# Testosterone 17β-*N,N*-Dimethylglycinate Hydrochloride: A Prodrug with a Potential for Nasal Delivery of Testosterone

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**ABSTRACT:** The purpose of this study was to examine the potential of the nasal route for the systemic delivery of the poorly water-soluble drug testosterone (TS) using a water-soluble prodrug, TS 17β-*N,N*-dimethylglycinate hydrochloride. The physicochemical properties of the prodrug, *in vitro* hydrolysis in human liver homogenate, and *in vivo* nasal and intravenous experiments were performed in rats. The aqueous solubility of the prodrug was more than 100 mg/mL, compared with 0.01 mg/mL for TS, and its log partition coefficient between 0.05 M, phosphate buffer (pH 6) and octanol was 2.4. The prodrug was found to generate TS in 33% human liver homogenate and was absorbed from the nasal cavity rapidly and quantitatively. The bioavailabilities of both the prodrug and TS after nasal administration of the prodrug were similar to that after equivalent intravenous doses. These studies in rats suggest that this water-soluble prodrug of TS may have therapeutic utility for the management of TS deficiency.  J Pharm Sci 91:785–789, 2002;
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**Keywords:** testosterone; bioavailability; water-soluble prodrug; nasal absorption

## INTRODUCTION

Testosterone (TS) is considered to be the most potent of the natural male sex hormones. Men with primary or secondary hypogonadism require lifelong androgen replacement to prevent osteoporosis and maintain normal muscle and bone mass, erythropoiesis, and sexual function.[1] Although TS administered orally is readily absorbed, it is ineffective because of extensive metabolism by the liver before reaching the systemic circulation. Esters of TS (enanthate and cypionate) given orally are similarly metabolized after hydrolysis. Consequently, TS and its esters are generally administered by intramuscular injection. In view of the fluctuations in serum TS concentrations associated with TS esters, alternative methods for TS delivery such as buccal/sublingual,[2] nasal,[3] and transdermal[4] have been investigated in search of a convenient and effective modality of delivering the drug systemically. Recently, two transdermal TS patches have become available commercially. Although this route of administration is effective, these patches have several drawbacks. The site of application in one transdermal system, i.e., scrotum, and the prerequisite special preparation of the site renders it undesirable to patients, resulting in a low rate of compliance with therapy regimens. Also,

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higher than normal concentration of dihydrotestosterone is reported due to the presence of the enzyme 5α-reductase in high concentrations in the scrotal skin.[4] The other TS transdermal system is intended for application to nonscrotal skin.[5] However, it is usually associated with skin irritation and contact dermatitis at the application site; some patients discontinued using the patches because of these rashes.[6]

In a previous study by Hussain et al.,[3] TS was shown to be efficiently and completely absorbed in rats intranasally with a half-life of elimination ~40 min. However, TS is poorly soluble in water (0.01 mg/mL) and it is not feasible to prepare an aqueous solution containing sufficient drug (~3 mg) for intranasal human use. Thus, if the solubility of TS can be increased, it would be an ideal candidate for nasal administration. One approach to achieve this objective is to use solubilizers. But, this approach is not always successful because of the complex interactions between these agents and the nasal mucosa that may lead to temporary or permanent damage to the tissues. However, the use of a TS prodrug that has an acceptable aqueous solubility is a more practical approach for the formulation of an intranasal dosage form. In this report, it is shown that a water-soluble ester of TS [TS 17β-*N*,*N*-dimethylglycinate hydrochloride (TSDG)] is absorbed rapidly and completely when administered intranasally in doses that are unattainable with TS itself. This prodrug was selected over other prodrugs carrying an amine promoiety, such as dimethylaminobutyrate, because it provided the fastest hydrolysis rates in different tissues (Hussain et al., unpublished data). Although conventionally a comparison is made between a prodrug administered nasally and the parent drug intravenously, it was desired in these experiments to compare the prodrug administered nasally to the prodrug administered intravenously to better assess its absorption and conversion potential. Relatively high doses of the prodrug were used in these studies in order to be able to measure the prodrug and TS by a high-performance liquid chromatography (HPLC) method.

## MATERIALS AND METHODS

### Synthesis of TSDG

The prodrug, TSDG, shown in Figure 1, was synthesized by a standard esterification method.[7] Briefly, dimethylglycine hydrochloride and oxalyl chloride were gently warmed at 40°C until evolution of HCl gas ceased. Nitrogen gas was then bubbled through the solution to remove unreacted oxalyl chloride. The resulting acid chloride was dissolved in dimethylformamide and added dropwise with stirring to a solution of TS in methylene chloride. The reaction mixture was refluxed for 3 h. Similar to what was previously reported,[7] the ester was isolated, converted to the HCl salt, and characterized by elemental analysis, nuclear magnetic resonance (NMR), infrared, and HPLC. Elemental analysis (C, H, N) results were within ± 0.4% of the calculated values. $^1$H-NMR ($CDCl_3$) showed the characteristic chemical shifts of the methylene group protons of the promoiety, δ 3.1 (s, 2H); and the *N*-dimethyl protons, δ 2.82 (s, 6H).

