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Technical Note

Scintigraphic Evaluation of the Ocular Disposition of ^{18}F-Imirestat in Rabbits

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INTRODUCTION

The enzyme aldose reductase has become the focus for numerous investigations into the underlying cellular pathophysiologic changes which occur as secondary abnormalities associated with diabetes mellitus (1–3). Aldose reductase is present in ocular tissues, with high levels especially found in the lens (4,5). Topical administration of aldose reductase inhibitors may provide drugs which bind directly to the target enzyme in eye tissue and blocks the sorbitol pathway for the metabolism of excess glucose. Imirestat (ALO1576, HOE 843), the hydantoin derivative of 2,7-difluorofluorenone, is an aldose reductase inhibitor currently under evaluation for the topical ocular treatment of cataracts, one of the complications of diabetes.

Previous studies have examined the ocular pharmacokinetics of imirestat following topical ocular instillation (6). However, these studies utilized ^{14}C-imirestat and necessitated the use of a large number of animals with a limited sampling schedule because of serial sacrifice. The use of an appropriate radiolabel would permit the determination of imirestat retention in the eye in a dynamic, noninvasive fashion using gamma scintigraphy. Besides decreasing the number of animals needed for a study, this technique would also provide an extensive sampling schedule for each subject. One isotope which could be utilized in this manner is fluorine-18, which decays with a half-life of 110 min by the emission of a positron. The positron subsequently undergoes annihilation to yield gamma photons with an energy of 0.511 MeV. In the present study, imirestat was radiolabeled with fluorine-18 and its ocular disposition was investigated in rabbits.

MATERIALS AND METHODS

Precursors for the synthesis of imirestat were kindly supplied by Dr. Mark DuPriest of Alcon Laboratories. Fluorine-18 in the form of NaF was produced in the University of Kentucky's Van de Graaff accelerator by irradiating a 65% enriched $H_2{}^{18}O$ target with 6-MeV protons with a beam of 3.5 μA. The target was constructed from a single piece of nickel which was machined for a 142-μl target cavity, a water cooled section, and loading/venting tubes.

Synthesis of [^{18}F]Imirestat. [^{18}F]Imirestat was synthesized by means of the Schiemann reaction. Fifty milligrams (0.180 mmol) of 1 was added to 936 μl of a 1:1 (v/v) mixture of tetrahydrofuran and water (Fig. 1). The suspension was cooled in an ice bath, after which 68 μl of HCl was added, followed by a solution of $LiBF_4$ (81 mg, 0.86 mmol) in 112 μl of water. A 10% solution of sodium nitrite in water (205 μl) was added and stirred for 10 min. The resulting precipitate was filtered, washed with cold 5% $LiBF_4$ and cold water, and dried under vaccum over P_2O_5 overnight. Yield: 63 mg of a yellow solid (92%); IR (KBr) had a strong peak at 2225 cm^{-1}.

The above diazonium salt (2) (25 mg, 0.07 mmol) was suspended in 1 ml of acetone in a siliconized flask to which was added the 10.3 mCi of fluorine-18. The exchange was allowed to proceed for 30 min. Acetone and water were removed in a rotary evaporator (aspirator first, then a pump). The residue was taken up in 4 ml of a 3:1 xylene:dioxane mixture and refluxed for 40–45 min. The mixture was filtered and washed with ethyl acetate. The ethyl acetate was removed and the residue was chromatographed (preparative silica gel TLC plate) using 1:1 hexane:ethyl acetate. The band corresponding to the authentic compound was isolated (3) and analyzed. Infrared, NMR, and mass spectra were identical to authentic compound. IR (KBr, cm^{-1}) 3300, 3060, 1780(w), 1715(s), 1470; NMR (DMSO-d_6) 7.25–7.48 (m, 4H), 7.85–7.95 (m, 2H), 8.62 (s, NH's); MS (m/z) 286, 215, 149, 57. Calc'd for $C_{15}H_8F_2N_2O_2$: 286.0553; found 286.0554. A total of 74 μCi of product (70.4 mCi/mmol) was recovered, which corresponds to an 8.3% radiochemical yield after decay correction.

In Vivo *Disposition Studies.* The [^{18}F]imirestat was dissolved in 1% arginine buffer (pH 10) and 4 × 15-μl doses (concentration, 0.5%) were administered at 3-min intervals to rabbits that had been placed in a restrainer and positioned

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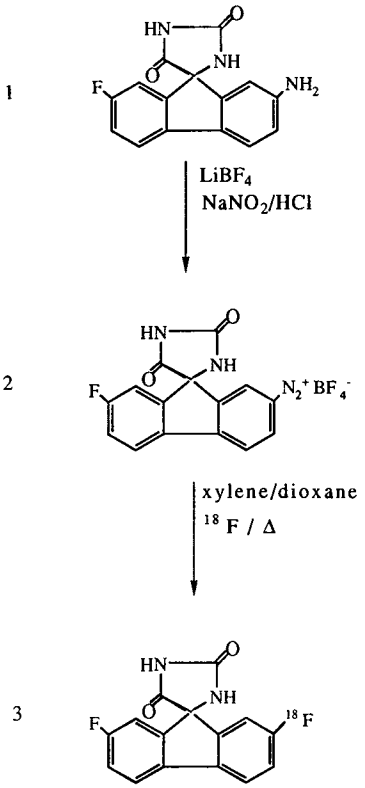

