
Synthesis and Evaluation of Fluorine-18 21-Fluoroprednisone as a Potential Ligand for Neuro-PET Studies

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No-carrier-added fluorine-18-labeled fluoroprednisone ($[^{18}\text{F}]21$ -fluoroprednisone) was synthesized by tosylate displacement in 2%–8% radiochemical yield in 80 min end of cyclotron bombardment (EOB), and its metabolism and distribution were investigated. After intravenous administration to rats, $[^{18}\text{F}]21$ -fluoroprednisone was rapidly cleared from the blood and biotransformed into $[^{18}\text{F}]20$ -dihydro-21-fluoroprednisone. The suitability of $[^{18}\text{F}]21$ -fluorocorticoids for receptor imaging in humans with positron emitting tomography will depend on the synthesis of compounds with high binding affinity and low rate of carbonyl reduction at C-20.

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Corticosteroids exert a variety of physiologic, biochemical, and behavioral effects, modulate homeostatic mechanisms and, in high doses, exhibit major antiinflammatory, antineoplastic, and antiedema potency. Steroids and their metabolites affect nerve cell activity in the brain (1), and fluctuations in regional brain concentrations have been implicated in the pathogenesis of diverse clinical entities such as anorexia nervosa and endogenous depression (2, 3).

Corticosteroids bind specifically to cortical and subcortical receptors, which are similar (if not identical) to the receptors found in peripheral glucocorticoid target tissues (4). Glucocorticoid receptors recently have been demonstrated in human brain tumors (meningioma, neurinoma, glioma), and their role in promoting tumor growth has been debated (5). Uptake and binding studies in the rat have demonstrated the existence of two distinct glucocorticoid receptor subtypes, one preferentially binding corticosterone and the other preferentially binding synthetic steroids such as dexamethasone; recent work strongly suggests the presence of a third receptor subtype, similar to kidney type 1 (mineralocorticoid) receptors (6). Corticosteroids probably exert their effects on neural tissues by binding to intracellular receptors followed by transcriptional modulation of protein synthesis. However, nongenomic effects may

explain many of the biochemical sequelae of corticosteroid administration, including the potentiation of ischemic neuronal injury (7).

Anticipating future positron emitting tomography (PET) studies in human subjects using fluorine-18 fluorine- (^{18}F) labeled corticosteroid ligands, we synthesized high-specific-activity $[^{18}\text{F}]21$ -fluoroprednisone and fluoroprednisolone and investigated their metabolism and biodistribution in vivo.

MATERIALS AND METHODS

Instrumental Methods

Nuclear magnetic resonance (NMR) spectra were determined in deuteriochloroform with tetramethylsilane (TMS) as internal standard; chemical shifts for assigned protons are quoted in parts per million downfield from TMS. Mass spectra were determined on a VG 70-250 magnetic sector instrument operating in alternating positive ion/negative ion desorption chemical ionization mode; ionizing reagents (8) were methane (for positive CI) and hydroxide (for negative CI); source temperature: 200°C; heating rate: 70 mA/sec up to 1.25 A; scan speed: 0.5 sec/decade in mass range 800–60 amu; scan cycle time: 1 sec. Scintillation counting was performed with a sodium iodide automated gamma counter cross-calibrated for ^{18}F against a dose calibrator. High performance liquid chromatography (HPLC) pumps and detectors were standard research-grade instruments. All temperatures are expressed in degrees Celsius.

Synthesis of Prednisone 21-Tosylate (1)

A solution of prednisone (803 mg, 2.24 mmol) in dry pyridine (15 ml) was cooled to -78° and treated rapidly

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dropwise with a solution of tosyl chloride (650 mg, 3.41 mmol) in dry chloroform (7 ml). The reaction was allowed to continue for 2 hr at -78° and then for 20 hr at -20° . Next, the reaction solution was concentrated in vacuo, reconstituted with chloroform (25 ml), and washed with hydrochloric acid (2 N, 2×10 ml), water (2×10 ml), and saturated aqueous sodium bicarbonate (2×10 ml). After being dried with sodium sulfate, the chloroform phase was evaporated to a tacky foam and purified by flash chromatography (1.5% methanol in chloroform) to afford the desired tosylate (9) as an amorphous solid (866 mg, 79%).

NMR (300 MHz): 0.585 (s, C(18)H₃); 1.422 (s, C(19)H₃); 2.458 (s, Ar-CH₃); 4.695 (d, J = 17.7, C(21)-H); 5.156 (d, J = 17.7, C(21)-H); 6.055 (bs, C(3)-H); 6.193 (dd, J = 1.8 and 10.2, C(2)-H); 7.671 (d, J = 10.2, C(1)-H); 7.802 (d, J = 8.2, Ar-H); 7.359 (d, J = 8.2, Ar-H).

Synthesis of Prednisolone 21-Tosylate

Using a similar procedure, prednisolone was converted to the corresponding tosylate in 88% yield.

