



Open labeled, uncontrolled pharmacokinetic study of a single intramuscular hCG dose in healthy male volunteers

A.W. Davidoff¹, M.D. Hill², S.C. Cramer³, Y. Yang¹ and A. Moore¹

¹*Stem Cell Therapeutics Corp.*, ²*University of Calgary, Calgary, Alberta, Canada* and ³*Departments of Neurology and Anatomy & Neurobiology, University of California, Irvine, USA*

Key words

human chorionic gonadotropin – serum – cerebrospinal fluid – pharmacokinetics – Pregnyl – Ovitrelle

Abstract. The current study was designed to compare blood and cerebrospinal fluid (CSF) pharmacokinetic characteristics of two forms of human chorionic gonadotropin (hCG): Pregnyl[®], derived from human urine, and Ovitrelle[®] a recombinant form. Two separate groups, each with six older male human subjects, were dosed with either form of the drug at 10,000 IU intramuscularly (IM), and followed over a 36-hour period. No significant difference in the serum level of hCG was observed for either preparation of hCG (Peak serum conc.: 316 ± 53 vs. 270 ± 60 at 12 hours, 311 ± 38 vs. 321 ± 60 IU/l at 24 hours; AUC: 10,053 ± 1,268 vs. 8,793 ± 1,768, Pregnyl and Ovitrelle, mean ± SD, respectively). Additionally, both forms of circulating hCG distributed to the central nervous system (CNS) as manifest by an increased number of subjects whose CSF samples showed detectable levels of hCG in their CSF over a 36-hour period. Similarly, there was no significant difference between the two forms when distribution to the CSF was compared at 36 hours (2.0 and 1.2 IU/l; range 1.9–2.1 and 1–1.4 IU/l for Pregnyl and Ovitrelle, resp.). This preliminary study in normal human volunteers suggests that the two forms of hCG tested, Ovitrelle[®] and Pregnyl[®], when administered IM, distribute in a similar fashion into the circulation and CSF. Consequently, we conclude that these two drugs demonstrate no statistical significant difference with respect to the CSF.

Introduction

Human chorionic gonadotropin (hCG) is a naturally occurring polypeptide hormone produced by the human placenta. It is detectable in serum ranging from > 2 IU/l in healthy female humans to concentrations > 40,000 IU/l [Braunstein et al. 1976] or more in the

first trimester of pregnancy. As a commercially available therapeutic agent, hCG has two approved classes of use, the most common of which is for the induction of ovulation, while the most common secondary uses are for the treatment of prepubertal cryptorchidism and selected cases of hypogonadotropic hypogonadism in the male. The two commercially available forms of hCG: urine-derived Pregnyl[®], manufactured by Azko-Nobel, Arnhem, The Netherlands, and the recombinant form, Ovitrelle[®], manufactured by Serono, Geneva, Switzerland, are considered similar for these classes of use. Typically the two forms of hCG are administered by either IM or subcutaneous injection, and distribute to the serum rapidly. In animals, hCG has been reported to distribute to CSF [Lukacs et al. 1995]. In several species, hCG can be detected in CSF. While it has been reported that urine-derived hCG and recombinant hCG demonstrate similar temporal distribution into the serum of rats when administered intramuscularly, differences in molecular structure of these two forms of hCG, as well as, peptide impurities in the urine-derived hCG, do not imply similar passage across the blood-brain barrier in man. Thus, a comparison of CSF pharmacokinetics would permit the selection of a preferable form of hCG for development of therapies aimed at driving recovery after neurological injury.

Several regions of the brain known to be enriched with populations of stem cells express receptors for luteinizing hormone/hCG [AL-Hader et al. 1997, Lei et al. 1993], suggesting that hCG signaling occurs within select segments of the brain although the physiologic effect is largely unknown. Prior to this

Received
September 24, 2008;
accepted
April 1, 2009

Correspondence to
Dr. A.W. Davidoff, PhD
Stem Cell Therapeutics
Corp., Suite 1000,
1520- 4th St. SW,
Calgary, T2R 1H5,
Canada
adavidoff@
stemcellthera.com

study, we conducted pre-clinical studies to demonstrate that a drug scheduling protocol, designed to administer a neural stem cell proliferative agent (hCG: 300 IU/day 1, 3, 5 following stroke onset; S.C.) followed by a neural stem cell differentiating agent (erythropoietin-EPO: 1,440 IU/day 7, 8, 9 following stroke onset; I.V.), can act as an effective therapy to significantly restore or regenerate motor function recovery after middle cerebral artery occlusion (MCAo) stroke in rats [Belayev et al. 2007]. Efficient translation of these results into the clinical setting in requires an understanding of the comparable serum and CSF pharmacokinetics of the two commercially available forms of IM-delivered hCG in humans.

