Pharmacokinetics of human chorionic gonadotropin after i.m. administration in goats (*Capra hircus*)

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Abstract

The present investigation addresses the pharmacokinetics of human chorionic gonadotropin (hCG), intramuscularly (i.m.) administered to goats. Nine pluriparous does of the Boer goat breed, 2–6 years of age and weighing 45–60 kg, were administered 500 IU hCG (2 ml Chorulon) deep into the thigh musculature 18 h after superovulatory FSH treatment. Blood samples were drawn from the jugular vein at 2 h intervals for the first 24 h, at 6 h intervals until 42 h, and at 12 h intervals until 114 h after administration. After centrifugation, plasma hCG concentrations were determined by electrochemiluminescence immunoassay. Pharmacokinetical parameters were as follows: lag time, 0.4 (S.E.M. 0.1) h; absorption rate constant, 0.34 (S.E.M. 0.002) h; absorption half-life, 2.7 (S.E.M. 0.5) h; elimination rate constant, 0.02 (S.E.M. 0.002) h; biological half-life, 39.4 (S.E.M. 5.1) h; and apparent volume of distribution, 16.9 (S.E.M. 4.3) l. The plasma hCG profile was characterized by an absorption phase of 11.6 (S.E.M. 1.8) h and an elimination phase of 70.0 (S.E.M. 9.8) h, with considerable individual variation in bioavailability and pharmacokinetical parameters. Biological half-life was negatively correlated ($P < 0.05$) with peak concentration ($r = -0.76$), absorption rate constant ($r = -0.78$), and elimination rate constant ($r = -0.87$). The results indicate that after rapid absorption, hCG remains in the circulation for an extended period. This has to be taken into account when assessing the stimulatory response to hCG treatment on an ovarian level.

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Introduction

Human chorionic gonadotropin (hCG) is a heterodimeric glycoprotein of 57 kDa consisting of a noncovalently bound α -subunit (92 amino acids) and a distinctive β -subunit (134 amino acids). It has the α -subunit in common with the glycoprotein hormones LH, FSH, and TSH. The β -subunit is unique for each of these hormones and responsible for their specific functions (Gupta & Dighe 2000, Senger 2005, De Rensis *et al.* 2010). The β -hCG chain shows 80% homology with the 121 amino acid subunit of the LH β -subunit (Norman & Litwack 1997). The prime source of hCG is the trophoblast of the human embryo and, after implantation, the villous syncytiotrophoblast of the placenta (Cole 2010). hCG contributes to maternal recognition of the developing human embryo at about 6–8 weeks of pregnancy (Senger 2005, Cole 2010). Furthermore, it initiates angiogenesis via growth factors to prepare the uterus for implantation (Zygmunt *et al.* 2002, Filicori *et al.* 2005, Cole 2010) and induces the initial production of progesterone by the corpus luteum until the placenta synthesizes adequate amounts to maintain gravidity (Norman & Litwack 1997).

Due to the structural similarity of hCG with LH, it exerts an effect by binding to LH receptors (Lei & Rao

1994, Birken *et al.* 1996). The prolonged activity and low clearance rate of hCG, in comparison to endogenous LH, are a consequence of the high glycosylation rate (Cole 2010). More than 75% of the hCG molecules are degraded in the liver; the remaining molecules are eliminated via the kidney (Cole 2010). hCG for clinical purposes is extracted from urine of pregnant women (Farrag *et al.* 2008). Alternatively, hCG may be derived via recombinant DNA technology from genetically engineered Chinese hamster ovary cells (Farrag *et al.* 2008).

In humans, hCG is extensively used as an ovulation-inducing agent *in lieu* of LH (Nader & Berkowitz 1990) and, after introduction of IVF and ICSI, in *in vitro* maturation of oocytes (Farrag *et al.* 2008). In men, hCG is used to stimulate the Leydig cells to synthesize testosterone to support spermatogenesis (Heller & Leach 1971). In domestic animals, hCG has a wide range of applications. It is used in the context of estrus synchronization in cattle and horses (Schmitt *et al.* 1996, Ginther *et al.* 2009) and ovulation induction in sheep and goats (Wani *et al.* 1997, Saleh *et al.* in preparation) as well as in fish (Kahkesh *et al.* 2010), to overcome the negative effect of premature regression of corpora lutea after superovulatory treatment in goats (Saharrea *et al.* 1998), and for improving pregnancy rate in cattle

and goats (Rajamahendran & Sianangama 1992, Fonseca & Torres 2005). There is a paucity of information on the pharmacokinetics of hCG in domestic animals with the exception of cats (Swanson *et al.* 1997). The objective of this study was to characterize the pharmacokinetics of hCG following an i.m. injection to induce ovulation in superovulated Boer goat does.

