Plasma Levels, Tissue Distribution, and Excretion of Radioactivity After Single-Dose Administration of (³H)-Oleic Acid Added to D-004, a Lipid Extract of the Fruit of *Roystonea regia,* in Rats

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ABSTRACT

Background: D-004, a lipid extract of the fruit of *Roystonea regia,* contains a mixture of fatty acids—mainly oleic, lauric, palmitic, and myristic acids, with oleic acid being among the most abundant—that has been found to reduce the risk for prostatic hyperplasia (PH) induced with testosterone (T) in rats. The pharmacokinetic profile of D-004 has not been reported.

Objective: The objective of this study in rats was to assess plasma levels, tissue distribution, and excretion of total radioactivity (TR) after single-dose administration of oral D-004 radiolabeled with (³H)-oleic acid, as a surrogate for the pharmacokinetics of D-004.

Methods: This experimental study was conducted at the Pharmacology Department, Center of Natural Products, National Center for Scientific Research, Havana, Cuba. Single doses of suspensions of (³H)-oleic acid 0.16 μCi/mg mixed with D-004 400 mg/kg (radioactive dose/animal 7.2 μCi) were given orally to male Wistar rats weighing 150 to 200 g assigned to the treated or control group. Three rats were euthanized at each of the following times: 0.25, 0.5, 1, 1.5, 2, 4, 8, 24, 48, 72, 96, and 144 hours after study drug administration. After administration, the rats euthanized at the last experimental time point were housed individually in metabolism cages. Urine and feces samples were collected daily. At each time point, blood samples were drawn and plasma samples were obtained using centrifugation. After euthanization, tissue samples (liver, lungs, spleen, brain, kidneys, adipose tissue, muscle, stomach, small and large intestines, adrenal glands, heart, testes, prostate, and seminal vesicles) were quickly removed, washed, blotted, and homogenized. Plasma (100 μL), tissue aliquots (100 mg), feces (10 mg), and urine (100 μL) were dissolved and TR was measured. Samples were assayed in duplicate. Results were expressed in μgEq of radiolabeled oleic acid per milliliter of plasma or urine or gram of tissue or feces. Plasma, tissue, feces, and urine samples of rats that did not receive (³H)-oleic acid were

used as controls. Excretion was expressed as the percentage of the radioactivity excreted via each route with respect to the total radioactive dose administered to each rat.

Results: A total of 50 rats were included in the experiment (mean age, 4 weeks; mean weight, 310 g). Absorption was rapid; mean C_{max} was 195.56 (31.12) µgEq/mL, and mean T_{max} was 2 hours. Thereafter, a biphasic decay of TR was found: a rapid first phase ($t_{1/2\alpha}$, 1.33 hours), followed by a slower second elimination phase ($t_{1/2\beta}$, 36.07 hours). Radioactivity was rapidly and broadly distributed throughout the tissues, with more accumulating in the prostate than elsewhere. In the first 8 hours, accumulation of TR was greatest in the prostate, followed by the liver, small intestine, and plasma. Subsequently, TR increased in the small intestine, while it decreased in the liver and plasma. In contrast, over the periods of 24 and 144 hours after administration, TR increased in the adipose tissue, while it decreased in the other tissues and plasma. During those intervals, TR was greatest in the prostate, followed by adipose tissue. Mean peak radioactivity in the prostate (562.41 µgEq/g) was reached at 4 hours and decreased slowly thereafter. The prostate had the highest values of $t_{1/2\beta}$ and cumulative AUC compared with the other tissues and plasma. Mean (SD) TR was similar in feces (33.48% [4.90%]) and urine (28.96% [5.32%]), with total excretion being 62.40% (5.90%) of the administered dose.

Conclusions: In this experimental study, after single-dose administration of oral D-004 radiolabeled with (^{3}H)-oleic acid in rats, TR was rapidly and widely distributed across the tissues, with the prostate having the highest accumulation of radioactivity. Excretion of TR was limited, with similar amounts being excreted in feces and urine. The broad distribution of radiolabeled oleic acid and/or its metabolites suggests a pharmacokinetic rationale for the effectiveness of D-004 in reducing the risk for PH induced with T in rats. (*Curr Ther Res Clin Exp.* 2006;67:406–419) Copyright © 2006 Excerpta Medica, Inc.