To our knowledge, characterization of systemically administered hCG, the pharmacokinetics of hCG transport into the serum then CSF has not been previously published in healthy human male volunteers. Similarly, quantitative analyses of hCG concentrations in CSF have not previously been validated, or published. The primary aim of this study was to demonstrate that systemically administered hCG achieves relevant concentrations in the CSF, understand the serum and CSF pharmacokinetics of the two available forms of hCG in healthy volunteers, and secondarily to examine issues of assay validity. This study describes two new sets of findings. First, we report here the pharmacokinetic properties of IM administered hCG into serum, passage of the two forms of commercially available forms of hCG into the central nervous system (CNS), and the temporal distribution and concentration of hCG in serum and CSF. Second, we describe initial steps towards validating a method for analyzing hCG in human CSF samples.

Materials and methods

Study design

Study objectives

The primary objective of the study was to characterize the pharmacokinetic profile of a single IM dose of hCG including examination of the ability of IM-administered hCG to penetrate into, and distribute within the CNS, in

healthy male volunteers, based on a dose allometrically scaled from animal models. Two different manufacturers' hCG products were evaluated. The trial design was an open label pharmacokinetic study.

The primary endpoints were individual serum hCG profiles, which include mean serum hCG concentrations as measured at 1, 2, 4, 8, 12, 24 and 36 hours post-dose, C_{max} , t_{max} , half-life and area under the curve (AUC), and CSF hCG concentration at 12, 24 and 36 hours post-dose.

One secondary objective was to monitor hCG safety for the dosing employed, using vital signs, weight, and adverse event (AE) reporting. An additional secondary objective was to validate the CSF hCG assay.

Selection of study population

Prior to the start of the study, Institutional Ethics Committee approval was obtained on the following documents: the study protocol, written subject information, subject consent form and advertisements for subject recruitment. The study was conducted at the Phase I clinic by Medicon, Denmark and subjects were recruited from the research unit's volunteer database via phone contact as well as through advertisements. Informed consent was obtained prior to performing any screening examinations. Screening examinations included a medical history, urinary drug screen, serology and hCG testing. Following screening, those subjects determined to be eligible for the study were asked to come to the clinic on Day -1 for the admission period of the study. The study in life period was 2 days, and subjects were received a physical exam and adverse event questionnaire at 7 days post dose. An arbitrary volunteer age range of 40 – 75 was selected as these are adults who are potential stroke victims and therefore potential candidates.

Inclusion criteria: Healthy males; age range 40 – 75, inclusive; mental competence sufficient to provide informed consent; normal platelet count.

Exclusion criteria: hCG $> 1 \times$ SD greater than normal distribution at screening; headache within prior 7 days before study Day 1; history of headache > 2 two episodes per week within the last 12 months; history of mi-

graine headaches or post-lumbar puncture headache; chronic skin disease or local skin infection at the puncture site; INR > 1.5 or active thromboembolic disorder; any sign of increased intracranial pressure, evaluated using an ophthalmoscope; systemic infection within the last 14 days prior to study Day 1; history of cardiovascular disease, choriocarcinoma, germ cell tumors (testicular cancer, seminoma, teratoma, dysgerminoma, and non-seminomatous germ cell tumor), bladder cancer, renal cancer, prostate cancer, gastrointestinal cancer (biliary, pancreatic, gastric, liver, and colorectal cancers), neuroendocrine tumor (carcinoid tumor, pituitary tumor, somatotroph adenoma), lung cancer, breast cancer, head and neck cancer, hematological cancer such as lymphoma; history of major psychiatric disease; current alcohol or drug abuse; known and recorded history of disease resulting in increased hCG levels, within 18 months prior to screening; or a hypersensitivity to the active substance or to any of the excipients.

Treatments allocated, dosed and administered

Subjects were assigned to each group sequentially, in order of their place on the screening list (i.e. first 6 eligible subjects on the screening log and who were able to participate were allocated to Group 1 and received Pregnyl® and the next 6 eligible subjects were allocated to Group 2 and received Ovitrelle®). Subjects were included in the study if they were still eligible on Day 1; otherwise, a replacement was sought among other eligible subjects on the screening list.

