIST OF INVESTIGATORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CHU de TOULOUSE Endocrinology PWS Reference Center</td>
<td>Principal investigator: Pr Maïthé TAUBER</td>
<td>Service: Centre de référence du SPW/ Equipe d’Endocrinologie Hôpital des Enfants/ 330 av de Grande Bretagne / TSA70034 / 31059 Toulouse Cedex 9 Email: <a href="mailto:tauber.mt@chu-toulouse.fr">tauber.mt@chu-toulouse.fr</a> Tel: 05 34 55 85 51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dr Gwenaëlle DIENE</td>
<td>Service: Centre de référence du SPW/ Equipe d’Endocrinologie Hôpital des Enfants/ 330 av de Grande Bretagne / TSA70034 / 31059 Toulouse Cedex 9 Email: <a href="mailto:diene.g@chu-toulouse.fr">diene.g@chu-toulouse.fr</a> Tel : 05 34 55 87 46</td>
</tr>
<tr>
<td>Service</td>
<td>Name</td>
<td>Address</td>
<td>Email</td>
</tr>
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</tr>
<tr>
<td>Centre de référence du SPW/Equipe d'Endocrinologie Hôpital des Enfants</td>
<td>Dr Sophie CABAL-BERTHOUMIEU</td>
<td>Centre de référence du SPW/Equipe d'Endocrinologie Hôpital des Enfants/330 av de Grande Bretagne/TSA70034/31059 Toulouse Cedex 9</td>
<td><a href="mailto:cabal-berthoumieu.s@chu-toulouse.fr">cabal-berthoumieu.s@chu-toulouse.fr</a></td>
</tr>
<tr>
<td>Equipe d'Endocrinologie Hôpital des Enfants</td>
<td>Pr Jean-Pierre SALLES</td>
<td>Service: Equipe d'Endocrinologie Hôpital des Enfants/330 av de Grande Bretagne/TSA70034/31059 Toulouse Cedex 9</td>
<td><a href="mailto:salles.jp@chu-toulouse.fr">salles.jp@chu-toulouse.fr</a></td>
</tr>
<tr>
<td>Réanimation et Surveillance Médicale Continue Hôpital des Enfants</td>
<td>Dr Marie-Claude BLOOM</td>
<td>Réanimation et Surveillance Médicale Continue / Hôpital des Enfants/330 av de Grande Bretagne/TSA70034/31059 Toulouse Cedex 9</td>
<td><a href="mailto:bloom.mc@chu-toulouse.fr">bloom.mc@chu-toulouse.fr</a></td>
</tr>
<tr>
<td>Service de Médecine Nucléaire Hopital Purpan</td>
<td>Pr Pierre PAYOUX</td>
<td>Service de Médecine Nucléaire Hopital Purpan / Place du Docteur Baylac - TSA 40031 / 31059 Toulouse cedex 9</td>
<td><a href="mailto:payoux.p@chu-toulouse.fr">payoux.p@chu-toulouse.fr</a></td>
</tr>
<tr>
<td>Service ORL</td>
<td>Dr Pascale FICHAUX-BOURIN</td>
<td>Service ORL Hôpital Larrey / 24 chemin de Pouvoirville / TSA 30030 / 31059 Toulouse cedex 9</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>CENTER</td>
<td>COMPLETE NAME AND ADDRESS</td>
<td>OTHER PARTICIPANTS</td>
</tr>
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</tbody>
</table>
| 1   | CHU de TOULOUSE | Catherine MOLINAS CAZALS, CRA | Service: Centre de référence du SPW/ Equipe d’Endocrinologie Hôpital des Enfants/ 330 av de Grande Bretagne / TSA70034 / 31059 Toulouse Cedex 9  
Email: molinas.c@chu-toulouse.fr  
Tel: 05 67 77 12 32 |