Bioavailability of hCG after intramuscular or subcutaneous injection in obese and non-obese women

Carina C.W.Chan[1,3], Ernest H.Y.Ng[1], Maureen M.Y.Chan[2], Oi Shan Tang[1], Estella Y.L.Lau[1], William S.B.Yeung[1] and Pak-chung Ho[1]

[1]Department of Obstetrics and Gynaecology, Queen Mary Hospital, The University of Hong Kong and [2]Department of Pharmacology, The University of Hong Kong, China

[3]To whom correspondence should be addressed at: Department of Obstetrics and Gynaecology, Queen Mary Hospital, The University of Hong Kong, 102 Pokfulam Road, Hong Kong SAR, China. E-mail: cwcchan@graduate.hku.hk

BACKGROUND: Obese women require higher gonadotrophin doses for ovarian stimulation and to trigger ovulation. The bioavailability of a drug is affected by its route of administration. Herein, the bioavailability of hCG was compared after intramuscular (i.m.) or subcutaneous (s.c.) injection in obese and non-obese women. METHODS: Twenty four Chinese women, 12 with a body mass index (BMI) $\geqslant$28 kg/m^2 and 12 with a BMI of 20–25 kg/m^2 were recruited as the obese and non-obese groups respectively. A single hCG injection was given intramuscularly on one occasion, and subcutaneously on a second occasion, separated by 4 weeks. Blood samples were taken at intervals for the pharmacokinetic study of hCG. RESULTS: Examination of the hCG plasma concentration–time curve showed the area under the curve (AUC) and maximum concentration (C_{max}) of hCG to be significantly higher after i.m. injection than after s.c. injection in both the obese and non-obese groups. However, the AUC and C_{max} values in obese women were significantly lower than in non-obese women, irrespective of whether i.m. or s.c. dosing was employed. CONCLUSIONS: Intramuscular dosing of hCG provided better bioavailability than s.c. dosing, but bioavailability was significantly less in obese women than in non-obese women.

Key words: hCG/intramuscular/obese/subcutaneous

Introduction

Human chorionic gonadotrophin is used to induce final oocyte maturation and to provide luteal phase support during IVF treatment. hCG is usually given via the intramuscular (i.m.) route, but subcutaneous (s.c.) administration has also been described. Although the pharmacokinetics after either route have been compared (Saal *et al.*, 1991; Wikland *et al.*, 1995; Weissman *et al.*, 1996; Mannaerts *et al.*, 1998; Elkind-Hirsch *et al.*, 2001; Sills *et al.*, 2001; Stelling *et al.*, 2003), the results obtained were inconsistent. Whilst variations in the bioavailability of hCG after s.c. injection may be accounted for by differences in drug absorption at different tissue layers, the lack of any difference between i.m. and s.c. injection might be the result of injecting the drug into the subcutaneous fat layer rather than the intended muscle layer. The failure of all these investigators to confirm the site of drug deposition leaves this controversy unresolved.

The pharmacokinetic behaviour of hCG may also be affected by obesity. In general, a larger distribution volume results in a lower serum concentration (Dobbs *et al.*, 1994). In a standard IVF programme, serum hCG levels at 12 h after injection were found to be significantly lower in obese women, regardless of the route of administration (Elkind-Hirsch *et al.*, 2001).

However, a direct comparison of the bioavailability of hCG in obese and non-obese women has not been reported. The aim of the present study was to make such as comparison after i.m. or s.c. injection of hCG.

Materials and methods

Patients

The study was conducted according to the Declaration of Helsinki, and approved by the Institutional Review Board of the hospital. All patients provided their written consent to join the study. Women of Chinese ethnicity with body mass index (BMI) $\geqslant$28 kg/m^2 who were awaiting IVF treatment were recruited as the obese group ($n = 12$), while those with BMI 20–25 kg/m^2 were recruited as the non-obese group ($n = 12$). This cut-off for obesity was adopted from the World Health Organization (WHO) recommendation that Asians be considered obese when the BMI was $\geqslant$25 kg/m^2 (World Health Organization, 2000). Women with a contraindication for the use of hCG and/or major medical conditions, including uncontrolled hypertension, uncontrolled diabetes or ischaemic heart disease, were excluded.

