EXCRETION BALANCE AND URINARY METABOLIC PATTERN OF [^{3}H]CABERGOLINE IN MAN

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SUMMARY

After oral administration of [^{3}H]cabergoline to man at a single nominal dose of 0.6 mg/subject radioactivity is mainly eliminated by the faecal route (72% of the dose after 10 days). Urine contains 18% of the dose after the same period. The unchanged drug and metabolites present in urine were identified by comparison with reference compounds and quantified by radio-TLC analysis.

Cabergoline is extensively metabolized. Unchanged drug in 0-24 h urine represents less than 14% of urinary radioactivity, reaching 20% in 0-96 h urine. The acid derivative FCE 21589 is the main metabolite, amounting to 38% and 30% of the urinary radioactivity in 0-24 h and 0-96 h urine, respectively. The amide derivative FCE 21590 appears to be present in only a small amount, accounting for no more than 4% of the urinary radioactivity in the urine of the first 24 hours after administration and increasing to about 8% in the 0-96 h urine.

KEY WORDS

cabergoline, excretion, metabolic pattern, man

INTRODUCTION

Cabergoline, 1-[(6-allylergolin-8β-yl)carbonyl]-1-[3-(dimethylamino)propyl]-3-ethylurea, [CAS-81409-90-7], (Figure 1), is a new ergoline derivative with potent and long-lasting inhibitory activity on basal secretion and pharmacological and physiological hypersecretion of prolactin in the rat /1, 2/. Comparative time-course analysis of the rat brain and hypophysis inhibition of [^{3}H]N-*n*-propylnorapomorphine binding showed that cabergoline was more potent than bromocriptine in inhibiting this binding and that it occupied the receptor for a longer period of time /3/.

Clinical studies indicate a marked prolactin lowering effect also in healthy male volunteers, lasting up to 4 days after single oral doses of 0.2-0.3 mg and up to 7 days after a single oral dose of 0.6 mg /4/.

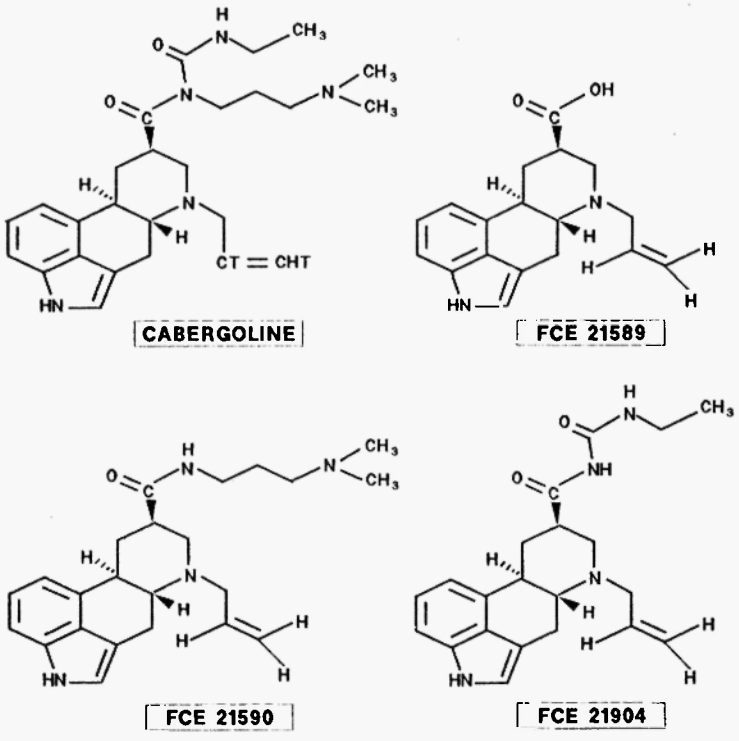

Fig. 1: Structure and labelling position (T = ^{3}H) of [^{3}H]cabergoline and some of its derivatives.

The maximal decrease of prolactin secretion in hyperprolactinaemic patients ranges between 49.2% and 55.2% and occurs between 2 and 5 days after a single oral dose of 0.3 mg /5/.

