Nasal Absorption of Testosterone in Rats

ANWAR A. HUSSAIN, RYOHEI KIMURA, and CHONG HENG HUANG

Received July 22, 1983 from the *College of Pharmacy, University of Kentucky, Lexington, KY 40536-0053.* Accepted for publication September 19, 1983.

Abstract □ Testosterone levels in the blood were determined in rats following nasal, intravenous, and intraduodenal administration of 25-μg and 50-μg doses. The results indicated that the drug levels after nasal and intravenous administration were similar, whereas intraduodenal administration resulted in considerably lower levels. The bioavailability of the nasally administered drug was calculated to be 99% and 90% at the 25-μg and 50-μg doses, respectively. The intraduodenal bioavailability was only 1% at the dose studied.

Keyphrases □ Testosterone—nasal absorption, rats □ Nasal absorption—testosterone, rats

Testosterone is considered to be the most potent of the natural male sex hormones. This androgen is readily absorbed orally, but it is ineffective when so administered because of extensive metabolism in the GI tract and the liver before reaching systemic circulation (1). The esters of testosterone are similarly metabolized (1). Consequently, testosterone and its esters are generally administered by intramuscular injection. Other routes of administration, for example, buccal, transdermal, and subcutaneous implantation, have been attempted, but success has been limited (1).

Previous studies by Hussain and co-workers have shown that selected drugs, for example, propranolol and progesterone, are efficiently and completely absorbed following nasal absorption in animals and man (2–5). To enhance testosterone bioavailability from non-parenteral routes, the nasal route was examined. This report presents results on the nasal administration of testosterone in rats as compared to intravenous and intraduodenal administration of the drug.

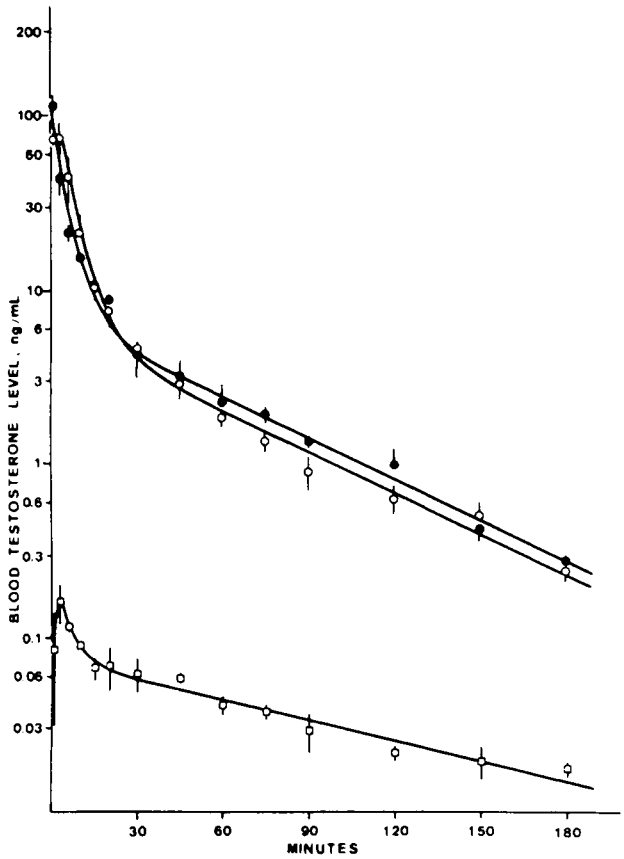

Figure 1—*Mean blood levels of testosterone in rats following nasal (○), intravenous (●), and intraduodenal (□) administration of 25-μg testosterone per rat. Points represent mean values of 3 animals ±SEM.*

Table I—Area Under Blood Level Curve (AUC) Following Intravenous, Nasal, and Intraduodenal Administration of Testosterone in Rats

Dose, μg	Route	AUC, ng·h/mL[a]	AUC (nasal, intraduodenal) / AUC (intravenous)
25	Intravenous	900.6 ± 90.9	—
	Nasal	890.5 ± 168.0	0.99
	Intraduodenal	8.9 ± 0.19	0.01
50	Intravenous	1716.3 ± 47.2	—
	Nasal	1545.7 ± 168.6	0.90

[a] Mean ± *SEM* (n = 3).