NMR (90 MHz): 0.92 (s, C(18)H₃); 1.45 (s, C(19)H₃); 2.45 (s, Ar-CH₃); 4.46 (bm, C(11)-H); 4.84 (d, J = 17.6, C(21)-H); 5.12 (d, J = 17.6, C(21)-H); 5.99 (bs, C(4)-H); 6.24 (dd, J = 10.1 and 1.2, C(2)-H); 7.26 (d, J = 10, C(1)-H); 7.35 (d, J = 8.1, Ar-H); 7.83 (d, J = 8.1, Ar-H).

Synthesis of 21-Fluoroprednisone (2)

Prednisone 21-tosylate (1) (100 mg, 0.21 mmol) in acetonitrile (7 ml, reagent grade, dried over 3A molecular sieves) was caused to react with anhydrous cesium fluoride (150 mg, 0.99 mmol) at gentle reflux for 50 min. Next, the reaction mixture was evaporated to dryness in vacuo and the residue was partitioned between dichloromethane and water. The organic phase was decanted, dried over sodium sulfate, and evaporated to a yellow oil. Analysis of this oil by HPLC (3:1 acetonitrile/water, 5-micron Spherisorb ODS column, ultraviolet detection at 254 nm) revealed 21-fluoroprednisone (2) and the rearrangement product (3) in a 24:55 ratio. These compounds were isolated by a combination of flash chromatography (3% methanol in chloroform) and preparative HPLC in 20% and 40% yields, respectively.

21-fluoroprednisone NMR (500 MHz): 0.728 (s, C(18)H₃); 1.444 (s, C(19)H₃); 5.022 (dd, J_{HH} = 17.1, J_{HF} = 52.6, C(21)-H); 5.315 (dd, J_{HH} = 17.1, J_{HF} = 47.7, C(21)-H); 6.088 (bs, C(4)-H); 6.215 (dd, J = 10.2 and 0.9, C(2)-H); 7.661 (d, J = 10.2, C(1)-H). Negative-ion MS: 361 (13) M+1, 360 (54) M, 341 (18), 340 (73), 339 (15), 338 (10), 322 (15), 299 (21), 298 (100). Positive-ion MS: 390 (18) M+C₂H₅, 362 (23) MH+1, 361 (100) MH, 360 (14), 325 (10). Rearrangement product NMR (300 MHz): 0.875 (d, J = 0.44, C(18)H₃); 1.581 (s, C(19)H₃); 2.294 (d, J = 12.2 (C(12)-H α ?); 2.959 (d, J = 12.2, C(12)-H β ?); 4.904 (d, J = 15.0, C(21)-H); 5.093 (d, J = 15.0, C(21)-H); 6.088 (t, J = 1.5, C(4)-H); 6.216 (dd, J = 10.3 and 1.9, C(2)-H); 7.650 (d, J = 10.2, C(1)-H). Negative-ion MS: 341 (23) M+1, 340 (100) M. Positive-ion MS: 370 (18) M+C₂H₅, 342 (23) MH+1, 341 (100) MH, 323 (8), 313 (6), 298 (9), 295 (6), 265 (10).

Synthesis of 21-Fluoroprednisolone

Similarly, a sample of 21-fluoroprednisolone was synthesized (7.5% yield) for mass spectral comparison.

Negative-ion MS: 361 (11) M-H, 343 (18), 342 (58), 341

(100), 327 (10), 326 (12), 319 (40), 301 (18), 300 (94), 299 (74), 285 (32), 235 (26), 233 (17), 221 (14). Positive-ion MS: 363 (11) MH, 345, (81), 343 (16), 325 (100), 307 (37), 297 (14), 295 (24), 283 (20).

Synthesis of [¹⁸F]21-Fluoroprednisone

Fluorine-18 was cyclotron-produced using the ¹⁸O(p,n)¹⁸F nuclear reaction (10), and the activity so generated was delivered into a borosilicate glass crucible containing tetramethylammonium hydroxide (2 μ mol). The solution was evaporated to dryness at 100 $^{\circ}$ under a stream of nitrogen, and further dried azeotropically with acetonitrile. Prednisone 21-tosylate (5 mg) in acetonitrile (0.6 ml) was added to the crucible, and heating was continued for 10 min. The reaction mixture was partitioned between dichloromethane (3 ml) and water (3 ml). The organic phase was decanted, dried over sodium sulfate, and chromatographed on silica gel (11 \times 200 mm, 3.5% methanol in dichloromethane). The radioactive fraction was collected and further purified by preparative HPLC (10 \times 250 mm Spherisorb 5-micron ODS column, 3 ml/min, 65:35 acetonitrile/water) to afford radiochemically pure [¹⁸F]21-fluoroprednisone (2%–8% radiochemical yield in 70–80 min EOB). Total mass associated with the preparation (\sim 20 μ g) was quantified for several runs using reverse-phase HPLC (uv 254 nm detection) against a standardized fluoroprednisone solution. However, normal-phase HPLC (2% methanol in dichloromethane, 1 ml/min, 4.6 \times 250 mm 5-micron Spherisorb silica column, uv (254 nm) detector) revealed that this material is not all cold fluoroprednisone, and the specific activity EOB was calculated at 2.5 Ci/ μ mol.