The purpose of this study was to determine the relative importance of the urinary and faecal routes of excretion for cabergoline and/or its metabolites in humans as well as to investigate the metabolic pattern of this compound as a function of time, in order to know whether unchanged cabergoline is present in the urine during the whole period of prolactin lowering. For the above-mentioned purpose it was essential to determine the stability of cabergoline in human urine, during both storage and processing of urine to separate metabolites.

MATERIALS AND METHODS

Chemicals

Reagents and solvents were of analytical grade (ACS specifications) and supplied by Farmitalia Carlo Erba S.r.l, Erbamont Group (Milan, Italy). Standard reference compounds used for metabolite indentification (Figure 2) were synthesized in the Chemistry Laboratories of Farmitalia Carlo Erba.

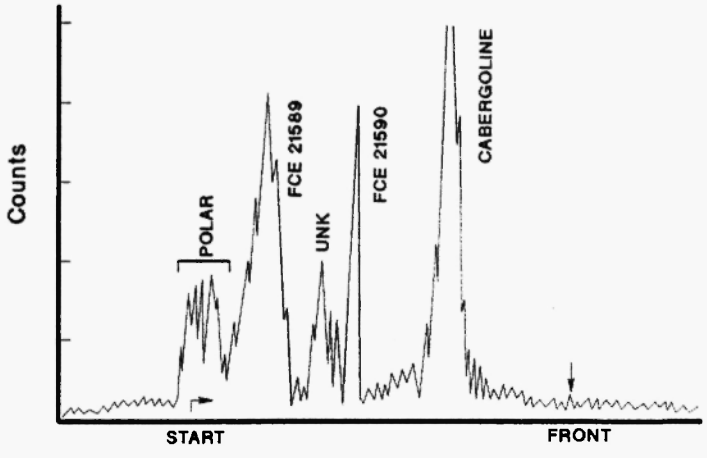

Fig. 2: Typical radio-TLC profile from XAD2 acetonic eluate of a 12-24 h human urine following oral administration of [^{3}H]cabergoline at a nominal dose of 0.6 mg (as base)/subject.

Radioactive compound

The study of excretion and metabolism was performed using tritium-labelled cabergoline /6/. The compound was labelled in position 2 and 3 of the allyl moiety (Figure 1). The radiochemical purity was >95% as determined by radio-thin layer chromatography (TLC) (Silicagel Merk F254 plates; (a) chloroform:methanol:30% ammonium hydroxide 70:30:0.25 by volume or (b) cyclohexane:ethyl acetate:diethylamine 20:70:10 by volume). The specific radioactivity of the compound, as free base, was found to be 23.7 MBq/mg (641.6 μCi/mg).

Human studies

Three healthy male volunteers, aged from 30 to 32 years and weighing from 71 to 93 kg, took part in the study.

The study was carried out under the supervision of Prof. P. D'Amico at the Department of Nuclear Medicine of "San Gerardo dei Tintori" Hospital, Monza, Italy.

The subjects were in good health on the basis of medical history, physical examination and routine laboratory tests on blood and urine. They gave their written consent to participate after the aim of the study and the pharmacodynamic effects of the treatment had been explained. On the day of administration the subjects fasted overnight before receiving orally [^{3}H]cabergoline at a dose of 0.6 mg (as base)/subject (14.2 MBq/subject, 383 μCi/subject) dissolved in aqueous phosphoric acid 1:2 (10 ml). The vial containing the drug was washed twice with 15 ml of water and the washes were ingested. A light breakfast and lunch were given 2 and 5 hours after drug administration, respectively. Urine and faeces were collected before dosing and at timed intervals after administration until 240 h. All biological samples were frozen at -30°C in the dark until analysis. The analysis was carried out as soon as possible, as it is known that with storage of at least 3 months under these conditions virtually no degradation of cabergoline occurs (Pianezzola and LaCroix, unpublished results).

Radiometric assay

Radioactivity in urine, water and organic extracts was measured by liquid scintillation spectrometry after mixing with the liquid scintillation cocktails Instagel (Packard, Illinois, USA) or Rialuma (Lumac B.V., Holland).