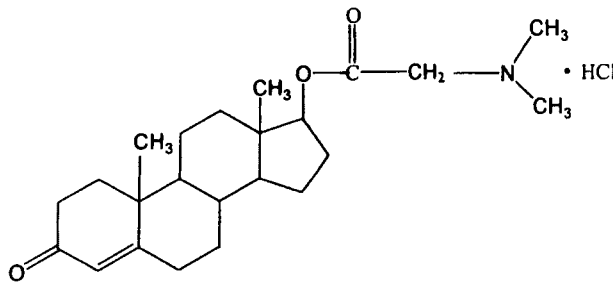


**Figure 1.** Structure of TSDG.

### Stability of TS Prodrug (TSDG) in Human Liver Homogenate

A preparation of human liver homogenate (33%) was prepared by homogenizing slices of human liver (Keystone Skin Bank, Exton, PA) in 0.05 M phosphate buffer (pH 6) at 0°C. A TSDG solution in the same buffer was prepared at a 1 mg/mL concentration and 0.1 mL of it was added to 0.9 mL of the liver homogenate and incubated at 37°C. At different time points, 0.1 mL of the incubate was sampled and added to 0.2 mL of acetonitrile. The mixture was vortexed for 1 min, centrifuged for 6 min, and 20 μL of the supernatant injected onto the HPLC column to analyze for TSDG and TS. The experiment was run in triplicate. Control experiments of only the prodrug in the buffer showed that the prodrug was stable for the duration of the experiment.

### Animal Studies

Male Sprague-Dawley rats, each weighing approximately 300 g, were anesthetized with pentobarbital sodium (Nembutal® sodium solution; Abbott Laboratories, Abbott Park, IL) (50 mg/mL using a 40 mg/kg dose intraperitone-

ally). The animals were prepared using a surgical procedure allowing intranasal administration.[3] Briefly, an incision was made in the neck, and the trachea was cannulated with a polyethylene tube. A closed-end tube was inserted through the esophagus to the posterior part of the nasal cavity. The nasopalatine passage was sealed with an adhesive agent to prevent drainage of the drug from the nasal cavity into the mouth. Each rat was administered 100 μL of a solution of the prodrug in 0.05 M phosphate buffer (pH 6) through one nostril using a microsyringe. For intravenous administration, the same volume was delivered into a cannulated jugular vein. Two dosing levels of TSDG equivalent to 25 and 50 mg of TS/kg were tested. Each experiment was conducted in triplicate. Blood samples (200 μL) were collected from a cannula inserted in the femoral artery at 0, 5, 10, 20, 30, 40, 60, 90, 120, 150, and 180 min. After immediate centrifugation, the plasma was separated and analyzed by HPLC. All animal procedures were conducted according to the *Principles of Laboratory Animal Care* (NIH publication no. 85-23).

### Analysis of TSDG and TS

Analytical HPLC measurements were conducted using the following: Spectroflow 400 solvent delivery system (Kratos, Manchester, UK); Spectroflow 77 absorbance detector (Kratos), SP4270 integrator (Spectra-Physics, Mountain View, CA); Rheodyne model 7125 injector, column: Ultrasphere octyl, 5 μ, 250 × 10 mm (Beckman, Fullerton, CA); guard column: filled with 25–37 μm glass beads with chemically bonded octadecyl groups, 70 × 2 mm; injection volume: 20 μL; mobile phase: 0.1 M sodium acetate (pH 4): acetonitrile 50:50 v/v; flow rate: 1 mL/min; detection wavelength 238 nm. Retention times for TSDG and TS were 4.6 and 7.7 min, respectively.

### Solubility and Partition Coefficient

The solubility of TSDG and TS in 0.05 M phosphate buffer (pH 6) was determined. The partition coefficient of TSDG between 0.05 M phosphate buffer (pH 6) and octanol was also measured following standard procedures.[8]

## RESULTS AND DISCUSSION

### Liver Homogenate Study

Figure 2 shows the time course for the disappearance of TSDG in 33% human liver homogenate at 37°C. The apparent half-life of hydrolysis of TSDG at this concentration of liver homogenate was estimated to be 10 min. The quantitative liberation of TS from the prodrug satisfies the basis prerequisite in prodrug design.