Synthesis of [¹⁸F]21-Fluoroprednisolone

Using the same conditions described above, prednisolone 21-tosylate was converted into the corresponding [¹⁸F]21-fluoro derivative (6% crude radiochemical yield) for use as a radio-HPLC standard.

In Vitro Incubation Experiments

Venous blood (2 ml) was withdrawn from a male Wistar rat via a femoral catheter and was mixed with [¹⁸F]fluoroprednisone (100–200 μ Ci dissolved in 20–50 μ l 40% DMSO-saline). The mixture was incubated in a 37 $^{\circ}$ water bath and aliquots were withdrawn at 15–20 min intervals. Each aliquot (100 μ l) of whole blood was deproteinized by adding ice-cold, saturated aqueous picric acid (600 μ l) and centrifuging the resulting precipitate. The supernatant, which contained $50 \pm 15\%$ of the original activity, was quantitatively analyzed by reverse-phase radio-HPLC. Fractions collected from the HPLC and compared with the amount injected showed 68%–106% radioactivity balance for the analysis.

Partition Coefficients

Test tubes containing 1-octanol (3.0 ml) and USP Ringer's solution buffered with 5 mM HEPES (3.0 ml) were spiked with 10–20 μ Ci [¹⁸F]fluoroprednisone in 10–20 μ l octanol. The tubes were capped, placed in a 37 $^{\circ}$ bath and manually shaken every 5 min. After 30, 60, and 120 min, three tubes each were centrifuged and aliquots of each phase were transferred to tared scintillation vials for counting. Activities were decay-corrected and converted to concentrations by applying density corrections. The calculation of the partition coefficient (11) was obtained from the ratio of μ Ci/ml in the octanol phase relative to the Ringer's phase.

For comparison, the partition coefficients of prednisone and hydrocortisone were determined as follows. First, a saturated solution of each compound in USP Ringer's solution buffered with 5 mM HEPES was prepared, and quadruplicate aliquots (3.0 ml) of this solution were allowed to equilibrate with 1-octanol (3.0 ml) as described above for 2 hr. Aliquots of each phase were analyzed by analytical reverse-phase HPLC (uv 254 nm detection), and partition coefficients were calculated from the integrated areas.

Animal Studies

Unilateral femoral arterial and venous catheters were inserted under Ethrane/nitrous oxide anesthesia in male Wistar rats (210–260 g). Postoperatively, the animals were loosely restrained with masking tape on a lead brick and were allowed to recover from general anesthesia for at least 1.5 hr, during which time their body temperature was maintained in the range of 35°–37° by means of a heat lamp connected through a thermostat to a rectal temperature probe. Heart rate and arterial blood pressure were monitored continuously.

Fluorine-18 fluoroprednisone (0.15–9.0 mCi/animal), dissolved in 0.55 ml 40% DMSO–saline, was infused intravenously over 45 sec. Arterial blood samples were collected at intervals in tared scintillation tubes and counted in a gamma counter. Animals were killed 60 min postinjection. Tissues were removed, blotted free of blood, weighed, and counted in a gamma counter. All activities were decay-corrected to EOB and normalized for animal weight and injected dose. Tissue radioactivity concentrations are expressed as percent injected dose per gram. Blood radioactivity concentrations are expressed as relative concentration ($\mu\text{Ci/g}$ sample divided by μCi injected per gram rat).

To examine the brain and liver for radioactive species, ~2-g samples of each organ were mixed with ice-cold saturated aqueous picric acid and homogenized with a Brinkmann Polytron. The resulting homogenate was centrifuged (10,000 g, 10 min, 4°), and the supernatant, which contained about one-half the original activity, was extracted with dichloromethane. The extract was dried over sodium sulfate and analyzed quantitatively by radio-HPLC. Fractions were collected from the HPLC and compared with the amount injected to determine the activity balance.