Fig. 1. Synthesis of ^{18}F-imirestat by the Schiemann reaction.

in front of a gamma scintillation camera (Siemens). This was done to reproduce the conditions of the comparative studies that had employed ^{14}C-imirestat. The camera, which had been fitted with a pinhole collimator, was interfaced with a computer that allowed for continuous monitoring of the radioactivity in a region of interest over the eye. Data was accumulated at 15-sec intervals for the first 15 min, after which the accumulation interval was increased to 60 sec. Data were corrected for radioactive decay and the counts in the eye region of interest were plotted as a function of time.

RESULTS AND DISCUSSION

Several approaches were attempted to synthesize imirestat with a fluorine-18 label. The Schiemann reaction provided the product with the greatest radiochemical yield. As a result of the labeling by exchange of F-18 fluoride with the tetrafluoroborate salt, the maximum radiochemical yield for this reaction is 25%. This limits the specific activity that can be achieved using this synthetic approach. We were able to achieve a specific activity of 70.4 mCi/mmol (246 μCi/μg), which was sufficient to allow us to administer the desired amount of drug and still have enough radioactivity to monitor with a gamma camera.

A representative profile from a single rabbit eye demonstrating the clearance of ^{18}F-imirestat is presented in Fig. 2. A biphasic disappearance of radiolabeled material is apparent during the 150 min over which it was followed. There was a 50% reduction in radioactivity after 12 min and a further decline to 20% of the initial measurement within 1 hr. Thereafter, the radioactivity remained relatively constant over the next 2 hr until the isotope has decayed. Because of

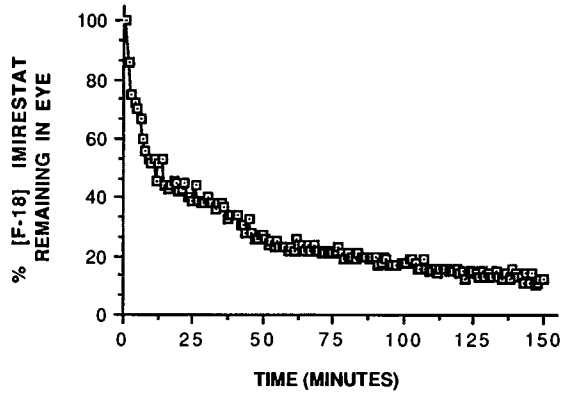

Fig. 2. *In vivo* clearance of ^{18}F-imirestat measured with a gamma camera following ocular administration to a rabbit.

the persistence of imirestat in the eye, an accurate terminal half-life could not be calculated.

The slower terminal elimination phase in the eye observed in these experiments is consistent with results obtained previously for imirestat following both topical ocular and intravenous administration. The disappearance of ^{14}C-imirestat equivalents following a 4-mg/kg intravenous dose is prolonged in several tissues, including the testes, kidneys, and eyes (7). The extensive retention of drug in these tissues, known to have high concentrations of aldose reductase, is suggestive of specific, high-affinity binding of imirestat to the aldose reductase enzyme.

There is also evidence for the slow clearance of imirestat from the eye following topical ocular administration. Following topical ocular dosing of ^{14}C-imirestat to rabbits, imirestat was subject to rapid uptake into the cornea followed by initial rapid decline, then very slow elimination with a half-life of approximately 130 hr. Drug was rapidly absorbed into aqueous humor, declining to nondetectable levels by 12 hr, but was retained in the lens similar to that in cornea with an apparent elimination half-life of 140 hr. With multiple-dose ocular instillation of 0.1% imirestat suspension to Brown–Norway rats, a long persistence of imirestat in lenses is observed during a 2-week washout period (8).

Accumulation of imirestat in the eye and the long-term retention of drug in selective tissues are important because of the potential for inhibition of aldose reductase at these sites. Imirestat has been shown to prevent cataract formation in experimental models of diabetes following systemic administration (9). The drug has also been shown to delay or prevent lens changes in nondiabetic oxidative cataract models following topical ocular administration (10) and is currently undergoing clinical investigation for the prevention of cataracts in man. Data concerning the disposition of this compound in the eye are therefore important in understanding the *in vivo* activity of the drug.

The results of this study provide evidence for the utility of gamma scintigraphy in studying the ocular disposition of drugs. As a noninvasive means to obtain multiple *in vivo* measurements, this technique could have broad applications in human studies as well as in animal models. Planar imaging of positron-emitting radionuclides with a conventional gamma camera does not provide adequate resolution to distinguish the location of drug in various parts of the eye. This

can be accomplished to a certain degree with positron emission tomographic imaging; however, this would require much larger amounts of radioactivity.

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