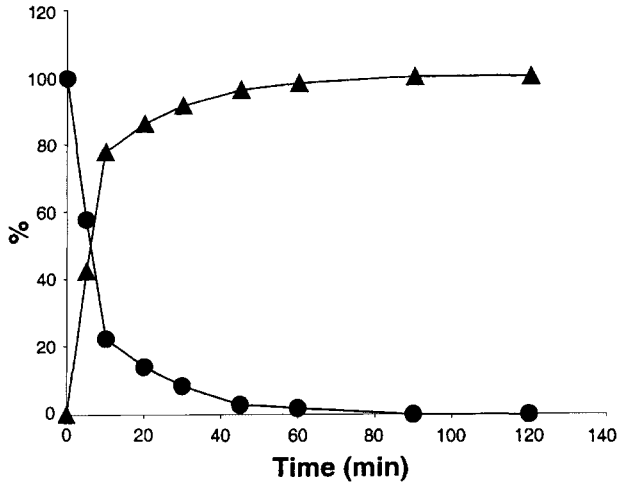


**Figure 2.** Hydrolysis of TSDG in 33% human liver homogenate. Disappearance of the prodrug (●), and appearance of TS (▲). Values represent average molar percentages.

### Solubility of TSDG and TS

Despite a relatively high partition coefficient of TSDG between octanol and 0.05 M phosphate buffer (pH 6), i.e., log $P = 2.4$, the solubility of TSDG in 0.05 M phosphate buffer (pH 6) is more than 100 mg/mL. The high solubility of this prodrug is attributed to its surface activity, with critical micelle concentration of ~3 mg/mL, similar to that of a similar prodrug, *N,N*-dimethylaminobutyrate, previously reported.[9] Log *P* of TS is 3.4[10] whereas its aqueous solubility is about 0.01 mg/mL. Such high solubility of the prodrug makes it feasible to formulate aqueous solutions containing a sufficient amount of TS for intranasal administration.

### Pharmacokinetics: Intranasal and Intravenous

Figures 3 and 4 show the plasma concentrations of TSDG and TS after the intravenous administration of TSDG in doses equivalent to 25 and 50 mg of TS/kg, respectively. The apparent half-life of TSDG disappearance (> 60%), as determined from the initial slope, is 10 min for the lower dose and 20 min for the higher dose,

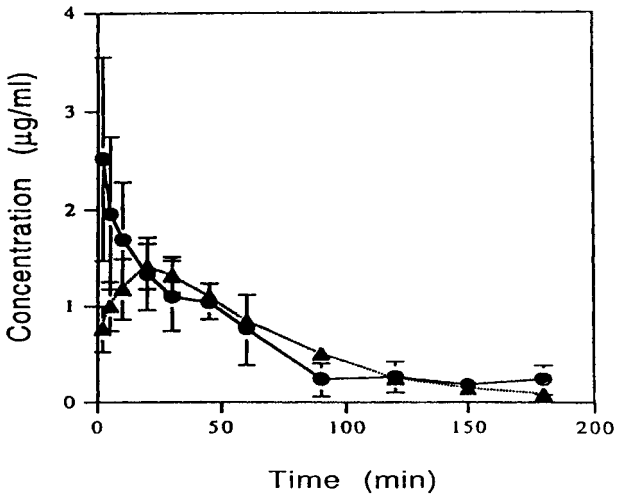


**Figure 3.** Plasma concentrations of TSDG (●) and TS (▲) after intravenous administration of the prodrug at a dose equivalent to 25 mg/kg of TS, mean and SE ($n = 3$).

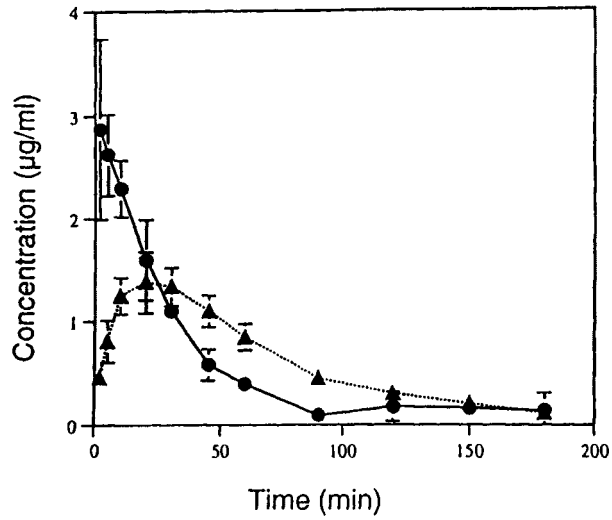


**Figure 5.** Plasma concentrations of TSDG (●) and TS (▲) after nasal administration of the prodrug at a dose equivalent to 25 mg/kg of TS, mean and SE ($n = 3$).

whereas the corresponding terminal elimination half-lives for TS are estimated from Figures 3 and 4 be about 50 min.