Isolation of the Major Metabolite by In Vitro Incubation

Fluorine-18 fluoroprednisone (17 mCi, at EOB+70 min) was dissolved in 1:1 DMSO–saline (0.15 ml) containing carrier fluoroprednisone (1 mg). To this was added freshly drawn rat blood (5 ml, obtained by cardiac puncture), and the mixture was allowed to react for 2 hr at 37° in a heparinized flask. Next, the reaction mixture was added to ice-cold saturated aqueous picric acid (30 ml) and centrifuged (5,000 g, 5 min). The pellet was resuspended in cold picric acid solution (5 ml) and recentrifuged, and the combined supernatants were extracted with dichloromethane (3 × 15 ml). The combined extracts (1.5 mCi at EOB+247 min, 45% radiochemical yield) were dried over sodium sulfate, concentrated, and subjected to flash chromatography (11 × 200 mm, 3.5% methanol in dichloromethane). A single radioactive band was collected from the column (0.57 mCi at EOB+320 min, 25% radiochemical yield), which consisted of an unseparated mixture of [¹⁸F]metabolite and [¹⁸F]fluoroprednisone. This fraction was evaporated and purified by preparative HPLC (10 × 250

mm 5-micron Spherisorb ODS column, 65:35 acetonitrile/water, 3 ml/min) affording [¹⁸F]metabolite (0.36 mCi at EOB+335 min, 17% radiochemical yield) and recovered [¹⁸F]fluoroprednisone (0.05 mCi at EOB+335 min, 5.7% radiochemical yield). Radioactivity in the major metabolite-containing fraction was allowed to decay and then the sample was evaporated to dryness. The residue (1 μg) was identified by mass spectroscopy as 20-dihydro-21-fluoroprednisone.

Negative-ion MS: 362 (27 M), 342 (100), 328 (11), 324 (48), 312 (23), 298 (76), 143 (11).

RESULTS AND DISCUSSION

Producing an ¹⁸F-Labeled Glucocorticoid

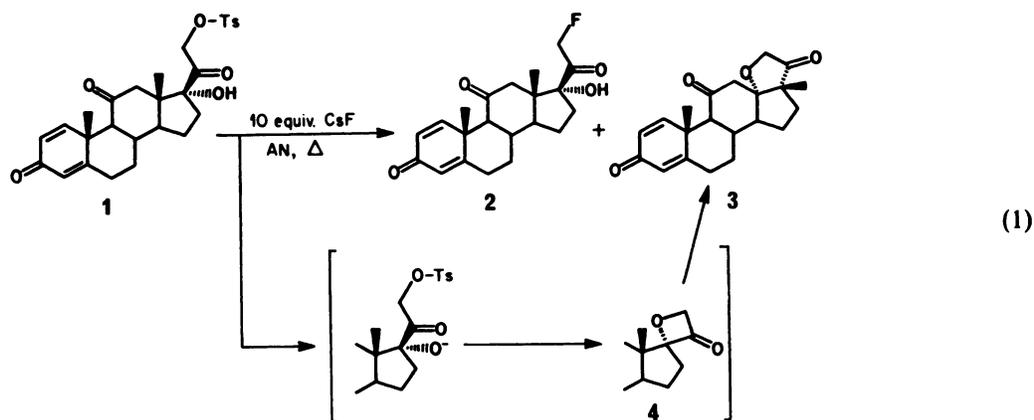
Since the serendipitous discovery by Fried and Sabo in 1954 (12) that 9 α -fluorocortisone acetate has several times greater biologic activity than cortisol, synthesis of structurally modified corticosteroids has been a fruitful area for research. A host of fundamental structural features have since been identified, which enhance pharmacologic potency (13). Certain fluorinated steroids, notably those with a fluorine at the 9 α and/or 6 α positions, such as dexamethasone, flucinolone, and triamcinolone, have been found to exhibit exceptional potency.

Initially, it was desired to select one of the frequently prescribed, fluorine-containing glucocorticoids as the target for radiosynthesis, but, in the course of reviewing the body of synthetic literature, it became apparent that the routes and reagents traditionally employed for introducing fluorine substituents into the steroid nucleus (14, 15) were incompatible with the constraints inherent in work with high specific-activity ¹⁸F. The principal reaction used to introduce fluorine into positions C-9, C-6, and elsewhere, is the opening of an epoxide in the presence of an excess of hydrogen fluoride and, if used, would produce a product of low specific activity.* An interesting alternative to fluorinate C-9 was proposed by Barton (17,19), in which 11-keto steroids are converted to $\Delta^{9,11}$ -enol benzoates for subsequent reaction with trifluoromethyl hypofluorite. Presumably this scheme could be adapted for use with [¹⁸F]acetyl hypofluorite, if only to produce low specific-activity products. The possibility of introducing ¹⁸F at C-6 by performing an S_N2 reaction on 6-bromo steroids (20,21) was considered unfeasible, due to the propensity for such conformationally rigid allylic bromides to undergo E₂ elimination. All these approaches additionally require steps to protect and subsequently deprotect the highly sensitive dihydroxyacetone sidechain.

