Selective and extremely long inhibition of prolactin release in man by 1-ethyl-3-(3'-dimethylaminopropyl)-3-(6'-allylergoline-8'- β -carbonyl)-urea-diphosphate (FCE 21336)

A. E. PONTIROLI¹, G. C. VIBERTI¹, R. MANGILI¹, L. CAMMELLI¹ & A. DUBINI²

¹Istituto Scientifico Ospedale San Raffaele, Cattedra di Clinica Medica, Università di Milano, Milano and

²Farmitalia Carlo Erba S.p.A., Research & Development, Milano, Italy

1 The effects on anterior pituitary function of FCE 21336 (1-ethyl-3-(3'-dimethylamino-propyl)-3-(6'-allylergoline-8'- β -carbonyl)-urea-diphosphate), a synthetic ergoline derivative with selective dopamine agonistic properties, were studied.

2 Circulating PRL, GH, TSH, cortisol and LH levels were determined up to 96 h after single oral doses of 50, 100, 200 and 300 μ g of the compound to eight healthy males, and up to 168 h after single oral doses of 400 and 600 μ g to six healthy males, according to double-blind, within subjects, experimental designs vs placebo. Vital signs, ECG, laboratory tests and the appearance of newly observed signs and symptoms were monitored.

3 A dose-related decrease of serum PRL in comparison with both basal and post-placebo levels was observed after 200 μ g and greater doses of the compound, with inhibition of spontaneous circadian rhythm. Maximal inhibition (PRL < 2 ng ml⁻¹) was observed in one out of five subjects after 200, three out of seven subjects after 300, four out of six after 400 and five out of six subjects after 600 μ g. The effect was of rapid onset and long duration; the maximum or nearly maximum decrease was observed within 3 h after dosing as well as up to 96 h (200 and 300 μ g) and up to 168 h (400 and 600 μ g). No modifications of GH, TSH, LH and cortisol as well as of vital signs, ECG and laboratory tests were apparent. No signs or symptoms of clinical relevance were reported; nasal stuffiness and headache occurred in four out of six and two out of six subjects after the highest dose, respectively. These data indicate that FCE 21336 is a long-lasting and selective inhibitor of PRL secretion in normal man.

Keywords prolactin ergoline derivatives pituitary growth hormone TSH LH cortisol

Introduction

During the last 15 years drugs able to inhibit prolactin (PRL) secretion both in animals and in man became available. Bromocriptine (Del Pozo *et al.*, 1972) was the first drug undergoing extensive clinical use for the prevention of puerperal lactation and for the management of idiopathic and tumoral hyperprolactinaemia, and

was followed later on by metergoline (Ferrari *et al.*, 1980); both drugs are active at daily dosages of some milligrams and require more than one administration per day. Lisuride (De Cecco *et al.*, 1978) was then developed, active at fractions of a milligram, but still its activity was relatively short-lived. Finally pergolide (Lemberger &

Crabtree, 1979) was characterized as a drug effective at fractions of a milligram and with a longer duration of action, which allows administration every 48 h.

In the last 3 years 1-ethyl-3-(3'-dimethyl-amino-propyl)-3-(6'-allylergoline-8'- β -carbonyl)-urea-diphosphate (FCE 21336, Figure 1) has been studied in rats and has shown a powerful PRL lowering activity, with ED_{50} varying from 3 to 30 $\mu\text{g kg}^{-1}$ p.o. in the different animal models and with a duration of action of more than 72 h, much longer than pergolide or bromocriptine. *In vitro*, FCE 21336 inhibits PRL release from monolayer cultures of PRL secreting cells, to which it adheres firmly. In addition FCE 21336 does not affect pre-ovulatory LH surges nor the LH response to LH-RH, thus showing a selective inhibition of PRL release (Di Salle *et al.*, 1982, 1983a,b, 1984b). Studies performed so far indicate FCE 21336 as a dopaminergic drug: in *in vitro* binding experiments for striatal dopaminergic turnover, it displaced labeled spiperone and labeled domperidone, with IC_{50} 11 and 20 nmol respectively (IC_{50} for pergolide 20 and 100 nmol, IC_{50} for bromocriptine 5 and 100 nmol respectively) (Di Salle *et al.*, 1984a).

In this paper we describe the endocrine effects and the clinical and laboratory tolerability of this compound administered to normal volunteers in acute experiments at rising oral doses. FCE 21336 appears a selective inhibitor of PRL release, while basal release of GH, TSH, LH and cortisol is not affected: the duration of action of one single oral dose of 600 μg is longer than 168 h, and the drug does not induce significant side effects.

