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PERCUTANEOUS PENETRATION AND METABOLISM OF TOPICAL [^{14}C]FLUTAMIDE IN MEN

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This study was designed to determine the fate of the nonsteroid antiandrogen flutamide in men following a single 6-hr topical application of 5 mg ^{14}C-labeled drug dissolved in 50% ethanol/50% propylene glycol. Analysis of 0–120 hr urine shows at least 16% of the applied flutamide is absorbed. Fifty-six percent of the dose is recovered from the site of application with cotton swabs moistened with 50% ethanol/50% propylene glycol. Flutamide plasma levels peak in 4 to 6 hr at about 1.3 ng/ml and then decline rapidly to about 0.08 ng/ml 24 hr after application. Only 13% of plasma ^{14}C is associated with flutamide 6 hr after drug application. There are at least 10 plasma metabolites, of which 6 have been tentatively identified. These are α,α,α-trifluoro-4'-amino-m-acetotoluidide (A); α,α,α-trifluoro-4'-amino-2-methyl-m-lactotoluidide (B); α,α,α-trifluoro-4'-nitro-m-acetotoluidide (C); α,α,α-trifluoro-2-methyl-4'-nitro-m-lactotoluidide (D); α,α,α-trifluoro-4'-amino-2-methyl-m-propionotoluidide (E); and α,α,α-trifluoro-6-nitro-m-toluidine (F).

(D) is the major plasma metabolite, and its concentration exceeds flutamide's between 8 and 24 hr after drug. All the plasma metabolites are found in 0–24 hr urine in minor amounts. An additional metabolite, α,α,α-trifluoro-amino-5-nitro-p-cresol (G), accounts for 27% of urine ^{14}C.

Flutamide is a potent, nonsteroid antiandrogen in animals [1,2] that is being tested clinically for treatment of prostatic carcinoma [3]. Its fate in men after a single oral 200-mg dose has been studied [4]. Because flutamide is active topically [5], and is potentially useful for treatment of acne and hirsutism, we studied its fate in 5 men after topical application of 5 mg of ^{14}C-labeled drug.

MATERIALS AND METHODS

Radiochemical assays and isolation and quantitation of metabolites have been described [4].

Preparation of ^{14}C-Labeled Flutamide

Labeled drug was prepared from [^{14}C]CF_3-m-aminobenzotrifluoride (Mallinckrodt, St. Louis, Mo.) by reaction with isobutyric anhydride to yield an anilide which was then selectively nitrated to yield [^{14}C]CF_3-flutamide (sp act approximately 50 μCi/mg). Chemical purity was >99% and radiochemical purity >98% by TLC in solvent systems B, C, D, and E (see section on *Chromatography* for composition).

Drug Administration and Sample Collection

The experiment was performed after informed consent was obtained from each of the subjects. Two hundred microliters of 50% propylene glycol/50% ethanol containing 5.16 mg [^{14}C]flutamide (49.77 μCi/mg) was applied to a 100-cm^2 (51.6 μg/cm^2) area on the upper back of each of 5 healthy male volunteers. A hemispherical, perforated plastic cup was taped over the treated area and removed after 6 hr. The treated area was then swabbed with cotton moistened with 50% propylene glycol/50% ethyl alcohol. ^{14}C in the cotton swabs was extracted with ethyl alcohol and, after drying, an aliquot of each swab was hydrolyzed in 6 N HCl and the hydrolysate was assayed for ^{14}C.

Ten milliliters of blood (in heparinized tubes) were collected immediately before (zero time) and 1, 2, 4, 6, 8, 12, 16, 24, 48, and 72 hr after drug. Total urine samples were collected commencing 24 hr prior to and continuing up to 120 hr after drug.