Faeces were thawed and homogenized with an adequate volume of water. Aliquots of each homogenate were lyophilized and then solubilized at 50°C using Soluene 350 (Packard); after addition of isopropanol, the samples were decolourized with hydrogen peroxide, heated at 50°C for two hours and added to Dimilume 30 (Packard). Tritium counting was performed in Packard 300 C or 460 CD liquid scintillation spectrometers. Efficiency was calculated by the "channel ratio method". Calibration curves were plotted for each scintillation cocktail used. The counting error was <3% (2 S.D.) for all samples.

Analysis of urinary metabolites

Urine samples of each subject at various time intervals up to 96 hours were percolated through an Amberlite XAD2 resin (pore diameter: 8.5-9.0 nm; specific surface area: 290-330 m^2/g; grain size: 20-50 Mesh ASTM) column with a volume ratio resin/urine of about 0.40. Metabolites absorbed on the resin were washed with water and eluted first with acetone and then with acetic acid.

The radioactivity of the three fractions obtained, the aqueous eluate containing both exhausted urine and washings, the acetonic eluate and the acetic acid eluate, was detected by liquid scintillation counting.

The organic eluates were reduced in volume and then analyzed by TLC.

The TLC plates (0.5 mm thick) were silicagel 60 F254 (Merck); the solvent system was chloroform:methanol:30% ammonium hydroxide 70:30:1 (by volume).

Radioactivity detection was performed by a radiochromatogram scanner (Berthold, LB 282 TLC analyzer) interfaced with an Apple IIe Grappler plus personal computer.

The labelled metabolites were identified by comparison with standard reference compounds co-chromatographed with each analyzed sample (Figure 1).

The standard reference compounds were observed under UV lamp at 254 nm. The indol ring was further revealed by spraying the plates with Ehrlich's reagent (solution of 1.5 g *p*-dimethylamino-benzaldehyde in a mixture of 75 ml methanol and 25 ml 37% hydrochloric acid) /7/.

Stability of cabergoline during processing of urine to separate metabolites

[^{3}H]Cabergoline, dissolved in phosphoric acid aqueous solution, was added to blank human urine at a final concentration of 276 ng (as base)/ml. Urine was then percolated through an Amberlite XAD2 resin column, processing the sample in the same way as the urine collected after oral administration of the compound.

The acetonic fraction eluted from the column was analyzed by TLC, using the solvent system described above. The stability of [^{3}H]cabergoline dissolved in phosphoric acid aqueous solution was also examined in aqueous solution and in acetone (2 ng/ml, as base). Different temperatures (+4°C, room temperature) and times (4 h and 24 h) were checked by TLC with the solvent system described above.

RESULTS

Excretion of radioactivity

Urinary radioactivity in the first 24 h (Table 1) accounted for 10% of the dose, while 240 h after the administration it reached 18%. The major part of radioactivity was present in faeces, being 72% of the dose at 240 h.

The total recovery in urine and faeces amounted to 90.72±6.33% (mean±SD) of the administered dose 240 h after treatment. It must be taken into account that the faeces were lyophilized to analyze their radioactive content; since loss of volatile radioactivity cannot be excluded during the process of lyophilization, the total recovery of the excreted radioactivity can be considered satisfactory.

TABLE 1

Urinary and faecal excretion of radioactivity in three healthy male volunteers following single oral administration of 0.6 mg [^{3}H]cabergoline. Data are expressed as mean (SD, n = 3) cumulative percent of dose.