Because of obstacles anticipated in introducing ¹⁸F in high specific activity at either C-9 or C-6, attention was focused on functionalizing the side chain at C-21. In fact, several 21-fluoroglucocorticoids are known, and their biologic activity is intermediate between the corresponding 21-deoxy- and 21-hydroxy

derivatives when screened by the rat liver glycogen assay (22). In this early work, 21-fluoro derivatives were conveniently prepared by treating the corresponding 21-iodo steroids with silver fluoride (23). Because C-21 is a primary center and is activated for nucleophilic substitution by the neighboring C-20 keto group, the possibility of displacing a suitable C-21 leaving group with no-carrier-added (NCA) [^{18}F]fluoride seemed attractive. Moreover, introduction of the label at this site could be performed in one step under mild conditions and presumably would obviate the need to protect the dihydroxyacetone group.

Initial experiments to substitute fluoride at C-21 were successfully carried out with prednisone. Although prednisone is not as potent as some other synthetic glucocorticoids (e.g., dexamethasone), it is considerably more potent than cortisol and has solubility properties more convenient to the envisioned chemical manipulations.[†] Tosylation of prednisone was routine (9). When prednisone 21-tosylate (**1**) was reacted with excess cesium fluoride or tetramethylammonium fluoride in acetonitrile at 45°–80°, only two products, produced in 1 to 2–4 ratio, were detectable by reverse-phase HPLC: the minor product (**2**) proved to be the desired 21-fluoroprednisone and the major one (**3**) was derived by intramolecular rearrangement as shown in Eq. (1). Similar rearrangements have been reported for related systems (22). It is uncertain whether **2** is derived directly from **1**, or whether **1** first suffers intramolecular displacement producing **4** and then **4** yields **2**.



Reaction of **1** with NCA [^{18}F]tetramethylammonium fluoride (TMAF) at gentle reflux produces a single radioactive species that coelutes with fluoroprednisone when analyzed by reverse-phase HPLC with uv and radioactivity detection. The starting tosylate is consumed and is largely transformed under these conditions into rearrangement product **3**. After purification by silica gel chromatography and reverse-phase preparative HPLC, [^{18}F]**2** can be cleanly separated from **3** and isolated in 2%–8% yield in 70–80 min after end of

cyclotron bombardment (EOB). In this way, 5–10 mCi (end of synthesis) can be prepared routinely, with as much as 37 mCi having been prepared in a single run. It was assumed at first that a more active leaving group would lead to improved radiochemical yields of [^{18}F]**2**. However, reaction of prednisone 21-mesylate with NCA [^{18}F]TMAF afforded only 0.2% incorporation of ^{18}F and almost exclusively rearrangement product **3**. The total mass of material associated with the product is $\sim 20 \mu\text{g}$ when quantified against a standardized fluoroprednisone solution. However, this material is not all cold fluoroprednisone, and the specific activity (EOB) was calculated at 2.5 Ci/ μmol .

Having developed a workable synthesis of [^{18}F]fluoroprednisone, experiments were undertaken to establish the pharmacologic effect, if any, of replacing the 21-hydroxyl group of prednisone with fluorine.

Metabolism and Distribution

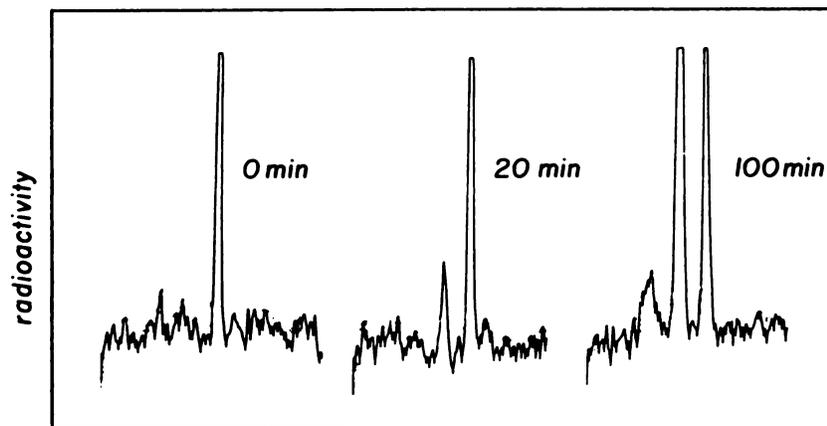
Quantitative PET measurements of tracer uptake and tissue binding presuppose a detailed knowledge of the metabolism *in vivo* of the injected radiopharmaceutical during the period of study. Similarly, accurate radiation absorbed dose estimates for studies *in vivo* involving human subjects are predicated on tissue biodistribution data obtained from prior animal experiments. Accordingly, a series of experiments was conducted *in vitro* and *in vivo* to determine (a) the metabolic stability of fluorine at C-21, (b) the time course of organ distribu-

tion and metabolism *in vivo*, and (c) the identity of the major metabolic products.