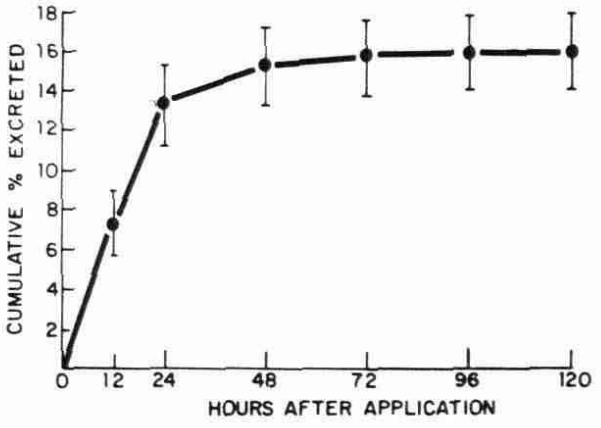

FIG. 1. Excretion of ^{14}C in urine by men following a single topical 5-mg dose of [^{14}C]flutamide. Values are average of 5 subjects ± SE.

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Abbreviations:
NMR: nuclear magnetic resonance
SE: standard error
TLC: thin-layer chromatography

Chromatography

All chromatographic separations were carried out on E. Merck 250-μ Silica gel GF plates (Brinkmann Instruments, Inc., Westbury, N. Y.) with solvent systems of the following composition (v/v): A, $CHCl_3$:ethyl acetate, 3:1; B, toluene:tetrahydrofuran, 9:1; C, toluene:

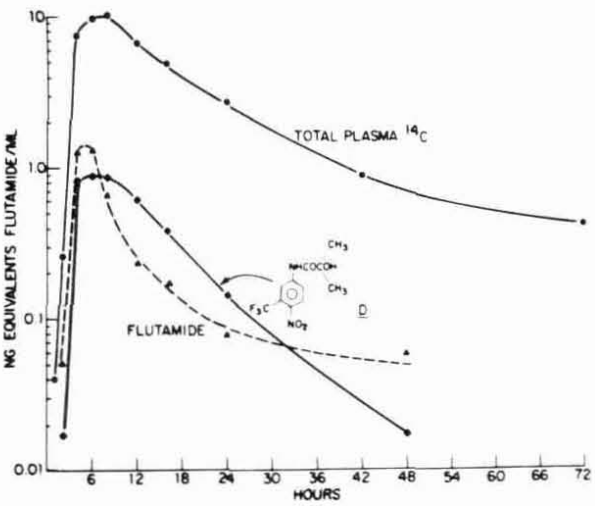

FIG. 2. Plasma radioactivity in men following a single topical 5-mg dose of [^{14}C]flutamide.

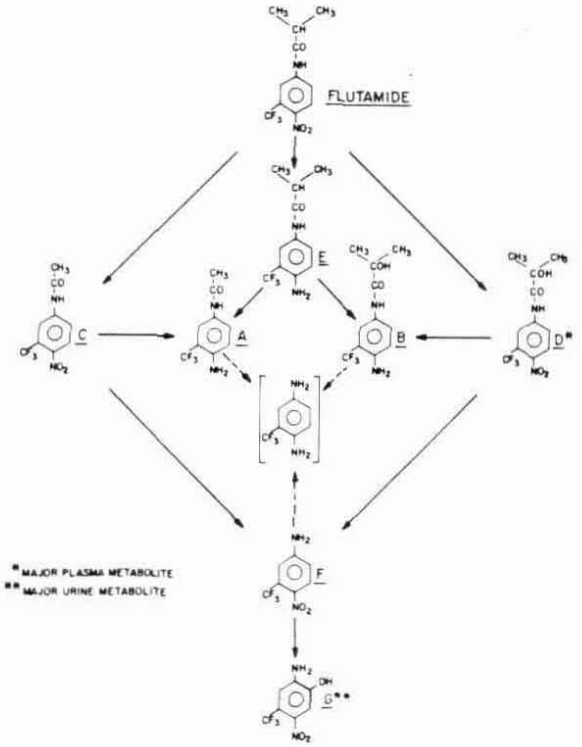

FIG. 4. Flutamide metabolism in men.