Figures 5 and 6 show the time dependence of TSDG and TS concentrations after intranasal administration of TSDG in doses equivalent to 25 and 50 mg/kg of TS, respectively. It appears that the prodrug is almost instantly absorbed and its conversion into TS at the two dosing levels commences immediately. The corresponding terminal elimination half-lives for TS are estimated from Figures 5 and 6 to be about 55 min, similar to those observed after intravenous administration. Half-lives of elimination of TS when administered iv and nasally to rats were also about 50 min.[3] The peak plasma concentration of TS was attained, both intranasally and intravenously, within 12 min at the lower dose and within 20 min at the higher dosing level.

In Table 1, the area under the curve (AUC) values for the prodrug and TS after intranasal

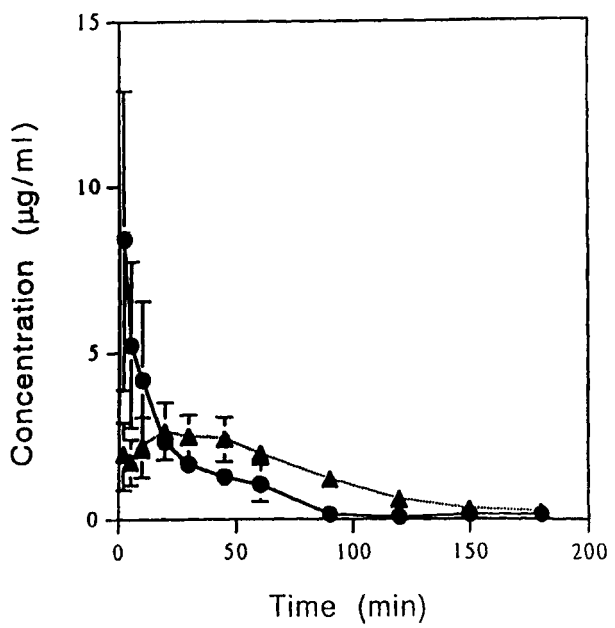


**Figure 4.** Plasma concentrations of TSDG (●) and TS (▲) after intravenous administration of the prodrug at a dose equivalent to 50 mg/kg of TS, mean and SE ($n = 3$).

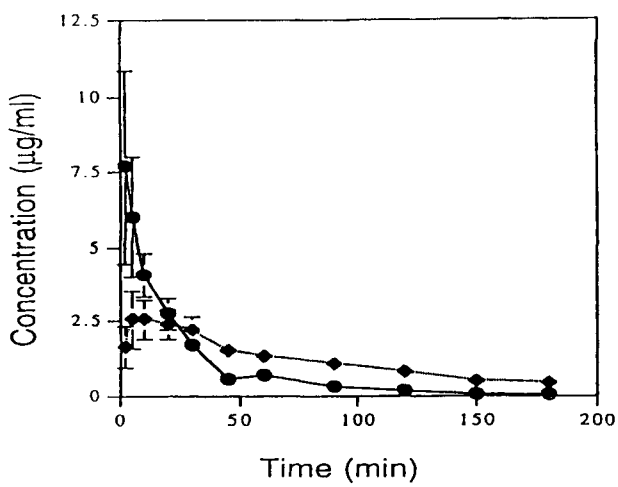


**Figure 6.** Plasma concentrations of TSDG (●) and TS (◆) after nasal administration of the prodrug at a dose equivalent to 50 mg/kg of TS, mean and SE ($n = 3$).

**Table 1.** AUC Values for TSDG and TS After Intravenous and Nasal Administration of the Prodrug at Doses Equivalent to 25 and 50 mg/kg of TS

| | Intravenous | Nasal |
|---|---|---|
| Dose, equivalent to 25 mg/kg of testosterone | | |
| AUC for TSDG (μg · h/mL) | 1.65 ± 0.33 | 1.63 ± 0.41 |
| AUC for TS (μg · h/mL) | 1.77 ± 0.12 | 1.76 ± 0.06 |
| Dose, equivalent to 50 mg/kg of testosterone | | |
| AUC for TSDG (μg · h/mL) | 2.83 ± 0.24 | 2.66 ± 0.35 |
| AUC for TS (μg · h/mL) | 3.78 ± 0.96 | 3.49 ± 1.05 |

and intravenous administration of TSDG at the two dosing levels are shown. The similarity in the AUC values indicated that the prodrug was completely absorbed after intranasal administration. Also, the AUC for TS is almost doubled upon doubling the dose of the prodrug intranasally or intravenously. In addition to its potential for nasal TS delivery, this prodrug may lend itself to parenteral delivery replacing painful intramuscular injection of oily formulations of TS derivatives.

## CONCLUSIONS

The prodrug TSDG is water-soluble to an extent that allows the formulation of drops, spray, or some other nasal dosage form, or even parenteral dosage forms, in which the required dose for humans is incorporated. Furthermore, it is hydrolyzed by human liver enzymes to TS rapidly and quantitatively and thus it may have a potential value in TS replacement therapy.

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