Initial experiments were performed *in vitro* to predict if significant metabolism would occur in the circulatory system, so this effect could later be distinguished from organ metabolism *in vivo*. After being incubated with cell-free rat plasma at 37° for 1 hr, [^{18}F]fluoroprednisone was recovered quantitatively. Next, [^{18}F]fluoroprednisone was incubated at 37° with heparinized rat whole blood. Under these conditions, [^{18}F]fluoroprednisone

FIGURE 1

Representative radio-HPLC elution profiles showing the radioactive species generated by incubating [^{18}F] fluoroprednisone with rat whole blood. For HPLC conditions, see text.



underwent transformation with a half-life of 53 ± 5 min to a new species (Fig. 1). From the radioactivity balance for the HPLC analysis, it was estimated that not more than 20% of the label was cleaved to [^{18}F] fluoride or [^{18}F]fluoroacetate after 1.5 hr incubation.

To determine the ease with which fluoroprednisone would cross the intact blood-brain barrier, the octanol-Ringer's partition coefficient was measured. As shown in Figure 2, introduction of a 21-fluoro group dramatically enhances the lipophilicity of prednisone. In fact, fluoroprednisone (PC = 131, log PC = 2.1) is more lipophilic than the blood flow tracer *n*-butanol (log PC = 0.88) (24), suggesting that the brain uptake of fluoroprednisone should be flow- rather than diffusion-limited (11).

Intravenous administration of [^{18}F]fluoroprednisone was initially problematic because the steroid appeared to be insoluble in aqueous solution. Glucocorticoids for parenteral administration invariably rely on 21-phosphate or 21-hemisuccinate esters to effect solubilization, a strategy not applicable to the present case. Although 50% aqueous ethanol did dissolve the radiotracer, the central nervous system depressant effect of ethanol at doses sufficient to solubilize the tracer for injection precluded its use for *in vivo* animal experiments. Finally, dimethyl sulfoxide/saline (40:60) was tested and proved an acceptable vehicle; when this mixture (0.5 ml) is administered intravenously to rats, blood pressure falls briefly but returns to normal levels within ~ 5 min. Recent studies in rats have shown that DMSO does not

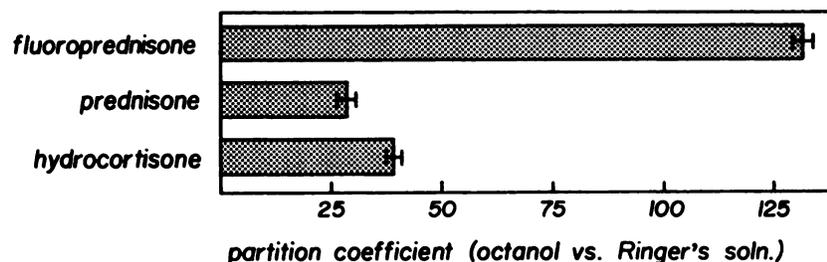
increase blood-brain barrier permeability to a variety of water-soluble tracers (25,26).

Tissue distribution and metabolic studies in rats revealed qualitatively similar plasma pharmacokinetics for fluoroprednisone and prednisone. However, strict interpretation of these data is rendered difficult by the paucity of published studies on prednisone metabolism. Nearly all reported studies focus on cortisone/cortisol (13,27), and invariably assume that synthetic congeners have qualitatively similar behavior. The biologic clearance of radioactivity (Fig. 3) was adequately described with a biexponential function ($T_{1/2} = 0.26 \pm 0.05$ and 60 ± 15 min), in which the exceedingly rapid initial phase resembles the behavior reported for prednisolone (28). The tissue distribution at 60 min postinjection (Fig. 4) is noteworthy for two reasons. Relatively high uptake in the small intestines may reflect hepatobiliary excretion of fluoroprednisone and/or its metabolites, a known route of biotransformation for prednisolone in rats (28). The low brain uptake may reflect the rapid metabolism of [^{18}F]fluoroprednisone, low binding affinity of membrane-bound and/or cytosolic receptors for the metabolites, and high receptor occupancy by endogenous corticosteroids.

Distribution studies alone do not predict the utility of [^{18}F]fluoroprednisone for positron emitting tomography (PET) measurement of glucocorticoid receptor binding *in vivo*, because they do not indicate the identity of the labeled species. Thus, the profile of radioactive metabolites in brain and liver was examined by

FIGURE 2

Comparison of octanol/Ringer's partition coefficients for fluoroprednisone, prednisone and cortisol. Note the dramatic enhancement of lipophilicity caused by introducing a 21-fluoro group in prednisone.