Thus 2 groups of 6 subjects received either one IM injection of hCG: i) Pregnyl® 10,000 IU, human chorionic gonadotropin, a solution reconstituted from lyophilized powder or ii) Ovitrelle® 10,000 IU, chorionic gonadotropin- α , solution for injection. Total Study size is n = 12.

Patient samples for pharmacokinetics

Blood samples were collected (8 blood samples/subject) pre-dose, 1, 2, 4, 8, 12, 24 and 36 hours post dose. Cerebrospinal fluid sampling: (3-3 ml samples/subject) were col-

lected using lumbar puncture 12, 24 and 36 hours post dose.

Prior and concomitant therapy

Concomitant medication was defined as all medication given in addition to the trial treatment during the trial. As a part of the lumbar punctures, the subjects were offered local anesthesia, which was not recorded as concomitant medication.

Safety measurements

The safety parameters measured were AEs, vital signs (blood pressure, pulse rate and body temperature), 12-lead ECG, clinical laboratory evaluations, body weight, and physical examination.

Validation of enhanced chemiluminescent immunometric assay for determination of hCG in CSF samples

The partial validation experiments were designed to evaluate the suitability of the analytical method for determining hCG concentration in CSF samples. The Immulite method is already validated for analysis of serum and urine samples (Bio-Rad, Denmark). Therefore, the purpose of the validation set of experiments was to determine whether there are any matrix differences between CSF and serum samples. In order to accomplish this, comparison of the results from analyzing the two different sample matrices (serum and CSF) for hCG concentrations were performed.

The determination of the hCG concentration was performed using a solid-phase, two-site automated enzyme enhanced chemiluminescent immunometric assay system, all work was conducted at Capio Diagnostic, Denmark, as described below. Calibration of the analytical system was performed by use of a stored master calibration curve which is adjusted at set intervals with bi-level adjusters. Accordance with the master curve is calculated by linear regression. Serum test samples (Seronorm Immunoassay L-2, Norway) and

CSF samples were obtained from Sero A/S, Norway, and test CSF sample and serum test samples were analyzed in dilution in duplicate experiments, LLOQ/detection limit ~ 1 IU/l. A LKCG hCG reagent kit was used for detection on LLOQ. For QC samples a Bio-Rad Lyphochek Plus Level 1 and 2 (Bio-Rad Laboratories, Denmark) was used.

A total of four CSF samples (denoted at A, B, C and D) were collected for the validation of the assay. Samples were identified by the birth year and letter for sex of each subject. All samples were kept in low temperature freezers (-70 °C) until analysis and returned there following analysis.

The CSF test sample and serum test sample were analyzed in dilution in duplicate experiments (analyzing the samples twice on consecutive days) to efficiently use sample materials.

CSF samples were mixed 1:5 with commercial reference material to a concentration of approximately 100 IU/l and this mixture was approximately 20% serum, which is reasonable to maintain the CSF matrix. The serum reference material was also diluted with a dilution reagent 1:5 to produce a test sample with the same concentration as the test CSF sample. Both the CSF and serum test samples were be serially diluted with a diluting reagent (1 + 1, 1 + 2, 1 + 3, 1 + 4, 1 + 5) in duplicate.

To evaluate the assay performance, quality control samples were analyzed with all samples in the validation. The serum reference material was analyzed in all analytical series to verify target value and uncertainty (imprecision). The three other CSF samples (B, C, D) were analyzed in all analytical series during validation to verify repeatability and reproducibility of CSF sample results were comparable to serum samples.

CSF test samples were prepared and analyzed as follows. CSF test sample A: CSF test sample A 100 1 + 100 1 diluting reagent (LCGZ dilution reagent, Sero A/S, Norway); CSF test sample 100 1 + 200 1 diluting reagent; CSF test sample 50 1 + 150 1 diluting reagent; CSF test sample 50 1 + 200 1 diluting reagent; CSF test sample 50 1 + 250 1 diluting reagent; serum QC sample (repeat analysis); CSF sample B (repeat analysis); CSF C (repeat analysis); CSF sample D (repeat analysis). The CSF samples A, B, C and

D were analyzed initially to determine initial concentration of hCG in samples.