Methods

Two studies were performed with fourteen healthy male physicians or undergraduate medical students (eight in the first study, six in the second

study) who volunteered and gave their written consent after being fully informed of the nature of the study and of the possible risks linked to the first administration of the drug in humans. Only subjects free of endocrine and metabolic disorders and with normal kidney, liver and bone marrow functions on the basis of physical examination and laboratory tests were admitted to the trial. Subjects taking drugs known or suspected to interfere with PRL release continuously in the 2 months or occasionally in the 2 weeks preceding the study were excluded. FCE 21336 was always administered orally.

In the first study placebo was administered to all eight subjects and FCE 21336 was administered at the dose of 50 (three subjects), 100 (three subjects), 200 (five subjects) and 300 μg (seven subjects), at 14 days intervals, under double-blind conditions. Doses were increased stepwise after verification of the clinical tolerance of each preceding dose level. Only one subject per session was exposed to each of the progressively increased doses. The drug was supplied as lyophilized powder reconstituted with water immediately before use. Tests began at 09.00 h, after an overnight fast and smoking withdrawal: treatments were administered together with 100 ml of milk, a small quantity of coffee and one piece of pastry to the subjects in the recumbent position. The position was maintained for 4 h except for heart rate and arterial blood pressure measurements (see below); thereafter subjects were allowed to move freely. On the days of the tests, a standardized diet of 2,500 kcal (60% carbohydrates, 22% proteins, 18% lipids) was given to each subject.

Blood samples for PRL, growth hormone (GH), thyroid-stimulating-hormone (TSH), luteinizing hormone (LH) and cortisol were taken at the intervals shown in Figure 2 for up to 96 h. At the same time intervals blood pressure and heart rate were assessed in lying conditions and 1 and 5 min after standing up.

A complete laboratory profile was performed before and 24 h after each treatment, and it included: haemochromocytometry, BUN, serum creatinine, uric acid, AST, ALT, γGT , alkaline phosphatase, prothrombin activity, cholesterol, triglyceride, total serum proteins and electrophoresis, amylases, LDH, Na^+ , and K^+ , and blood glucose levels; urinalysis, including 24 h excretion of Na^+ , K^+ , Ca^{2+} was also performed before and after each treatment. Resting ECG was done at admission and 24 h after each treatment.

Newly appearing signs and symptoms, spontaneously referred or specifically questioned for by the investigators, were recorded. In the second

Figure 1 Chemical structure of FCE 21336 [1-ethyl-3-(3'-dimethylamino-propyl)-3-(6'-allylergoline-8'- β -carbonyl)-urea-diphosphate].

Figure 2 Serum prolactin (hPRL) concentrations after placebo and after different doses of FCE 21336 in normal subjects. Means $\pm$ s.e. mean are indicated. Numbers in brackets indicate subjects who, at different periods, showed serum PRL levels < 2 ng ml⁻¹. Limit of sensitivity 2 ng ml⁻¹ indicated by dotted line.

study, which was carried out following the results of the first one, six normal subjects received placebo, and FCE 21336 400 and 600 µg at 14–21 days intervals.

In each subject the higher dose was administered always after the lower dose, and the placebo was randomized according to a within subject double-blind experimental design. The same experimental procedure of the first study was followed and the same assessments were performed; the observation period was extended to 168 h after treatment.

Blood samples were obtained through an indwelling needle inserted into a forearm vein kept patent by a slow 0.9% NaCl saline drip. Sera for the evaluation of PRL, TSH, GH, LH and cortisol levels were frozen at -20° C until assayed by radioimmunoassay. In our hands interassay coefficients of variation are 4.6, 8.0, 5.8, 6.8 and 9.6% respectively, and intra-assay coefficients of variation are 2.7, 3.5, 3.7, 5.5 and 5.4% for the five hormones considered; the limits of sensitivity are 1.0 ng ml⁻¹ for PRL, 0.5 µU ml⁻¹ for TSH, 0.25 ng ml⁻¹ for GH, 1.5 mIU ml⁻¹ for LH and 4.0 ng ml⁻¹ for cortisol, and recoveries of added doses are 102.0, 98.9, 98.0, 105.0 and 99.5% respectively.

Statistical analysis was performed on pre- and post-treatment laboratory data by paired Student's *t*-test; for PRL, GH, TSH, LH and

cortisol net secretory areas under the curves (Δ AUC) were calculated for each dose by the trapezoidal method and compared with the corresponding data after placebo.