Time (h)	Urine	Faeces (*)
4	3.55	
8	5.73	
12	7.57	
24	9.68	0.06
48	11.58	14.27
72	13.45	25.87
96	14.80	41.08
120	15.78	66.96
144	16.57	70.11
168	17.18	71.83
192	17.69	72.10
216	18.02	72.17
240	18.48 (0.49)	72.24 (3.51)
Total recovery	90.72 (6.33)	

(*)lyophilized samples

TABLE 2

Rf values of cabergoline and some standard reference derivatives

Compound	Rf
Cabergoline	0.67
6-demethyl-6-allyldihydrolysergic acid or 6-allyl-8'β-carboxyergoline (FCE 21589)	0.14
N-(3-dimethylaminopropyl)-6-allylergolin-8'β-carboxyamide (FCE 21590)	0.37
1-[(6-allylergolin-8'β-yl)carbonyl]-3-ethylurea (FCE 21904)	0.75

Analysis of the urinary metabolites

The Amberlite XAD2 extraction of human urinary metabolites enabled the analysis of about 90% of the radioactivity present in the samples: about 80% and 10% were in the acetonic and acetic acid eluates, respectively.

Rf values of cabergoline and standard reference derivates are shown in Table 2.

A representative metabolic profile following TLC chromatographic analyses of the urinary acetonic eluate shows the presence of several peaks, named, in order of decreasing polarity, Polar, FCE 21589, UNK, FCE 21590 and cabergoline, taking into account their Rf values (Fig. 2).

The radiochemical impurity of cabergoline, whose structure is unknown, has an Rf value higher than cabergoline in both solvent systems (a) and (b). Therefore one can assume that it is highly improbable that this impurity co-migrates with any metabolite. Chromatographic analyses of rat urine (results not shown) with two different techniques (radio-TLC with the solvent system described above and radio-HPLC) suggest that no other compounds have the same chromatographic behaviour in TLC as the standard reference compounds FCE 21589, FCE 21590 and cabergoline.

The chromatographic analyses of 0-24 h urinary organic eluates (acetonic and acetic acid) showed that in man the main peak was that migrating like the reference compound FCE 21589: it amounted to 3.71% of the administered dose (Table 3). The peak migrating like cabergoline represented also an important fraction of the 0-24 h urinary organic eluates, accounting for 1.31% of the dose. The peak migrating like the standard reference compound FCE 21590 was also present, amounting to 0.41% of the dose.

The remaining radioactivity present in the organic extracts was due to a fraction more polar than the acid derivative FCE 21589 and to a minor fraction migrating between the acid derivative FCE 21589 and the amide derivative FCE 21590. The former amounted to 2.47% and the latter to 0.47% of the dose.

The metabolic profile was similar in the urine of 0-96 h after administration; nevertheless, the unchanged drug and peak UNK substantially increased compared to the 0-24 h urine, reaching 2.91% and 0.80% of the dose, respectively; the amide derivative FCE 21590 proportionally increased even more, reaching 1.15% of the dose.

TABLE 3

Radioactive peaks detected by radio-TLC analysis in urine up to 96 h following oral administration of [^{3}H]cabergoline to three healthy male volunteers at a nominal dose of 0.6 mg/subject. Data are expressed as mean (SD, n = 3) cumulative percent of dose.

		Radioactive peaks in the acitonic + acetic acid eluates				
Time (h)	NA (*)	Polar	FCE 21589	UNK	FCE 21590	Cabergoline
4	0.24 (0.10)	1.09 (0.11)	1.83 (0.54)	0.16 (0.01)	0.04 (0.06)	0.20 (0.01)
8	0.67 (0.22)	1.70 (0.11)	2.60 (0.33)	0.27 (0.05)	0.10 (0.09)	0.40 (0.08)
12	1.03 (0.09)	2.06 (0.08)	3.25 (0.46)	0.35 (0.07)	0.21 (0.11)	0.66 (0.17)
24	1.31 (0.10)	2.47 (0.25)	3.71 (0.27)	0.47 (0.08)	0.41 (0.14)	1.31 (0.26)
48	1.61 (0.07)	2.85 (0.21)	4.01 (0.37)	0.58 (0.09)	0.67 (0.16)	1.85 (0.38)
72	1.78 (0.10)	3.25 (0.10)	4.26 (0.45)	0.71 (0.07)	0.94 (0.15)	2.50 (0.56)
96	1.93 (0.13)	3.53 (0.02)	4.47 (0.55)	0.80 (0.07)	1.15 (0.16)	2.91 (0.53)

(*)NA = Aqueous eluate from Amberlite XAD2 resin column, not analyzed by TLC

The acid derivative FCE 21589 and the polar fraction increased slightly, reaching 4.47% and 3.53% of the dose, respectively.