Serum test samples were prepared and analyzed as follows. Serum test sample A: serum test sample A 100 1 + 100 1 diluting reagent; serum test sample 100 1 + 200 1 diluting reagent; serum test sample 50 1 + 150 1 diluting reagent; serum test sample 50 1 + 200 1 diluting reagent; serum test sample 50 1 + 250 1 diluting reagent; serum QC sample (repeat analysis); CSF sample B (repeat analysis); CSF C (repeat analysis); CSF sample D (repeat analysis). The preparation of CSF test sample A:

CSF sample A – 400 μ l

Seronorm Immunoassay L-2 N03542 – 100 μ l

Preparation of serum test sample: hCG dilution reagent – 400 μ l

Seronorm Immunoassay L-2 N03542 – 100 μ l

The linearity, correlation, and recovery of hCG were calculated both for the CSF test sample and for the serum reference material. For repeat analyses, standard deviation and coefficient of variation were calculated.

The acceptance criterion for the coefficient of correlation was set at > 0.95 for serum and CSF test samples. In addition, the slope of the linear regression curve for serum and CSF upon comparison must show that CSF does not differ by more than 20% from the serum slope.

Finally, recovery of hCG from the CSF and serum-diluted matrices was calculated both for the CSF and serum test sample and the value was expected to be within 25%, i.e. 75 – 125% of the expected hCG concentration.

hCG quantification: drug concentration and endpoint measurements

Determination of hCG concentration was performed using a solid-phase, two-site automated enzyme-enhanced chemiluminescent immunometric assay (manufactured by Diagnostic Product Corporation-DPC). The samples (serum or CSF) were introduced into a sampling cup containing a solid-phase (polystyrene bead) coated with monoclonal murine anti-hCG antibody. Alkaline phosphatase conjugated to polyclonal ovine anti-hCG was

added and hCG in the sample was bound in an antibody sandwich complex. Excess sample material and reagent were removed by centrifugation. Chemiluminescent substrate was added and the following reaction led to the emission of light of an intensity that was directly proportional with the amount of hCG in the sample. Validation of this technique was performed to determine if there were any matrix differences in CSF compared to serum that may have an effect on accurate determination of hCG concentration. The validation protocol is detailed above.

Data quality assurance

All assays used to measure drug concentration and safety laboratory analyses were performed using validated assays. All blood and CSF samples taken during the study were analyzed at the same laboratory, using the same assays. Both partial validation of hCG testing in human CSF and testing of human CSF samples were conducted at Capio Diagnostic a.s. Laboratories in Copenhagen, Denmark.

Data sets analyzed

Three analysis sets were defined for this study, the Intention to treat (ITT); Per Protocol (PP). Analysis sets were identical all analyses.

Statistics

Throughout the analyses, an $\alpha = 0.05$ was used, unless otherwise specified, without correction for multiple comparisons. In all analyses the null hypothesis was that the two hCG treatments were equal, as opposed to the alternative hypothesis that they differed.

All subjects were in the PP analysis set and as such only the ITT analysis was used.

To accomplish the primary objective of the study – characterization of the pharmacokinetics of hCG – the following analyses were employed:

AUC calculated on the basis of the individual serum hCG concentration profiles. AUC was calculated by the trapezoid method,

as the elimination phase was not reached (Figures 1, 2).

The mean AUC for the two products was summarized by mean and standard deviation. The two means were compared by a two-sample t-test under the assumption that the data were normally distributed. Secondly, a Wilcoxon two-sample test was performed.

The CSF hCG concentrations for both products were to be summarized at each time point by mean and standard deviation. The two products were to be compared at each time point by a two-sample t-test under the assumption that the data were normally distributed. A Wilcoxon two-sample test was also performed. However, given the observation that too few detectable hCG measurements exist, determining the frequency of detecting hCG or not, was made the main descriptor, rather than hCG concentration levels. As such, the test was changed from a t-test/Wilcoxon two-sample test to a Fisher's exact test, appropriate for evaluating proportions.

The sample size for this study was based upon precedent set by other Phase I studies of a similar nature rather than any formal power calculations.

Results and discussion

Disposition of subjects and protocol deviations

All 12 subjects completed the trial, and all 12 subjects are in the per protocol analysis set. As the ITT, PP and safety analysis sets were identical, the wording ITT analysis set is used consistently for all analyses. Overall, there were two protocol deviations in the study in which two CSF samples were not taken, one because it was not possible to gain access to the CSF and one subject did not want to have the CSF sample taken. No significant protocol violations occurred.