EXPERIMENTAL SECTION

Animal Studies—Male Sprague-Dawley rats, each weighing approximately 300 g, were anesthetized with pentobarbital (50 mg/kg). The surgical operation carried out on the rats was that described by Hussain *et al.* (2). For nasal administration of [^{3}H]testosterone[1], an incision was made in the neck of the animals, and the trachea was cannulated with a polyethylene tube. A closed tube was inserted through the esophagus to the posterior part of the nasal cavity. The nasopalatine passage was closed with an adhesive agent to prevent drainage of the drug from the nasal cavity to the mouth. Two doses of 25 and 50 μg of [^{3}H]testosterone, each containing 20 μCi of [^{3}H]testosterone in 0.1 mL of 1% polysorbate 80-saline solution, were administered to the nasal cavity by means of micropipet, and the nostrils were then closed with an adhesive agent. For intravenous administration, the same doses were injected through the femoral vein. For intraduodenal administration, the abdomen was opened by a midline incision, and the 25-μg dose in 0.1 mL of 1% polysorbate 80-saline solution was injected directly through the duodenum.

Analytical Method—The blood samples were placed in polyethylene tubes, to which 0.2 g of NaCl, 200 μL of nonradioactive testosterone solution (1.25 mg/mL) in ethyl acetate, and 5 mL of ether were added. After shaking and centrifugation, the tube was frozen in a dry ice–acetone mixture. The ether layer was decanted into another tube and evaporated to dryness. The residue was then dissolved in 60 μL of methanol and 40 μL was spotted on TLC plates and developed using chloroform–acetone (9:1, v/v). The spot that corresponded to unchanged testosterone was then scraped off the plate, and the powder was suspended in 10 mL of scintillation cocktail for a radioactivity count. The developing solvent system was shown to separate unchanged testosterone from its metabolites.

RESULTS AND DISCUSSION

The levels of testosterone in the blood after nasal administration increased rapidly and attained the peak level within 2 min, whereas the intraduodenal administration resulted in considerably lower blood levels. The blood levels following nasal administration were similar to those following intravenous administration, and the half-life of elimination for the two routes was ~40 min.

The nasal bioavailability, calculated from the ratio of the area under the blood level–time curve [(nasal/intravenous) × 100], was 99% at the 25-μg dose and 90% at the 50-μg dose; the intraduodenal bioavailability was only 1% of that of the intravenous bioavailability at the dose studied (Table I; Fig. 1).

The results of this study strongly suggest that the natural male sex hormone, testosterone, is rapidly absorbed from the nasal mucosa into systemic blood

[1] [1β,2β-^{3}H]Testosterone (40 Ci/mmol); New England Nuclear, Boston, Mass.

0022-3549/84/0900-1300$01.00/0
© 1984, American Pharmaceutical Association

without first-pass metabolism. The nasal route may be of practical value for the administration of this hormone.

REFERENCES

(1) "The Pharmacological Basis of Therapeutics," 6th ed., A. G. Gilman, L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1980, Chap. 62.

(2) A. Hussain, S. Hirai, and R. Bawarshi, *J. Pharm. Sci.*, **68**, 1196 (1979).

(3) A. Hussian, S. Hirai, and R. Bawarshi, *J. Pharm. Sci.*, **69**, 1411 (1980).

(4) A. Hussian, S. Hirai, and R. Bawarshi, *J. Pharm. Sci.*, **70**, 466 (1981).

(5) A. Hussain, T. Foster, S. Hirai, T. Kashihara, R. Batenhorst, and M. Jones, *J. Pharm. Sci.*, **69**, 1240 (1980).

Colorimetric Determination of Gentamicin, Kanamycin, Tobramycin, and Amikacin Aminoglycosides with 2,4-Dinitrofluorobenzene

JAMES A. RYAN

Received March 29, 1983, from the *Department of Pharmaceutical Research and Development, Merck Sharp and Dohme Research Laboratories, Division of Merck and Company, Inc. West Point, PA 19486.* Accepted for publication August 3, 1983.

Abstract □ The reaction of 2,4-dinitrofluorobenzene (Sanger's reagent) is used to form colored products with aminoglycoside antibiotics. Stopping the progress of the reaction with acid after a fixed time allows aqueous solubility to be maintained while discharging any color due to excess reactant. The choice of an appropriate analytical wavelength results in adherence to Beer's law. Although this colorimetric method is not expected to be stability-indicating, it is convenient and should be useful in content uniformity determinations for pharmaceutical dosage forms (*e.g.*, ointments).