Study design

The study was performed in the early follicular phase up to day 7 of the start of a spontaneous or progestogen-induced menstruation. A

Table I. Comparison of obese and non-obese groups[a]

Parameter	Obese group ($n = 12$)	Non-obese group ($n = 12$)	P[b]
Age (years)	34 (32–38)	34 (30–35)	NS
Body mass index (kg/m²)	29.6 (28.2–32.8)	21.3 (19.8–22.0)	<0.001
Waist circumference (cm)	88.0 (83.0–95.0)	69.5 (58.0–73.8)	<0.001
Hip circumference (cm)	105.0 (103.0–111.0)	91.0 (89.3–94.8)	<0.001
Waist:hip ratio	0.83 (0.81–0.86)	0.77 (0.75–0.78)	<0.001
Triceps skin fold (cm)	38.0 (37.0–42.0)	32.0 (26.8–35.0)	<0.001
AUC (i.m.) (IU·h/l)	16 050 (11 886–18 858)	25 080 (23 670–27 828)	<0.001
C_{max} (i.m.) (IU/l)	331 (239–400)	541 (492–644)	<0.001
AUC (s.c.) (IU·h/l)	10 134 (8838–14 112)	20 178 (16 500–23 370)	<0.001
C_{max} (s.c.) (IU/l)	175 (135–229)	306 (229–392)	0.001

[a]Values are median (inter-quartile range).
[b]Mann–Whitney U-test.
NS = not significant.
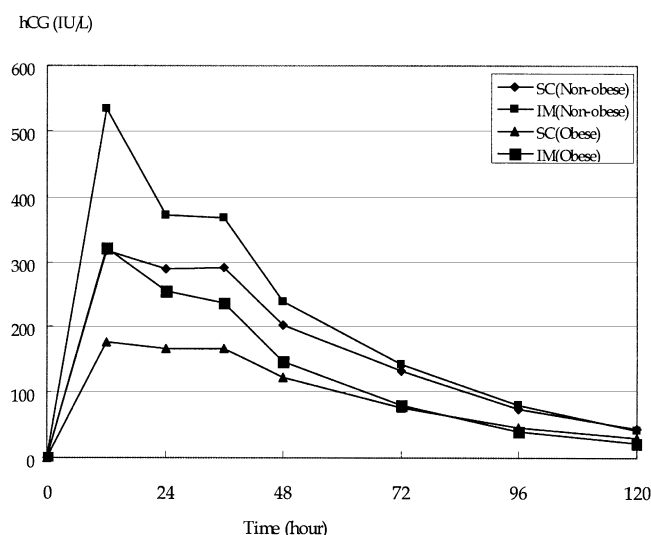
Figure 1. Pharmacokinetics of hCG after a single dose of 10 000 IU hCG after intramuscular and subcutaneous injections in obese and non-obese women.

blood sample was taken 5 min before hCG administration to ensure that there was no endogenous hCG present. An i.m. injection of 10 000 IU hCG (Pregnyl®; N.V. Organon, The Netherlands) was given under ultrasound guidance to ensure that the needle was correctly placed in the muscle layer of the deltoid muscle. Blood was taken at intervals (12, 24, 36, 48, 72, 96 and 120 h) for determination of hCG concentration by immunoassay using direct chemilluminometric technology (Bayer, New York, USA). These sampling intervals were based on a study conducted on the pharmacokinetics of hCG in normal patients (Mannaerts *et al.*, 1998). The mean (± SD) time to reach maximum serum concentration (t_{max}) after i.m. injection of 10 000 IU hCG was reported as 20.78 ± 9.68 h, and the half-life ($t_{\frac{1}{2}}$) was 33.55 ± 4.14 h. The maximum concentration (C_{max}) and area under the plasma concentration-time curve (AUC) from zero to infinity after i.m. administration were determined.

At 4 weeks after the initial (i.m.) administration of hCG, the same patients each received a s.c. injection of 10 000 IU hCG. The 4-week interval was utilized to allow clearance of hCG after i.m. dosing, and also to allow drug administration during the early follicular phase of the cycle. The injections were given using an injection pen and insulin needle to ensure that the drug was deposited in the subcutaneous layer of the periumbilical region. Blood was taken at the same intervals

as after i.m. injection. The C_{max} and AUC of hCG after s.c. administration were determined. All hCG used in the present study was from the same batch in order to avoid batch-to-batch variability of the drug.