Demographics and other baseline characteristics

There was no indication in the demographic or baseline data to suggest any source of imbalance between the two treatment

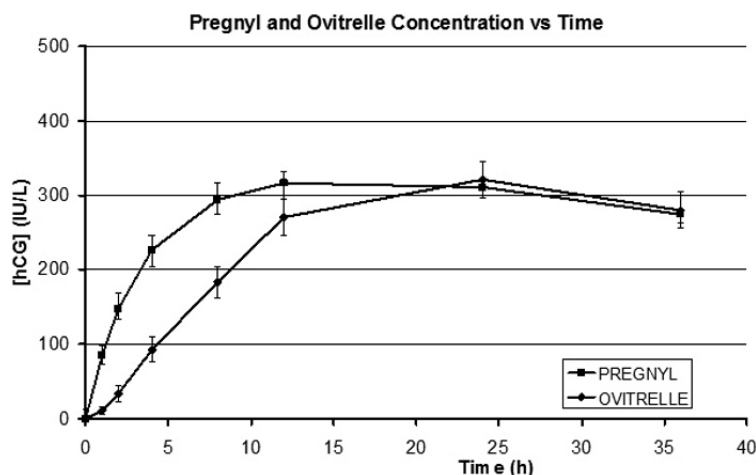


Figure 1. Mean plasma hCG concentration and s.e.m. of 10,000 IU hCG administered intramuscularly caused comparable increases in the plasma of 6 patients who received Ovitrelle and in 6 patients that received hCG. Patients that received hCG showed earlier increases in plasma hCG compared to those patients that received Ovitrelle at 1, 2, 4 and 8 hours after the initial dose, thereafter plasma concentrations were equivalent. It is notable that plasma AUC was equal for these two forms of hCG.

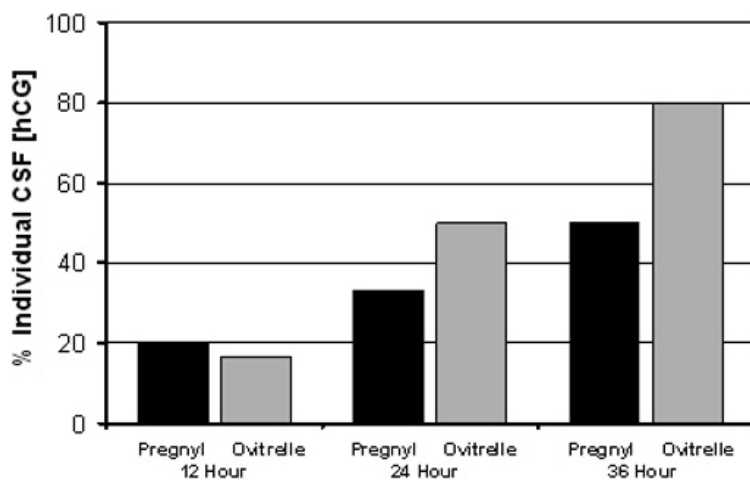


Figure 2. The frequency with which hCG could be detected in the CSF of patients at 12, 24 and 36 hours after a single, intramuscular, 10,000 IU dose of hCG. hCG Ovitrelle (n = 6) or hCG Pregnyl (n = 6) was administered at time 0, samples of cerebrospinal fluid were taken at three time points and CSF concentrations of hCG were noted with increasing frequency at 36 hours compared to 12. Pregnyl administration resulted in earlier and more individuals with detectable CSF hCG than Ovitrelle.

groups, although the mean age in the Pregnyl treatment group showed a trend to be higher than in the Ovitrelle treatment group. All patients were Caucasian males with a similar body mass index.

PK evaluation

Serum hCG concentration profiles

The results of the mean serum hCG concentration and individual serum hCG concentration profiles measured after taking patient samples at 1, 2, 4, 8, 12, 24 and 36 hours post-dose (Pregnyl or Ovitrelle) can be seen in Figure 1.

No statistical difference between the two treatment groups can be seen in Figure 1, although the mean serum hCG concentration of Pregnyl lies above the mean serum hCG concentration of Ovitrelle during the first 24 hours. This is also reflected in the individual profiles (not shown). Here it is clear that there is no difference between the two groups when C_{max} is compared but the data also suggests that hCG seems to be lower in subjects treated with Ovitrelle than Pregnyl during the first 24 hours after drug administration. No significant difference was observed when serum levels of hCG were compared for either preparation of hCG (Peak serum conc.: $3.16 \times 10(2) \pm 5.30 \times 10$ vs. $2.70 \times 10(2) \pm 6.00 \times 10$ at 12 hours, $3.11 \times 10(2) \pm 3.80 \times 10$ vs. $3.21 \times 10(2) \pm 6.00 \times 10$ IU/l at 24 hours (t-test; $p = 0.81$); Pregnyl and Ovitrelle, mean \pm SD, respectively).