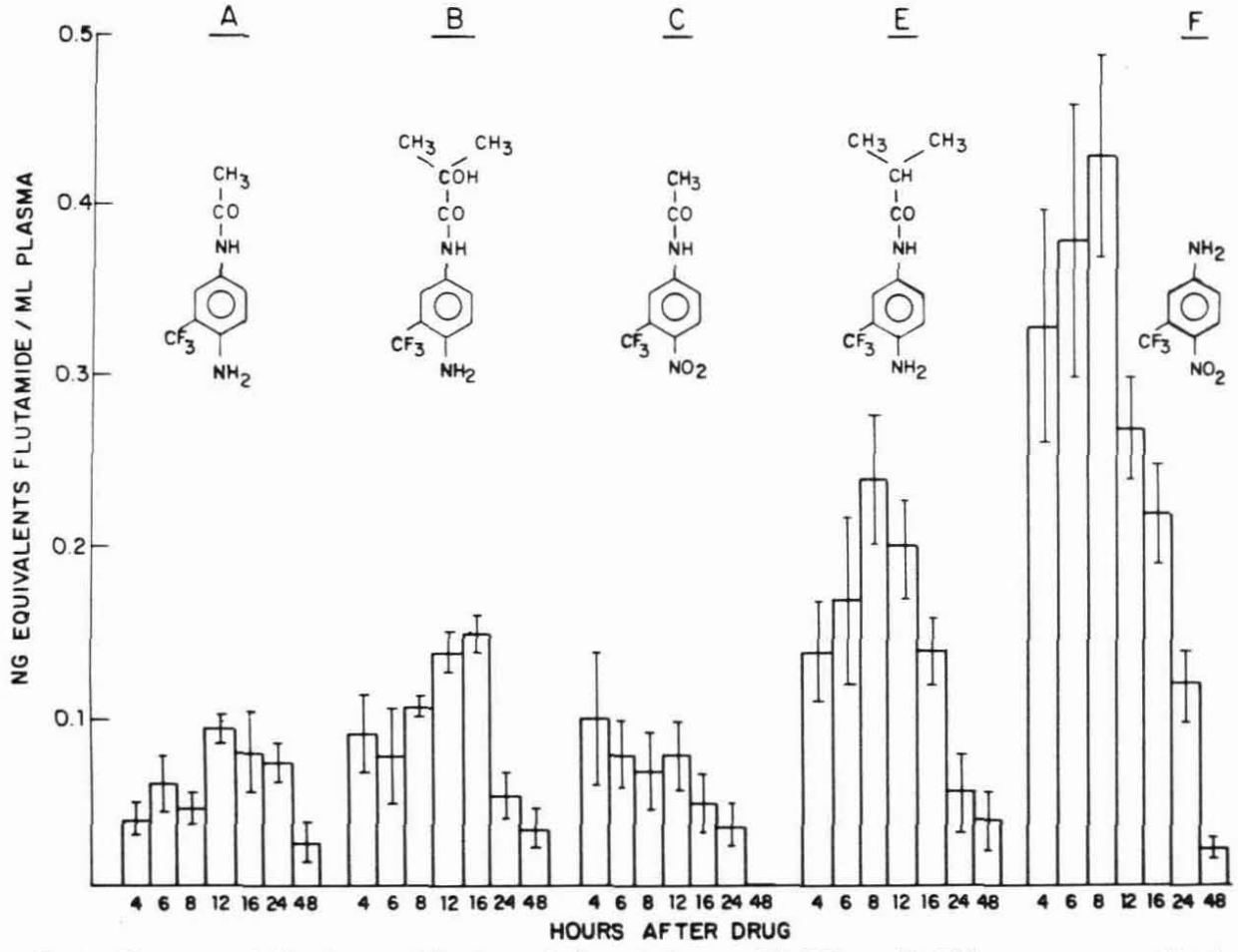

FIG. 3. Plasma metabolites in men following a single topical 5-mg of [^{14}C]flutamide. Values are average of 5 subjects ± SE.