Results

Figure 2 shows serum PRL levels after increasing doses of FCE 21336 and placebo in the first study. The dose of 50 µg (not shown) and the dose of 100 µg had no clear effect; only one out of five subjects had PRL levels < 2 ng ml⁻¹ after 200 µg, while after 300 µg PRL levels < 2 ng ml⁻¹ were observed in three out of six subjects. Mean serum PRL levels were depressed to a similar degree, 3 and 96 h after administration of 300 µg of FCE 21336. Statistical analysis performed on AUC showed the dose of 300 µg but not the dose of 200 µg to be significantly more active than placebo ($P < 0.01$). The figure does not include one subject who had (Figure 3) spontaneously raised serum PRL levels in basal conditions on two out of three test days: while no modifications were observed after placebo, both 200 µg and 300 µg were fully active in lowering PRL levels irrespective of the baseline levels.

Figure 4 shows that the doses of 400 µg and 600 µg of FCE 21336 were effective in suppressing PRL release in the subjects studied, the latter

Figure 3 Serum prolactin (hPRL) concentrations after placebo (○) and after two different doses of FCE 21336 (200 and 300 µg ●) in subject 5 (G.R.) who had spontaneously raised serum PRL levels in two out of three instances. The dotted line indicates the limit of sensitivity.

dose being on average slightly but not significantly more active, as confirmed by statistical analysis performed on Δ AUCs (400 µg vs placebo: $P < 0.01$; 600 µg vs placebo: $P < 0.01$; 400 vs 600 µg: NS). Mean serum PRL levels were to a similar extent depressed 3 as well as 168 h after administration of FCE 21336 at both dosages.

GH, TSH, LH and cortisol release were unaffected by FCE 21336 (not shown).

No changes of heart rate, blood pressure and ECG were observed. No changes were observed for laboratory examinations performed before and 24 h after administration of different doses of FCE 21336: a slight increase of urinary excretion of Na^+ and Ca^{2+} was apparent after FCE 21336 200 and 300 µg, but not after the higher doses.

Table 1 shows the signs and symptoms encountered during the studies. No signs and

symptoms of clinical relevance were reported; frequency of signs and symptoms appeared dose-related in the 300–600 µg range. Nasal stuffiness was reported with dose-related frequency after 400 and 600 µg. Two subjects at the highest dose complained of headache.

Discussion

FCE 21336 proved effective in lowering serum PRL levels in normal individuals up to 96 h after single oral doses of 300 µg and up to 168 h after single oral doses of 400 and 600 µg, corresponding to the last assessment interval in both studies.

Therefore it is possible that the effect of a 300 µg dose is longer than 96 h and that the effect of a 600 µg dose is longer than 168 h. No effects on basal release of other hormones, namely GH, TSH, LH and cortisol were observed.

These results confirm in humans the long duration of action of the compound observed in animals (Di Salle *et al.*, 1982, 1983a,b, 1984b): in particular, FCE 21336 was as selective in inhibiting PRL release as pergolide, but with a much longer duration of action. It remains to be evaluated whether the response of these hormones to appropriate stimuli is affected by FCE 21336.

At present the reasons why the effect of FCE 21336 is so long are unknown: a reliable assay of plasma levels of FCE 21336 is not yet available, and the kinetics of FCE 21336 in humans are not known. However radioactivity distribution studies carried out in the rat with the compound administered orally, showed extensive tissue distribution in highly perfused organs, pituitary included, and a particularly slow elimination

Figure 4 Serum prolactin (hPRL) concentrations after placebo and after two different doses of FCE 21336 (400 and 600 µg) in six normal subjects. Numbers in brackets indicate subjects who, at different periods, showed serum PRL levels $< 2 \text{ ng ml}^{-1}$, the limit of sensitivity indicated by the dotted line.

Table 1 Absolute frequencies of signs and symptoms after treatment with placebo and with different doses of FCE 21336

Symptoms/signs	Placebo	50	Doses of FCE 21336 (μg)				
			100	200	300	400	600
Anisocoria	0	1	0	0	0	0	0
Increased skin temperature	1	0	0	0	0	0	0
Diarrhoea	0	0	0	1*	1*	0	0
Nausea	0	0	0	0	1**	0	0
Nasal stuffiness	1	0	0	0	0	2	4
Headache	1	0	0	0	0	0	2
Dry mouth	0	0	0	0	0	1	1
Stipsis	0	0	0	0	0	0	1
Irritability	2	0	0	0	0	1	0
Somnolence	0	0	0	0	0	1	0
Vivid dreams	0	0	0	0	0	0	1
Increased appetite	0	0	0	0	0	0	1
Postural hypotension (asymptomatic)	1▲	0	0	0	1△	0	0
Number of subjects with at least one of indicated signs or symptoms	5	1	0	1	3	3	5
Number of subjects at risk	14	3	3	5	7	6	6

*Occurring 12 h later; ** occurring 3 days later.