Comparison of the mean AUC between groups was not statistically different $1.01 \times 10(4) \pm 1.27 \times 10(3)$ vs. $8.79 \times 10(3) \pm 1.77 \times 10(3)$ (T-test; Wilcoxon rank sum test, p value = 0.19 and 0.18, resp.), median AUC was $1.04 \times 10(4)$ vs. $8.81 \times 10(3)$ and the min-max range was $7.90 \times 10(3) - 1.176 \times 10(4)$ vs. $6.45 \times 10(3) - 1.15 \times 10(4)$ for Pregnyl vs. Ovitrelle, respectively. Thus, there is no statistical difference in the AUC when the two treatment groups were compared although treatment differences in individual profiles can also be seen in the AUCs. The exposure to hCG seems to be lower for subjects treated with Ovitrelle, but the apparent difference is not statistically significant as indicated by either p value

Partial validation results

The mean coefficients of correlation from the performed experiments were $1.0 \times 10(-1)$ for CSF and $1.0 \times 10(-1)$ for serum, respectively. The mean slopes of the linear regres-

Table 1. Plasma and CSF pharmacokinetic data for a single 10,000 IU intramuscular dose of Pregnyl or Ovitrelle.

	Pregnyl (n = 6)	Ovitrelle (n = 6)
Serum pharmacokinetic parameters		
C _{max} (IU/l)	3.16 × 10(2) ± 5.30 × 10	3.21 × 10(2) ± 6.00 × 10
t _{max} (h)	12	24
Half-life (h)	NA	NA
*AUC (IU h/l)	1.01 × 10(4) ± 1.27 × 10(3)	8.79 × 10(3) ± 1.77 × 10(3)
CSF pharmacokinetic parameters		
CSF concentration (IU/l)	1.3 – 2.1	1.0 – 1.4
C _{max} (IU/l)	NA	NA
t _{max} (h)	NA	NA
Half-life (h)	NA	NA
AUC (IU h/l)	NA	NA

*t-test, Wilcoxon rank sum test; p value is 0.19 and 0.18, respectively. NA = not available; values are mean ± SD.

sion line were 1.13 for CSF and 1.15 for serum – a difference of –1.9% between the slopes calculated relative to the slope of the serum linear regression. The dilution of the CSF and serum test samples show matrices that have complete commutability.

For a summary of plasma and CSF pharmacokinetic data for Pregnyl and Ovitrelle, see Table 1.

CSF hCG concentration determination

There is clear tendency over time whereby samples transitioned from being non-detectable towards having a measurable level. There is a clear tendency toward increased CSF hCG concentration over time as samples transitioned from being non-detectable towards showing measurable levels of hCG. For the other four subjects in this group, the levels went from being non-detectable at the first time point to being detectable at a later time. The measurable levels in this group were within the range 1.0 – 1.4 IU/l. In the Pregnyl group three subjects had non-detectable levels at all time points. Also in this group the levels went from being non-detectable at the first time point to being detectable at the later time points. The levels of hCG

measured in the CSF in this group were within the range 1.3 – 2.1 IU/l.

Figure 2 shows the percentages of detectable hCG in CSF samples at each time point for both Pregnyl and Ovitrelle. Subject sample size was 6 subjects for ITT analysis for both Pregnyl and Ovitrelle treated. CSF hCG was detected in 1 of 5 or 1 in 6 individuals treated with Pregnyl or Ovitrelle at Day 1 – sampled 12 hours after hCG administration, respectively; Fisher's exact test $p > 0.99$. At 24 hours post dose, hCG was detected in 2 in 6 and 3 in 6 individuals treated with Pregnyl or Ovitrelle; Fisher's exact test $p > 0.99$. 36 hours post dose, hCG was detected in 3 in 6 and 4 in 5 subjects CSF; Fisher's exact test $p = 0.55$. Although this study was conducted in healthy male volunteers, it is well-known that blood-brain barrier permeability may increase after neurological injury and thus, even higher CSF and intraparenchymal levels might be anticipated using this dose of hCG.

Safety evaluation

Overall, there appeared to be more subjects having treatment emergent adverse events in the Pregnyl treatment group than in the Ovitrelle treatment group and this was also reflected in the number of treatment adverse events. There were no drug-related adverse events, and only one event was classified as both severe and serious. The serious event was a headache, and determined to have no relation to the investigational product. This serious adverse event occurred in the Pregnyl treatment group.