Results

The plasma profile arising from the hCG injection is depicted in Fig. 1. Individual variation is reflected by the dimension of the range (shaded area) and s.d.. Parameters characterizing the profile are presented in Table 1. Observed and calculated peak concentrations (C_{peak}) of 64.6 and 70.9 mIU/ml were arrived after 11.6 and 11.0 h, respectively, with substantial individual variation ranging from 40 to 85 mIU/ml for observed and 50 to 99 mIU/ml for calculated peak concentrations. Individual differences in pattern and amplitude of plasma hCG concentration are also reflected by the formidable range in area under the curve (1632–3118 h mIU/ml). The pharmacokinetic parameters are presented in Table 2. Absorption from the site of administration was rapid, as indicated by the lag time (T_0) of 0.4 h and the absorption half-life ($T_{0.5abs}$) of 2.7 h. The mean absorption rate constant (K_a) was 0.34 and the biological half-life ($T_{0.5elm}$) was 39.4 h. Both absorption and elimination of hCG took place in an almost linear fashion (Fig. 2). However, whereas absorption took place rapidly, the elimination phase was characterized by a gradual decline extending to, on the average, 70 (S.E.M. 9.8, range 30–106) h (Table 1); traces of hCG were found in the system after 5 days.

The correlation between absorption rate constant and elimination half-life was $r = -0.78$ ($P = 0.01$). Pearson's correlation coefficients between peak concentration on the one hand and half-life ($r = -0.76$, $P = 0.02$),

Figure 1 hCG concentrations recorded after single i.m. administration of 500 IU hCG (arrow) (mean, s.d., minimum and maximum) to nine adult Boer goat does.

Table 1 Characterization of plasma hCG profile (observed and calculated time of maximum concentration: T_{peak} ; observed and calculated peak plasma concentration: C_{peak} ; duration of absorption and elimination phases; and the area under the curve: AUC) after i.m. administration of 500 IU hCG in nine adult Boer goat does.

Parameter	Mean	S.E.M.	Range
T_{peak} (h)			
Observed	11.6	1.8	4–20
Calculated	11.0	1.9	5–20
C_{peak} (mIU/ml)			
Observed	64.6	4.9	40–85
Calculated	70.9	4.6	50–99
Duration (h) of			
Absorption phase	11.6	1.8	4–20
Elimination phase	70	9.8	30–106
AUC (h mIU/ml)	2427	177	1632–3118

elimination rate constants ($r = 0.67$, $P = 0.05$), and apparent volume of distribution ($r = -0.97$, $P = 0.0001$) on the other hand were significant; however, clearance rate was not related ($r = 0.06$, $P = 0.90$).

Discussion

This study addresses the pharmacokinetics of intramuscularly injected hCG in goats. The plasma hCG profile following injection was characterized by rapid absorption (11.6 h) and slow elimination (70.0 h) with substantial individual variation in bioavailability.

Fitting the plasma hCG profile to the most appropriate model revealed that hCG follows a two-compartment pharmacokinetic model (Jambhekar & Breen 2009). The pattern of essentially linear absorption and elimination phases resembles that of humans (Weissman *et al.* 1996, Chan *et al.* 2003) and domestic cats (Swanson *et al.* 1997).

The substantial individual variation in peak levels might be associated with individual differences in the hypothetical volume of distribution which ranged from 12–25 l. The more hCG molecules are bound to target tissue, the lower the plasma concentration is expected to be. Hence, this is an indication of the hypothetical volume into which hCG is distributed. The biological half-life of, on average, 39.4 h is in close agreement with the 36 h reported for humans (Cole 2010) but longer than the 26.1 h observed in domestic cats (Swanson *et al.* 1997).

The negative correlation between absorption rate constant and elimination half-life suggests that, if absorption of hCG is rapid, so will be clearance from the circulation. In does with low peak concentrations, half-life was longer; accordingly, elimination rate was lower and apparent volume of distribution was higher. The clearance rate was unaffected.

Elimination from the circulation follows the pattern typical for a two-compartment model (Jambhekar & Breen 2009). The average duration of the elimination phase of 70 h reflects the time hCG molecules reside in the circulation. The elimination phase of hCG, being

Table 2 Pharmacokinetic parameters: lag time (T_0), absorption rate constant (K_a), absorption half-life ($T_{0.5abs}$), elimination rate constant (K_e), elimination (biological) half-life ($T_{0.5elm}$), apparent volume of distribution (V_d), and clearance rate after i.m. administration of 500 IU hCG in nine adult Boer goat does.