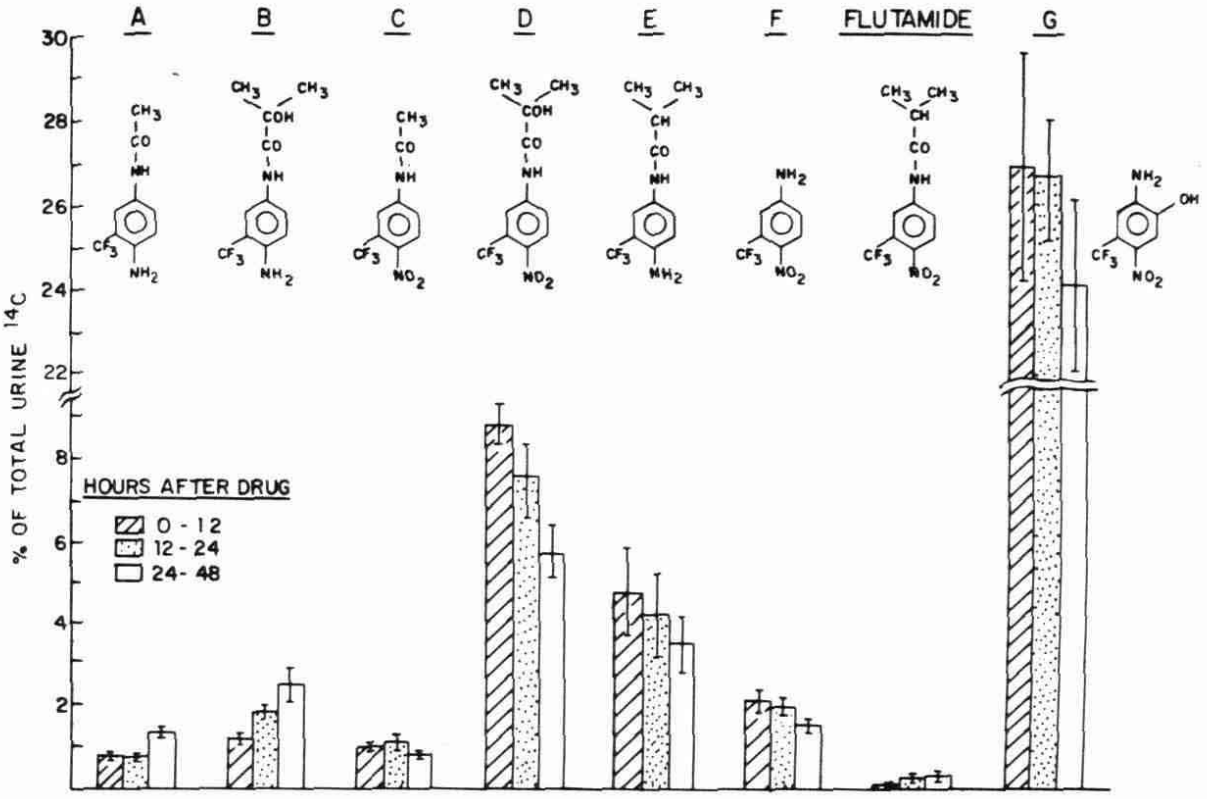

FIG. 5. Urine metabolites in men following a single topical 5-mg dose of [^{14}C]flutamide. Ethyl acetate extract of Glusulase-treated urine was cochromatographed with reference standards in system A. Values are average of 5 subjects ± SE.

ethyl acetate, 5:1; D, CH_2Cl_2; E, $CHCl_3$:diethyl ether, 8:2.

Identification and Quantitation of Metabolites

All metabolites were previously isolated and identified in pilot animal experiments by comparison with authentic standards via mass spectrometry. (G) was also identified by its NMR spectrum*. Detailed proofs of structure will be presented (J. McGlotten, J. Morton, and E. Gold, unpublished data).