Discussion of serum hCG and CSF hCG PK data

No clear difference between the two hCG treatments was apparent for the serum hCG concentration. A trend towards early higher concentrations of serum hCG was noted in Pregnyl-treated subjects during the first 24 hours, as compared to Ovitrelle-treated subjects, but did not reach significance, though this fact might in part be influenced by small sample size and thus power. The same pertains to the AUC, which was higher for Pregnyl-treated subjects but again this difference also did not reach significance.

It is generally accepted that the physicochemical, immunological, and biological activities of recombinant hCG are comparable to those of placental and human pregnancy urine-derived hCG [EMD Serono Inc. 2008].

One can speculate that higher hCG concentrations in subjects who received Pregnyl compared to Ovitrelle during the first 24 hours could be related to a key difference in the structure of the two molecules. While, both urine-derived and recombinant hCG (r-hCG) share identical amino acid sequences, the latter is expressed in Chinese hamster ovary cells. At least one previous study has described that while the glycoform pattern of the α -subunit of r-hCG is closely comparable to urinary-derived hCG (u-hCG), the structural differences between the two forms of hCG are mainly due to the branching and sialylation extent of the oligosaccharides. The β -chain of hCG possesses both O- and N-glycosylation sites and its structure and glycosylation pattern are also very similar to that of u-hCG [EMD Serono Inc. 2008]. Hence, the simplest explanation for the early observed difference in the serum hCG absorption might best be explained by differences in glycosylation and sialylation differences between the structure of these two forms of hCG.

The lack of a statistically significant difference observed for serum hCG concentration was also reflected in the CSF hCG concentration. A larger sample size may have permitted demonstration of a statistical difference in CSF hCG concentration at each of the time points, for example at 36 hours when 80% of the Ovitrelle versus 50% suggests a trend indicating a difference. Note that only 14 out of the 34 CSF samples had a detectable level of hCG (detection limit > 1.0 IU/l). All measurable levels were close to the detection limit (Ovitrelle, range 1.0 – 1.4 IU/l, Pregnyl, range 1.3 – 2.1 IU/l). The detection of hCG in CSF support the premise that hCG distributes to CSF into the CNS, via either the blood-brain barrier or the blood-CSF barrier [Pardridge 1989], and as expected the amount of hCG that can be found in the CSF is much lower than what can be found in the serum at the same time point. Lukacs et al. [1995] showed that in rats about 1/100th of peripherally injected hCG crossed the blood-brain barrier in an intact form and was found in the

CSF and hippocampus. The serum concentrations at time point 36 hours post dose are within the range of 219 – 359 IU/l, the CSF concentrations at the same time point are within the range > 1.0 – 2.1 IU/l. To our knowledge, no published study has investigated the passage of IM administered hCG into CSF in man. Only one study in rats, describing where passage approximately 1/100th circulating of levels of hCG into CSF was described using radioisotope methods of detection at 30 min following ^{125}I -u-hCG injection into the tail vein [Lukacs et al. 1995]. The results found in this study are in agreement with those of Lukacs et al. [1995]. Although this study was conducted in healthy male volunteers, patients suffering from acute ischemic stroke frequently experience increased blood brain-barrier permeability. As this is the first study of its kind showing detectable levels of hCG in the CSF in man, it can be speculated that in the setting of acute injury increased CSF and intraparenchymal concentrations hCG might translate into increased therapeutic benefit.

Several human studies have investigated the pharmacokinetics of Pregnyl or Ovitrelle following IM administration. One study by Mannaerts et al. [1998] showed IM administration of 10,000 IU of Pregnyl resulted in a peak serum concentration of 307 IU/l at approximately 20 hours. In this study we show similar peak concentrations of hCG but peak concentrations of the two drugs appeared to differ with the peak concentration with Pregnyl reached between 8 and 24 hours whilst serum concentrations of Ovitrelle were reached sometime during the period between 12 and 36 hours, though neither was statistically different. A second study by Chan et al. [2003] that administered IM u-hCG (Pregnyl) in women showed peak serum concentrations of 331 IU/l occurred at approximately 20 hours. While a study by Trincharde-Lugan et al. [2002] in 12 healthy male and female volunteers showed that 5,000 IU Ovitrelle or Pregnyl administered IV demonstrated that u-hCG tended to be distributed and eliminated slightly more slowly than the recombinant form of hCG (Ovitrelle). Overall serum pharmacokinetics results from our study are in agreement with these previously published studies. Mannaerts et al. [1998] investigated the bioavailability of SC and IM administra-