Parameter	Mean	S.E.M.	Range
T_0 (h)	0.40	0.06	0.33–0.66
K_a (per h)	0.34	0.06	0.13–0.62
$T_{0.5abs}$ (h)	2.70	0.54	1.1–5.3
K_e (per h)	0.02	0.002	0.01–0.03
$T_{0.5elm}$ (h)	39.4	5.1	25–66
V_d (l)	16.9	4.3	12–25
Clearance rate (l/h)	0.2	0.02	0.2–0.3

about seven times as long as the absorption phase, reflects the extremely slow clearance process especially when contrasted with a clearance rate of 1.9 l/h for endogenous LH released during the preovulatory surge recorded in the same group of goats (Saleh *et al.* in preparation). Compared with humans (Weissman *et al.* 1996) and domestic cats (Swanson *et al.* 1997), the retention time of hCG in the circulation of goats falls within an intermediate range. According to Schmitt *et al.* (1996), the increase in plasma concentration in hCG-injected cows lasted for 30 h, which is much shorter than in goats. The slow rate of degradation is said to be due to sialic acid residuals on the β -subunit of the hCG molecule (Kalyan *et al.* 1982, Nisula *et al.* 1989, Kobata 2010). In humans, it has been shown that these residuals are enzymatically dissociated (Apparailly & Combarous 1994, Zygmunt *et al.* 2002, Chan *et al.* 2003), followed by the binding of the molecule to hepatic receptors and degradation. This pathway takes care of more than three quarters of the biologically active hCG; the remaining is cleared via the kidney. Approximately, 21% is excreted via the urine as heterodimeric hCG, nicked heterodimeric hCG, free subunits (some nicked), and, predominantly, hCG β core fragment (Cole 2010). Elimination rates via liver and kidney might differ depending on the physiological state (Rao 1985), interactions with other hormones or substances (Liu *et al.* 1995), route of administration (Saal *et al.* 1991, Wikland *et al.* 1995, Stelling *et al.* 2003), body mass index (Chan *et al.* 2003, Detti *et al.* 2007), and the presence of large ovarian follicles (Detti *et al.* 2007).

In the present experiment, ultrasound monitoring of the ovaries revealed that ovulation occurred ~34 h after hCG treatment. Taking this into consideration, it may be surmized that the prolonged bioavailability of hCG, lasting until well after ovulation, might possibly be supportive of corpus luteum function, but might, on the other hand, also be responsible for interference with postovulatory ovarian function. A study, involving possible functional aspects of hCG, including possible interaction with LH and direct or indirect effect on ovulation, is about to be completed and will be published in due course.

In conclusion, hCG injected i.m. to goats will rapidly make its appearance in the circulation, whereas clearance from the blood occurs gradually lasting, on average, for 70 h. There is considerable individual variability in time course and amplitude of these events.

Materials and Methods

Animals

The experiment was conducted on pluriparous Boer goat does, 2–6 years of age and weighing 45–60 kg, from the departmental breeding flock at Goettingen, Germany (9° 41' E, 51° 46' N) during the breeding season (October to January). The does were group-housed in open barns with straw-bedding and outdoor concrete runs. They were fed a daily ration of 600 g concentrate, consisting of equal parts of a pelleted diet for breeding ewes (16% crude protein, 10.2 MJ ME/kg, supplemented with 43 mg/kg Se, 12 mg/kg I, and 5000 mg/kg Zn), oats, and dried sugar beet pulp and had free access to wheat or barley straw, salt lick, and water. Once daily, the does were routinely tested for estrus with an aproned male.

Donor preparation and superovulation

From 4 days after observed estrus, daily blood samples were drawn from does to be tested for plasma progesterone concentration. Once the concentration exceeded 5 ng/ml, an i.m. injection of 5.0 mg dinoprost (1 ml Dinolytic; Pfizer, Karlsruhe, Germany) was administered. Seven days later,

Figure 2 Absorption (top) and elimination phase (bottom) of the plasma hCG profile in the wake of i.m. administration of 500 IU to nine adult Boer goat does (fitted with polynomial trend lines).

an i.m. injection of 0.004 mg of the GnRH analog buserelin (1 ml Receptal; Intervet, Unterschleissheim, Germany) was administered followed, 5 days later, by a series of six s.c. injections of 4, 4, 2, 2, 2, and 2 armour units of pFSH supplemented with 40% pLH (Nowshari *et al.* 1995), administered at 12 h intervals. Along with the last two pFSH injections, 1 ml Dinolytic was administered intramuscularly. To determine the time of ovulation, 18 h after the last dinoprost injection, does were administered an i.m. injection of 500 IU hCG (2 ml Chorulon; Intervet) into the thigh musculature (m. biceps femoris and m. semitendineus).