▲ 20 mm Hg, △ 30 mm Hg

from the pituitary (Valzelli, unpublished data). It is possible, as it happens in rats, that the molecule adheres firmly to dopaminergic receptors on the pituitary gland (Di Salle *et al.*, 1984b).

The tolerability of these pharmacodynamically active doses was appreciable. Among the side-effects characteristic of the pharmacological class, only nasal stuffiness and headache were reported after 400 and/or 600 μ g. However, only acute administrations of FCE 21336 were performed, and the tolerability of repeated administrations has to be investigated.

Further studies appear therefore warranted in order to evaluate whether such a prolonged activity also applies to hyperprolactinaemic patients or to normal subjects who have serum PRL levels raised by stimulating agents, although preliminary evidence obtained in case 5 (G.R.) suggests that the effect of FCE 21336 takes place

independently of basal PRL levels. Based on our results, a pilot study with single oral doses of FCE 21336, 300 and 600 μ g, has been performed in hyperprolactinaemic patients. Also in that study, PRL levels were significantly depressed ($-57.4 \pm 8.3\%$ and $-78.6 \pm 2.7\%$, respectively), and the serum PRL fall was uniformly present at 3 h as well as 14–21 days later. In the same study, in hyperprolactinaemic patients, repetitive, once a week, 300 μ g FCE 21336 administration was associated with constant PRL suppression, and no adverse reactions were observed (Ferrari *et al.*, 1986).

If this drug will confirm its safety and activity, over prolonged treatments, it could represent a major advance in the field of drugs inhibiting PRL release.

The drug used in these studies, FCE 21336, was supplied by Farmitalia Carlo Erba, Milano, Italy.

References

- De Cecco, L., Foglia, G., Ragni, N., Rossato, P. & Venturini, P. L. (1978). The effect of lisuride hydrogen-maleate in the hyperprolactinemia-amenorrhea syndrome: clinical and hormonal responses. *Clin. Endocrinol. (Oxf.)* 9, 491–496.
- Del Pozo, E., Brun Del Re, R., Varga, L. & Friesen, H. (1972). The inhibition of prolactin secretion in man by CB-154 (2-Br- α -ergocriptine). *J. clin. Endocrinol. Metab.*, 35, 768–771.
- Di Salle, E., Giudici, D., Ornati, G., Carfagna, N., Caccia, C., Moretti, A., Pegrassi, L. & Rossi, A. (1984a). Prolactin lowering and central dopaminergic activities of the new ergoline derivative FCE 21336 *in vivo* and *in vitro* in the rat. *Proceedings of the First European Neuroendocrine Association*, Basel, 4–7 March, Abstract Volume, p. 122.
- Di Salle, E., Ornati, G. & Briatico, G. (1982). FCE 21336, a new ergoline derivative with a potent and

- long-acting lowering effect on prolactin secretion in rats. *J. Endocrinol. Invest.*, **5** (Suppl. 1), 45.
- Di Salle, E., Ornati, G. & Giudici, D. (1984b). Confronto della durata dell' effetto inibitorio sulla secrezione di prolattina *in vivo* ed *in vitro* nel ratto da parte di FCE 21336, pergolide e bromocriptina. *J. Endocrinol. Invest.*, **7** (Suppl. 1), 32.
- Di Salle, E., Ornati, G., Giudici, D. & Briatico, G. (1983a). Prolactin lowering effect of a new ergoline derivative, FCE 21336, in the rat: a comparison with bromocriptine. *Acta Endocrinol.*, **103** (Suppl. 256), 265.
- Di Salle, E., Ornati, G., Giudici, D. & Ragazzi, P. (1983b). Effect of a new ergoline derivative FCE 21336 on prolactin and LH secretion in the rat. *J. Endocrinol. Invest.*, **6** (Suppl. 1), 6.
- Ferrari, C., Paracchi, A., Boghen, M., Baroldi, P., Galparoli, G., Zavaglia, C. & Crosignani, P. G. (1986). Long-lasting prolactin-lowering effect of FCE 21336 in hyperprolactinemic patients: dose response and comparison with standard dose of bromocriptine. *J. Endocrinol. Invest.*, **9** (Suppl 1), 229.
- Ferrari, C., Reschini, E., Peracchi, M. & Crosignani, P. G. (1980). Endocrine profile and therapeutic employment of a new prolactin-lowering drug, metergoline. *Gynecol. Obstet. Invest.*, **11**, 1-11.
- Lemberger, L. & Crabtree, R. E. (1979). Pharmacologic effects in man of a potent, long-acting dopamine receptor agonist. *Nature*, **205**, 1151-1153.

(Received 7 July 1986,
accepted 9 December 1986)