Flutamide and its metabolites were quantitated as follows: One ml of plasma or urine from each subject was buffered at pH 5.0 and incubated 16 hr at 37°C with 0.1 ml of a mixture of β-glucuronidase and sulfatase (Glusulase, Endo Laboratories, Garden City, N. Y.). The metabolites were extracted with 10.0 ml of ethyl acetate, and an 8.0-ml aliquot of the ethyl acetate extract was evaporated under a stream of dry nitrogen to about 0.1 ml, which was then spotted on a silica gel thin-layer plate. The tube containing the residue was rinsed with 2 0.05-ml portions of methanol which were spotted on top of the first spot. Appropriate carriers were cochromatographed with the unknown in solvent system A. After drying, the spots were scraped from the plate into counting vials for assay of their ^{14}C content. Samples containing 10 cpm or less above background (equivalent to plasma levels of about 0.1 ng/ml or less) were counted for 120 min. Sufficient counts were collected to distinguish sample from background at $p < 0.05$.

* Copies of the NMR and mass spectra are available on request from the authors.

RESULTS

Excretion Pattern

Figure 1 shows that 16.1% (SE = 1.9%) of the applied flutamide is excreted in urine. About 97% of the excretion occurs in the first 24 hr after drug application and then rapidly declines.

Recovery of ^{14}C from Site of Application

Cotton swabs of the site of application 6 hr after drug application contain 56% (SE = 4.7%) of the applied dose. Recovery of ^{14}C from urine and the site of application accounts for 72% (SE = 5.3%) of the dose.

Plasma Metabolites

Total plasma ^{14}C peaks 8 hr after drug at 10.2 ng equivalents flutamide/ml and then declines multiexponentially (Fig. 2). Flutamide levels peak about 6 hr after drug at 1.31 ng/ml and also disappear multiexponentially. (D), the α-OH derivative of flutamide, is the major plasma metabolite after 6 hr and disappears with about a 6-hr half life (Fig. 2).

At least 5 other compounds are in the extractable fraction (Fig. 3), but they account for no more than 30% of total plasma ^{14}C up to 8 hr after drug. (D) and flutamide account for about 70% of the identified compounds.

A possible metabolic scheme for flutamide is shown in Figure 4.

Urine Metabolites

Following hydrolysis with Glusulase, 78 to 81% of the radioactivity is extractable with ethyl acetate. Thirty-five to forty-four percent is extractable before Glusulase hydrolysis. TLC of the fraction extractable after Glusulase hydrolysis shows about half of the ^{14}C is present as polar metabolites ($R_f < 0.15$). The compounds with $R_f > 0.15$ are present in small amounts, except for (G), which accounts for about 30% of the extractable radioactivity (Fig. 5).

DISCUSSSION

Flutamide is rapidly and almost completely converted to other compounds by men 1 hr after oral administration [4]. The plasma concentration of (D) is about 10 times greater than the concentration of flutamide. This suggests it is largely metabolized on its first pass through the liver. In contrast, 4 to 8 hr after topical administration, flutamide and (D) plasma concentrations are roughly equal.

The same metabolites are present in urine and plasma following oral and topical doses, which suggests that topical administration affects only the rate at which flutamide is metabolized. The metabolic scheme (Fig. 4) we have proposed for flutamide suggests that the isobutyryl side chain is removed by stepwise oxidation rather than hydrolysis. Once the side chain is removed, the resulting nitro aniline or phenylenediamine is hydroxylated to (G) and other unidentified compounds.

Recovery of radioactivity from urine and the site of application account for 72% of the dose. However, studies in men following oral administration of 200 mg of [^3H]flutamide show the drug is completely absorbed and that 9% of the dose is excreted in feces [4]. Assuming the excretion pattern is similar after topical administration, we would expect to find about 1.6% of the dose in feces. This means 17.7% (16.1% in urine + 1.6% in feces) of the applied flutamide penetrated in 6 hr. Presumably, the remainder is at the site of application.

Meaningful comparison of our findings with those of other percutaneous absorption studies in the literature is difficult because of wide differences in the test systems that were employed. Only one study [6] used a test system similar to ours. Of 21 organic chemicals, covering a wide range of structures and solubilities, just one, diethyltoluamide, resembled flutamide in rate and extent of percutaneous penetration.

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