The analysis of the metabolic pattern from the urinary organic extracts did not show the presence of a radioactive peak with a chromatographic behaviour similar to the standard reference compound FCE 21904, the urea derivative (Fig. 2), although this compound has an Rf value in the system used not very different from that of cabergoline (Table 2).

Stability of cabergoline during processing of urine to separate metabolites

Cabergoline showed good stability when dissolved in phosphoric acid aqueous solution, added to human urine and then percolated through an Amberlite XAD2 resin column.

The compound dissolved in phosphoric acid aqueous solution was also stable at 4°C for 24 h in water and in acetone, as well as at room temperature for 4 h in acetone.

DISCUSSION

Owing to the high biological activity of cabergoline /1, 4/ at low dosage, it appeared important to have a labelled compound with high specific activity available. The use of the tritium labelled molecule enabled us to obtain satisfactory information on the excretion balance and preliminary information on the urinary metabolic pattern in man. In contrast with terguride /8/, cabergoline belongs to the group of ergolinic compounds that are excreted mainly via the faecal route /9/. The large faecal excretion of radioactivity and the persistence of radioactivity in urine and faeces even after prolonged times after drug administration suggest biliary elimination of radioactivity and the possibility of enterohepatic recycling. Nevertheless, the kidney contributes to the elimination of the drug and its metabolites more than in the case of bromocriptine /10/ and approximately as much as in the case of lergotrile /11/. Despite the slow elimination, the recovery of the excreted radioactivity is satisfactory.

Cabergoline is extensively metabolized in man, at least after oral administration. During its metabolism cabergoline might lose some tritium, following N-deallylation and/or further metabolism of the

allyl group, as has been described for *n*-allyl chlonidine /12/ and eugenol /13/ in man. If tritiated water is produced by metabolism, it must be taken into account that its half life is considerably longer than that of cabergoline /14, 15/.

Although the tritium labelled molecule enabled us to obtain satisfactory preliminary information, cabergoline disposition and metabolism need to be further investigated by a ^{14}C-labelled molecule. Synthesis with ^{14}C in a metabolically stable position has already been carried out /6/.

The radioactivity excreted in urine is still appreciable ten days after administration, even though it amounts to no more than 3.7% of the dose in the 5-10 days interval, whereas it accounts for 14.8% of the dose 96 h after treatment. For this reason, the study of the urinary metabolic pattern was carried out on the urinary samples recovered up to 96 h.

Cabergoline might give artefacts in metabolic studies when the biological samples are not stored or analyzed under proper conditions. Direct analyses of the urinary samples would be preferable in principle. However, the direct analysis of human urinary samples was impossible, because of the low specific concentration of radioactivity. The method applied to concentrate the urinary radioactivity and to separate metabolites appeared to be satisfactory and to produce no artefact.

Cabergoline has already been extensively metabolized by 24 h after oral treatment. Cabergoline amounts to less than 14% of the urinary radioactivity in this time interval, whereas it reaches 20% of the urinary radioactivity in the urine of 0-96 h after administration.

The radioactive peak migrating like the standard reference compound FCE 21589 appears to be the main metabolite, accounting for almost 40% of the total urinary radioactivity in the 0-24 h interval and decreasing to 30% in the 0-96 h interval.

The amide derivative FCE 21590 amounts only to 4% of the total urinary radioactivity in the first 24 h and reaches 8% in the 0-96 h interval after treatment. The urea derivative FCE 21904 seems not to be present in the metabolic pattern of cabergoline, although further chromatographic analysis is necessary to make a definitive statement. Unfortunately cabergoline hydroxylated in position 13 was not available as a reference standard; this, in either the free or a conjugated form, might be a metabolite of cabergoline in man, as observed for lergotrile /11/. Hopefully this reference standard might

be prepared biosynthetically, as it was in the case of lergotrile /16/, thus allowing further investigation of the metabolic pathway of cabergoline in man.

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