Statistical analysis

Based on the results of a previous study (Mannaerts *et al.*, 1998), the mean AUC after i.m. hCG administration (10 000 IU) was 28130 ± 5870 IU·h/l. In order to detect a 20% reduction in AUC after s.c. injection, nine patients were required in each group to provide a power of 80% and P-value of 0.05. In order to allow a drop-out rate of 20%, 12 patients were recruited for each group; such a sample size also allowed detection of a 27% difference in the AUC of hCG between the obese and non-obese groups, with the same power.

Distributions of the variables were given as median and inter-quartile range. The C_{max} and AUC of hCG between the two different routes of administration in each group were compared by the Wilcoxon Signed Rank test. Similar comparisons between the obese and non-obese groups were made with the Mann–Whitney U-test. A P-value < 0.05 was considered statistically significant.

Results

Among the 24 patients recruited, one obese patient withdrew her consent after the i.m. cycle, and one non-obese patient conceived spontaneously after the i.m. cycle. Hence, 24 i.m. cycles with 12 patients in each group, and 22 s.c. cycles with 11 patients each group, were available for analysis. The age and height of the two groups were comparable, whereas the BMI, waist:hip ratio and triceps skinfold of the obese group were significantly higher than those of the non-obese group (Table I). These anthropometric parameters remained similar between the two cycles for each individual ($P > 0.05$, data not shown).

The AUC and C_{max} of hCG in the obese group were significantly lower than those of the non-obese group, whether hCG was given by either the i.m. or s.c. route (Table I; Figure 1). The AUC and C_{max} of hCG were significantly higher after i.m. injection than after s.c. injection in both the obese and non-obese groups ($P < 0.001$, Wilcoxon Signed Rank test; Figure 1).

Discussion

Generally, hCG injections are given intramuscularly, and are directed toward the buttock or the deltoid muscles. However, in

obese patients the needle may not be long enough to reach the muscle layer, especially when the subcutaneous fat is thick, and consequently the intended i.m. injection becomes a s.c. injection. In clinical terms, such an error would not have any clinical implications if absorption of the drug by these two routes of administration were comparable. In fact, s.c. administration of hCG has been used in both Europe (Wikland *et al.*, 1995) and North America (Elkind-Hirsch *et al.*, 2001; Sills *et al.*, 2001; Stelling *et al.*, 2003) during ovulation induction and IVF treatment. The s.c. route has certain advantages over the i.m. route, mainly as the patient or her husband can be instructed to give the injections themselves. This is especially relevant when hCG administration, which is usually timed, is to be given late at night and nursing staff must be available outside normal office hours to administer the i.m. injection. Clearly, it would be more convenient to the patient if the drug could be self-administered.

Several studies have compared the pharmacokinetics of hCG after i.m. and s.c. administration, but the results obtained have been controversial. In a preliminary study performed in male subjects (Saal *et al.*, 1991), the bioavailability of hCG was significantly higher after i.m. than s.c. injection. However, when the study was repeated in female subjects of normal body weight, no difference was observed (Mannaerts *et al.*, 1998). Another group (Wikland *et al.*, 1995) studied hCG levels at 12 and 36 h after hCG injection to induce oocyte maturation during IVF cycles, and found serum levels of hCG to be significantly higher in women receiving i.m. compared with s.c. injections. By contrast, and using the same model, no difference in the serum concentration of hCG could be found at either 12 h (Elkind-Hirsch *et al.*, 2001) or 24 h (Sills *et al.*, 2001) after injection when the study was repeated. A recent study reported an even higher serum hCG level at 36 h after a single i.m. injection than after a s.c. injection in an IVF programme (Stelling *et al.*, 2003), though the comparison of a single serum concentration does not reflect the true bioavailability of the drug. The absorption of hCG after s.c. injection appeared to be delayed (Saal *et al.*, 1991), and this cast doubt on the validity of comparing serum levels at a particular time point. It was also noticed that the difference in serum hCG levels between the i.m. and s.c. administration was smaller at 36 h, despite the serum level still being higher after i.m. injection.