tion of 10,000 IU u-hCG (Pregnyl; Organon) in 18 healthy pituitary-suppressed volunteers. The subjects were assigned to single hCG injections of 10,000 IU IM or 10,000 IU SC. IM and SC injections of 10,000 IU hCG were bioequivalent with respect to AUC_0 (2.813 ± 0.587 vs. 3.048 ± 0.532 IU h/l). The C_{max} and t_{max} were also similar between the two administration routes. Serum mean peak concentrations of hCG were 307 IU/l with 10,000 IU IM and 339 IU/l with 10,000 IU SC, which peaked 20 hours after injection. The elimination half-life was on average 32 – 33 hours, irrespective of the treatment regimen. It was concluded that SC u-hCG is bioequivalent to IM u-hCG with respect to the extent of absorption.

The study had several limitations that decreased the degree to which results could be interpreted. Sequential recruitment of subjects into the study groups has the potential to confound study outcomes. Small study group size, while sufficient to demonstrate the passage of an intramuscular injection of hCG, may be too small to differentiate pharmacokinetic changes observed between the two drugs during the early sampling periods. For compassionate and ethical reasons the number of spinal punctures was limited to three and total study duration was limited to 36 hours. In both circumstances this limited available data and so the resolution, evaluation and interpretation of hCG serum and CSF pharmacokinetics.

Conclusion

This pilot study describes a novel partially validated method for analyzing hCG in human CSF and provides new evidence that hCG can be detected and distributes into the CSF after peripheral administration of 10,000 IU in a limited number of healthy male subjects with an intact blood-brain barrier and yet, as expected, the concentration found in the CSF is much lower than what was found in the blood at the same time point. Examination of the CSF levels of individuals reveals a trend going from a not detectable level towards a detectable level with time, suggesting a lag time for the transport into the CNS, as the highest level of hCG in serum does not necessarily give a measurable amount in the

CSF (i.e. there is a marked augmentation in the amount of detectable samples after 36 hours). Finally, in the aftermath of a stroke a regional increase in diffusion across a damaged blood-brain barrier is anticipated, which could lead to higher concentrations of exogenously administered hCG in CSF than what was demonstrated in the present study in healthy subjects. In this study, there was no significant difference between blood levels in terms of 24-hour AUC or peak serum concentration between the two forms of hCG, and an interesting trend suggesting a difference in hCG serum concentration the first 24 hours after drug administration may exist if a larger number of subjects were to be studied.

References

- AL-Hader AA, Lei ZM, Rao CV.* Novel expression of functional luteinizing hormone/chorionic gonadotropin receptors in cultured glial cells from neonatal rat brains. *Biol Reprod.* 1997; 56: 501-507.
- Belayev L, Khoutorova L, Zhao KL, Zuo FM, Salzman A, Davidoff AW, Moore AF, Cramer SC.* A novel therapeutic strategy improves functional recovery after MCAo stroke in rats. *Stroke.* 2007; 38: 467.
- Braunstein GD, Rasor J, Danzer H, Adler D, Wade ME.* Serum human chorionic gonadotropin levels throughout normal pregnancy. *Am J Obstet Gynecol.* 1976; 126: 678-681.
- Chan CC, Ng EH, Chan MM, Tang OS, Lau EY, Yeung WS, Ho PC.* Bioavailability of hCG after intramuscular or subcutaneous injection in obese and non-obese women. *Hum Reprod.* 2003; 18: 2294-2297.
- EMD Serono Inc.* Ovidrel Prescribing Information 2008.
- Lei ZM, Rao CV, Kornyei JL, Licht P, Hiatt ES.* Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. *Endocrinology.* 1993; 132: 2262-2270.
- Lukacs H, Hiatt ES, Lei ZM, Rao CV.* Peripheral and intracerebroventricular administration of human chorionic gonadotropin alters several hippocampus-associated behaviors in cycling female rats. *Horm Behav.* 1995; 29: 42-58.
- Mannaerts BM, Geurts TB, Odink J.* A randomized three-way cross-over study in healthy pituitary-suppressed women to compare the bioavailability of human chorionic gonadotropin (Pregnyl) after intramuscular and subcutaneous administration. *Hum Reprod.* 1998; 13: 1461-1464.
- Pardridge WM.* Strategies for drug delivery through the blood-brain barrier. *Neurobiol Aging.* 1989; 10: 636-637.
- Trinchard-Lugan I, Khan A, Porchet HC, Munafò A.* Pharmacokinetics and pharmacodynamics of recombinant human chorionic gonadotropin in healthy male and female volunteers. *Reprod Biomed Online.* 2002; 4: 106-115.