Keyphrases □ Colorimetry—aminoglycosides, 2,4-dinitrofluorobenzene □ Aminoglycosides—2,4-dinitrofluorobenzene, colorimetric determination

Most assays for monitoring aminoglycoside antibiotics which are mentioned in the Federal Register (1) are plate-cup bioassays. A number of chemical assays (2) have been developed for use with pharmaceutical dosage forms and content determinations. These assays are useful, in some cases, as rapid control procedures featuring high precision, but do not actually measure bioactivity; most are amine reagents (3). We found in our preliminary work that certain pharmaceutical formulation excipients do not react with 2,4-dinitrofluorobenzene (Sanger's reagent) (4–6); therefore, this reagent could be useful when determining aminoglycosides. Sanger's reagent was originally used to detect terminal amino groups in insulin, and was utilized recently (7) for postcolumn HPLC-derivatization and detection of neomycin sulfate.

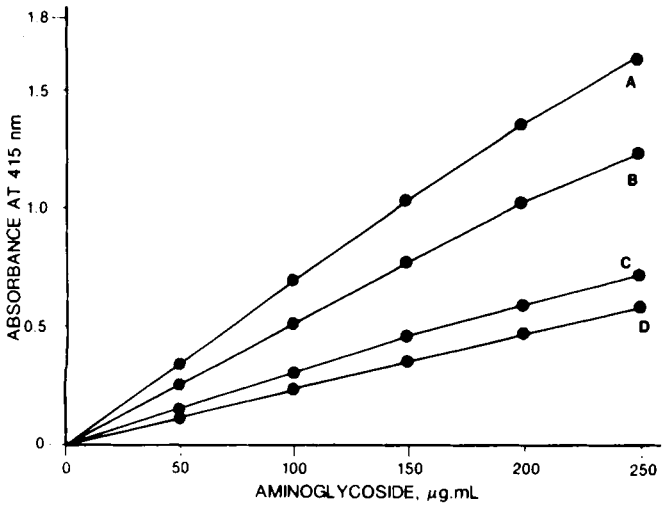

Figure 1—*Absorbance of aminoglycosides at 415 nm with 20-min reaction time. Key: (A) amikacin, (B) tobramycin, (C) kanamycin, (D) gentamicin.*

This paper reports the direct one-phase determination of gentamicin sulfate, kanamycin sulfate, tobramycin, and amikacin. The reaction was allowed to proceed under ambient conditions and then it was stopped, after an appropriate time, by acidification. The analytical wavelength chosen was not the absorption maximum, but one which permitted measurement in the aqueous-alcoholic medium without problem precipitation and with adherence to Beer's law over the range of 0–1000 μg/mL (Fig. 1). A typical reaction profile of gentamicin sulfate is outlined in Fig. 2.

EXPERIMENTAL SECTION[1]

Colorimetric Measurement—Exactly 5.0 mL of an aqueous solution containing ~500 μg of the aminoglycoside being tested was transferred to a 10-mL glass-stoppered flask or tube. Similarly, a series of flasks or tubes containing

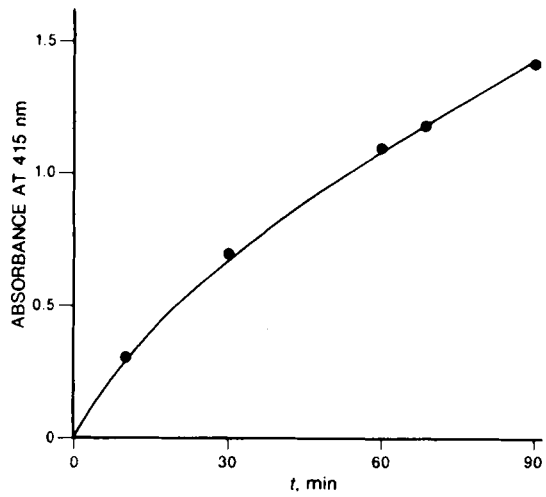

Figure 2—*Gentamicin sulfate reaction profile.*

[1] Gentamicin sulfate (lot GMC-6M-6024, potency 581 μg/mg) was obtained from the Schering Corp., Kenilworth, N.J. Kanamycin sulfate (lot 76F-140, potency 780 mg/mL) and amikacin (lot 74F-1805, potency 934 mg/mL) were both obtained from Bristol Labs., Syracuse, N.Y. The tobramycin (lot OCU55, potency 960 μg/mg) was made available through the courtesy of Eli Lilly and Co., Indianapolis, Ind. Sanger's reagent, 2,4-dinitrofluorobenzene (lot 101547, purity 98%), was obtained from the Aldrich Chemical Co., Milwaukee, Wis. Measurements were made on a Cary Model 15 UV-visible spectrophotometer.

0022-3549/84/0900-1301$01.00/0
© 1984, American Pharmaceutical Association