Furthermore, all of these studies failed to confirm the site of drug deposition, which might explain the discrepancy in observation. For example, when studying the pharmacokinetics of hMG, one group (Dobbs *et al.*, 1994) confirmed correct placement of the needle—and thus the site of drug deposition—by using electromyography. These authors noticed that it was often necessary to insert the needle considerably further than 4 cm (1.5 in) to reach the muscle layer. Undoubtedly, the relative obesity of their subjects contributed to the depth of needle placement required to reach this depth. Nevertheless, it was possible that some of these intended i.m. injections were given subcutaneously, even in women of normal body stature, thereby diluting any possible difference between the two routes of administration. During injection in the present study, the needle was confirmed—using ultrasound guidance—to be

correctly placed in the muscle layer. Moreover, the women were used as their own control, so that any difference in drug bioavailability was attributable to absorption from the injection site rather than to inter-individual variation. The deltoid muscle was chosen as the site for i.m. injection because the subcutaneous fat layer there appeared to be thinner than that overlying the gluteus muscle. Unfortunately, the depth of the subcutaneous layer was not measured as this was not an intended observation to be made when the study was designed. In retrospect however, this information may have been helpful.

Obese women have a larger volume of distribution than non-obese women, and this may lead to a lower serum concentration after drug administration (Dobbs *et al.*, 1994). A negative correlation between the BMI and serum hCG levels at 12 h after injection was observed in patients treated by IVF (Elkind-Hirsch *et al.*, 2001). In the present study, it was confirmed that after single injection, obese women had a lower bioavailability of hCG compared with non-obese women, irrespective of the route of administration. If this information can be extrapolated to infer the pharmacokinetics of other gonadotrophins (namely hMG), it may help to explain the observation that obese patients show less favourable responses to ovarian stimulation. For example, patients with polycystic ovary syndrome (PCOS) who were overweight required more ampoules of hMG in order to achieve ovulation (Chong *et al.*, 1986; Hamilton-Fairley *et al.*, 1992; McClure *et al.*, 1992). Even in women without PCOS who were undergoing IVF treatment, the odds ratio of a negative response both on day 7 and at the end of treatment increased with BMI (Crosignani *et al.*, 1994). Furthermore, in a more recent study a negative association between BMI and the number of oocytes retrieved in IVF cycles was found (Ng *et al.*, 2000). Only one investigation—a case-controlled retrospective study involving women with a BMI ≥ 28 kg/m²—reported a comparable ovarian response, though the peak estradiol level was still significantly lower in the heavier women (Lashen *et al.*, 1999). Direct pharmacokinetic data on hMG is difficult to obtain because of the presence of endogenous gonadotrophins. Even with the use of combined oral contraceptives or GnRH analogues, the suppression of endogenous FSH and LH secretion is often not complete (Dobbs *et al.*, 1994; Duijkers *et al.*, 1995). hCG is absent in non-pregnant women, and therefore serves as the ideal surrogate marker to study the pharmacokinetics of gonadotrophins. The low bioavailability of hCG (and perhaps also of other gonadotrophins) in obese women bears an important clinical implication that these women should either be given a higher dose or, more effectively, be advised to reduce their body weight before embarking on such treatment. The latter factor is especially important when the cost of treatment must be considered.

Different doses of hCG have been used in various IVF treatment protocols to induce final maturation. There is as yet no agreement on the minimum dose required, but evidence has suggested that different patients have different thresholds for their response to hCG. In one study, single i.m. administration of either 5000 or 10 000 IU hCG was enough to stimulate final oocyte maturation in the majority of patients (Abdalla *et al.*, 1987), though unfortunately the body weights of the patients were not reported. Later studies showed comparable clinical

outcomes in non-obese patients after 10 000 IU hCG given by either the i.m. or s.c. route in IVF programmes (Wikland *et al.*, 1995; Stelling *et al.*, 2003). These observations indicate that i.m. administration of 10 000 IU hCG probably exceeds the minimum threshold for non-obese patients. A lower bioavailability of hCG after s.c. administration still gives an adequate serum level in non-obese women. In the present study, the AUC of hCG after i.m. injection in obese patients was slightly lower than that after s.c. injection in non-obese patients. Such an approach may still reach the minimum hCG threshold for final oocyte maturation in most obese patients, although the dosage may need to be individualized in extremely obese women. Given its poor bioavailability, the s.c. administration of hCG in obese women is not advisable.

In conclusion, the higher bioavailability of hCG after i.m. injection than after s.c. injection was demonstrated, and a significantly lower bioavailability of hCG was observed in obese women, irrespective of the route of hCG administration. These findings support the advice that obese women should reduce their body weight before embarking on subfertility treatment, as the poor bioavailability of gonadotrophins is one of the many problems that these women might encounter.

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