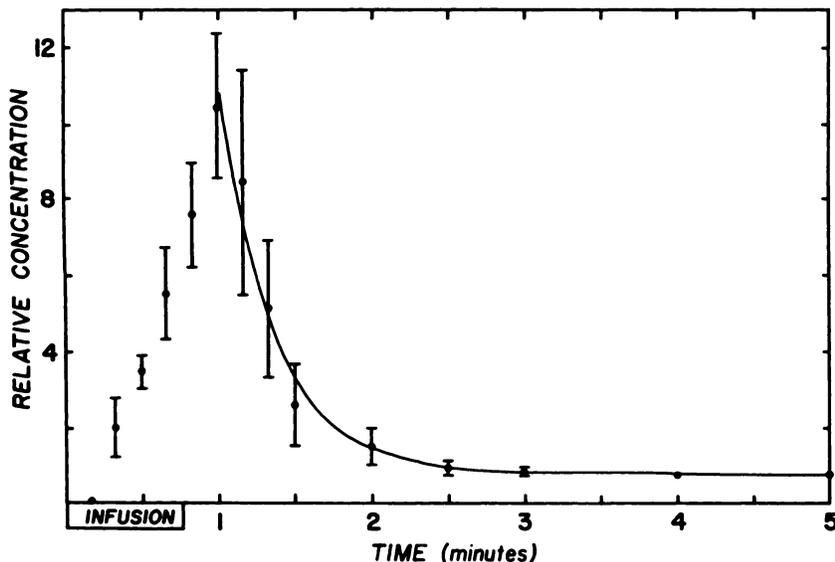


FIGURE 3
Time course of blood radioactivity concentration following i.v. infusion of [¹⁸F]fluoroprednisone in male Wistar rats. Data points show mean (\pm s.d.) for nine animals. A least squares fit of the data to a double exponential function with half-lives 0.26 ± 0.05 and $60. \pm 15$ min is illustrated.

radio-HPLC (Fig. 5). Biotransformation is remarkably selective for a single new species, which corresponds in retention time to that observed during the blood incubation experiments in vitro. Originally, it was thought that the new species might be [¹⁸F]fluoroprednisolone, because reversible oxidation-reduction of corticoids at C-11 is facile and generally favors the reduced form (29,30). However, comparison of the radio-HPLC retention time of the new species with independently synthesized [¹⁸F]fluoroprednisolone demonstrated conclusively that this is not the case.

The metabolite was prepared in vitro with rat whole blood, isolated, and subjected to mass spectroscopy

(Fig. 6), by which it was identified as *20-dihydro-21-fluoroprednisone*. Reduction at C-20 is a recognized and well-characterized metabolic pathway for cortisol (31). In vivo, oxidation-reduction at C-11 is the predominant process, with C-20 reduction, ring-A reductions, and side-chain cleavage occurring less rapidly. In a single human study of prednisone and prednisolone metabolism, conducted with carbon-14- (¹⁴C) labeled substrates, compounds derived from all these processes were detected in 24-hr urine samples (32). It is reasonable to speculate that with 21-fluoroprednisone an inductive effect from the fluorine enhances the susceptibility of the adjacent keto group to reduction, making

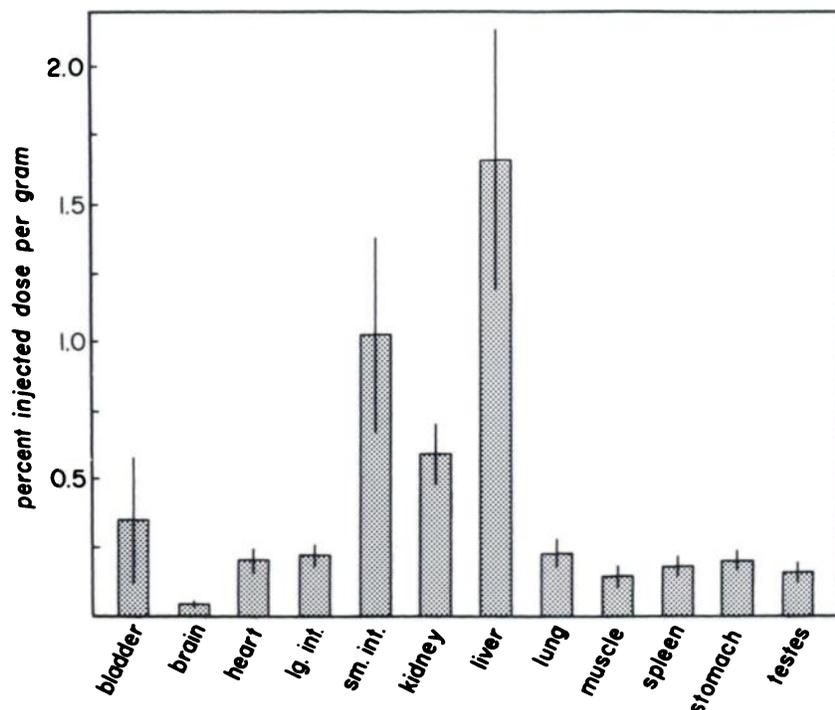
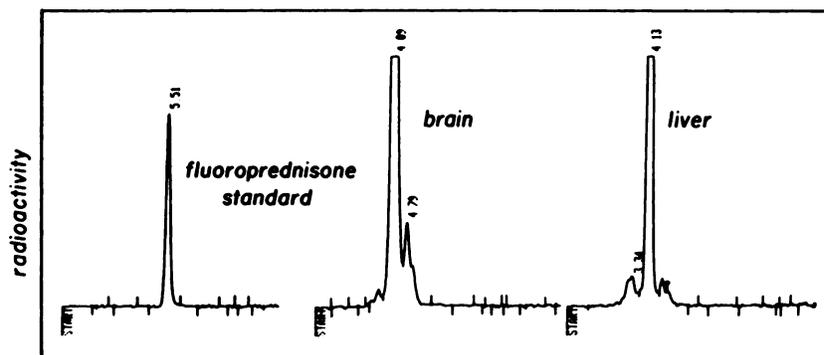