Blood sampling and determination of hCG concentration

Throughout the experiment, blood samples of 5 ml were drawn daily via jugular venipuncture. Before hCG administration, does were provided with indwelling jugular catheters, so samples could be drawn at 2 h intervals for the first 24 h and at 6 h intervals for the subsequent 18 h. Thereafter, a routine of daily sampling was resumed. Collection tubes contained three drops of Na-citrate to prevent clotting. After centrifugation at 1000 g for 10 min, plasma samples were stored at -20°C until being analyzed for hCG concentration by Electrochemiluminescence Immunoassay (ECLIA, ELECSYS; Roche) as described elsewhere (Forest *et al.* 1998). The assay is based on an electrochemiluminescent label (Ruthenium (II) tris (bipyridyl) $^{2+}$; $\text{Ru}(\text{bpy})_3^{2+}$), which can undergo multiple oxidation–reduction cycles when immobilized at the surface of an electrode in the presence of a co-reactant included in the assay buffer. The co-reactant tripropylamine (TPA), when oxidized at the electrode, produces a radical cation, which acts as a reducing agent. After the release of a proton, a TPA radical is formed that reacts with the oxidized form of the label to generate $\text{Ru}(\text{bpy})_3^{2+}$ in an excited state. After emission of a photon, the label can undergo another cycle and, therefore, generate multiple photons for each labeled molecule. Immobilization of the labeled complex is achieved by magnetic microparticles coated with streptavidin, which bind to biotin, linked, covalently, to reaction antibodies. A magnet immobilizes the bound fraction at the electrode to allow elimination of the unbound label before proceeding to the electrochemiluminescent detection.

Statistical analysis and calculation of biological half-life

To characterize the pharmacokinetics, the following parameters for hCG were assessed (Jambhekar & Breen 2009): lag time T_0 (time from treatment to first detection), absorption rate constant K_a ($0.693/T_{0.5\text{abs}}$), absorption half-life $T_{0.5\text{abs}}$, elimination rate constant K_e ($0.693/T_{0.5\text{elm}}$), elimination half-life $T_{0.5\text{elm}}$, and the apparent volume of distribution V_d . To assess the absorption half-life $T_{0.5\text{abs}}$ and the biological half-life $T_{0.5\text{elm}}$, plasma hCG concentrations of individual does were plotted against time. Absorption half-life was determined as the time required from administration to the point when half of the maximum plasma hCG level was reached, and biological half-life was determined as the time from maximum plasma concentration to half of that level. The apparent volume of

distribution, the volume to which a given dose of hCG would have to be diluted to have a concentration equal to the concentration detected in blood, was calculated as $V_d = \text{dose}/\text{hCG concentration}$.

To characterize the bioavailability parameters for hCG, observed and calculated peak concentration C_{peak} , observed and calculated time of peak occurrence T_{peak} , and the area under the hCG curve AUC_{0-114} were determined. These parameters were calculated on the basis of individual plasma concentrations, whereas the calculated values for the peak concentration and the time of peak occurrence T_{peak} were determined by the formulae $T_{\text{peak}} = (\ln(K_a/K_e))/(K_a - K_e)$ and $C_{\text{peak}} = I \times (e^{-K_e T_{\text{peak}}} - e^{-K_a T_{\text{peak}}})$, respectively, where I stands for intercept. The intercept was assigned after the individual elimination curves had been a subject to linear regression analysis. The area under the hCG curve AUC_{0-114} was calculated by means of the linear trapezoidal approximation using $(\text{AUC})_{t_1}^{t_2} = ((C_{p1} + C_{p2})/2) \times (t_2 - t_1)$, where C_{p1} and C_{p2} are hCG concentrations at the corresponding times t_1 and t_2 respectively. Pearson correlation coefficients were calculated to identify relationships between the pharmacokinetic parameters using the procedure CORR (SAS 9.2, Cary, NC, USA). Mean and standard error were calculated using the MEANS procedure (SAS 9.2).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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