FIGURE 4
Biodistribution of [¹⁸F]fluoroprednisone in rats 60 min after i.v. infusion. Values represent mean (\pm s.d.) for six animals.

FIGURE 5
Radio-HPLC elution profiles showing the fluorine-18-containing metabolites in rat brain and liver tissue 60 min after i.v. administration of [¹⁸F] fluoroprednisone. A single metabolite is produced in both organs with high bioselectivity (brain 95%, liver 90%). The major metabolite (4.1 min) corresponds in retention time to the metabolite produced during the in vitro blood incubation experiment. For HPLC conditions, see text.



dihydro-reduction at C-20 the dominant pathway for biotransformation.

CONCLUSIONS

The synthesis of an ¹⁸F-labeled glucocorticoid has been demonstrated by the preparation of [¹⁸F]21-fluo-

roprednisone. Experiments conducted in rats show that metabolism of this compound follows a pathway typical of other glucocorticoids—dihydro reduction at C-20. Brain radioactivity following [¹⁸F]21-fluoroprednisone infusion is low, a probable consequence of rapid hepatic biotransformation and biliary excretion. Because enterohepatic clearance quantitatively is not as important in humans as in the rodent, the suitability of [¹⁸F]21-fluorocorticoids for receptor imaging in humans with PET will depend on the synthesis of compounds with high binding affinity and a low rate of C-20 reduction.

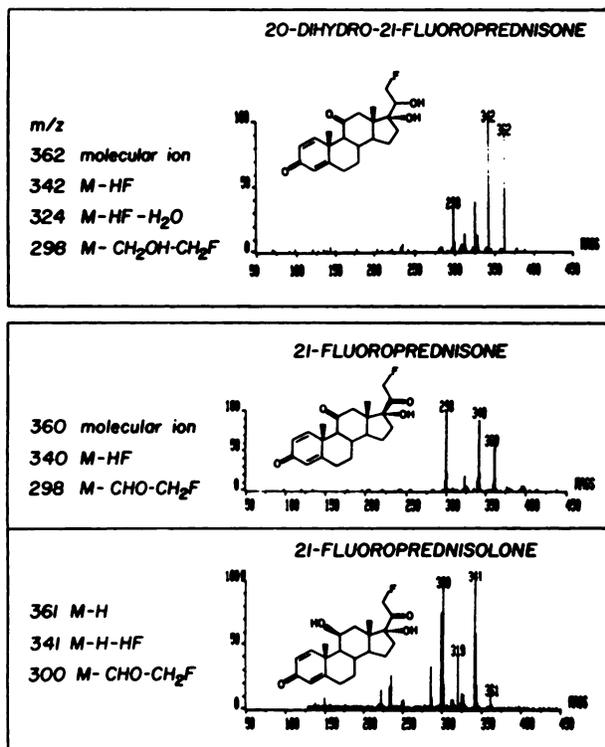


FIGURE 6
Top: Mass spectrum of the major radioactive metabolite produced by incubating carrier-added [¹⁸F]fluoroprednisone with rat whole blood. Bottom: Mass spectra of fluoroprednisone and fluoroprednisolone, for comparison. Note production of the same daughter ion (m/z 298) from fluoroprednisone (mol wt 360) and the metabolite (mol wt 362), demonstrating that the metabolite has undergone reduction on the side chain. Fluoroprednisolone (mol wt 362), on the other hand, which has the same side chain as fluoroprednisone but is reduced at C = 11, yields a daughter ion at m/z 300.

NOTES

* These epoxide openings are conducted with excess anhydrous hydrogen fluoride in the presence of a proton acceptor (typically, tetrahydrofuran or pyridine). The ratio of hydrogen fluoride to proton acceptor moderates the Lewis acidity of the reagent combination, allowing donation of a fluoride ion by hydrogen fluoride and protonation of the oxirane oxygen to occur (14-16). It is unlikely that this reactivity pattern could be reproduced with NCA anhydrous [¹⁸F]hydrogen fluoride.

† For example, dexamethasone 21-mesylate and triamcinolone acetonide 21-mesylate were difficult to work with because of limited solubility in common organic solvents and gave poor yields of [¹⁸F]fluoride-incorporated product.

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