Key words: oleic acid pharmacokinetics, D-004, *Roystonea regia* lipid extract, royal palm lipid extract.

INTRODUCTION

Benign prostatic hyperplasia (BPH), the uncontrolled, benign growth of the prostate that often results in difficulty urinating, is observed frequently in men aged ≥50 years.[1–5] Drug therapy, mainly with prostate 5α-reductase inhibitors and α_1-adrenoreceptor blockers, is the most common treatment of BPH.[1–7] Herbal medicines are also commonly used to treat BPH. In most cases, the effectiveness of herbal products is due to multiple mechanisms because they contain a mixture of compounds.[8]

The lipidosterolic extract (LE) of saw palmetto berries (*Serenoa repens*), a palm of the Arecaceae family, is the main alternative treatment for BPH, having fewer associated adverse effects compared with conventional drugs.[9–16] The LE of saw palmetto consists of ≥80% free fatty acids (eg, lauric acid [16%–38%],

oleic acid [9%–24%], capric acid [0.8%–3.2%], caprylic acid [0.9%–2.7%], myristic acid [6.5%–15%], palmitic acid [4%–8.8%], stearic acid [0.4%–1.2%], linoleic acid [0.7%–3.4%], and linolenic acid [0.2%–0.7%]), while other compounds are present in lesser proportions.[9,10,12,13]

Despite the extensive use of saw palmetto LE, published pharmacokinetic studies are scarce, based on a MEDLINE search (key terms: *Serenoa repens, pharmacokinetics,* and *distribution;* years: 1990–2006).[9,10,17,18] A study conducted in rats treated orally with saw palmetto LE radiolabeled with (^{14}C)-oleic and -lauric acids has found that the prostate contained the highest concentration of radioactivity compared with other organs,[16] supporting good bioavailability of the medication in the target organ.

D-004, a lipid extract of the fruit of the Cuban royal palm (*Roystonea regia*), also contains a reproducible mixture of fatty acids, with oleic acid being the most abundant, followed by lauric, palmitic, and myristic acids. In rodents, oral treatment with D-004 was found to reduce the incidence of prostatic hyperplasia (PH) induced with testosterone (T) but not with dihydrotestosterone, suggesting that it may act by inhibiting prostate 5α-reductase activity.[19–22] The pharmacokinetic profile of D-004 has not been reported.

Oleic acid, the most abundant component of D-004, has been found to inhibit prostate 5α-reductase activity in rats.[23] Therefore, the pharmacokinetic properties of oleic acid can be used as a surrogate for the pharmacokinetic properties of the components of D-004. The present study explored plasma levels, tissue distribution, and excretion of radioactivity after single-dose administration of oral D-004 radiolabeled with (^{3}H)-oleic acid in rats.

MATERIALS AND METHODS

This experimental study was conducted at the Pharmacology Department, Center of Natural Products, National Center for Scientific Research, Havana, Cuba.

Animals

Male Wistar rats weighing 150 to 200 g were obtained from the National Center for Laboratory Animal Production (CENPALAB, Havana, Cuba) and adapted for 14 days to laboratory conditions (temperature 25°C ± 2°C, humidity 60% ± 5%, and 12-hour dark–light cycles). Free access to food (rodent chow purchased at CENPALAB) and tap water was allowed.

The study protocol was reviewed and approved by both an independent institutional review board and the Quality Assurance Unit of the Center of Natural Products (Havana, Cuba), and animals were handled according to the Cuban Ethical Code for Care of Laboratory Animals.

Study Drug Administration

(^{3}H)-oleic acid 0.16 μCi/mg (Amershan GmbH, Stockholm, Sweden) was used as the labeled substrate. Fresh suspensions in Tween 36/water (2%) vehicle (Merck

GmbH, Berlin, Germany) were prepared by mixing (^{3}H)-oleic acid with D-004 2 hours before dosing. Rats were treated with D-004 400 mg/kg (radioactive dose/rat 7.2 µCi). Since D-004 administered orally in rats has been found to be effective in reducing the risk for PH induced with T in vivo,[19–22] we determined that this dose should reach the prostate, the target organ for such an effect.

Because we were using the pharmacokinetic data for oleic acid as a surrogate for the pharmacokinetic properties of the components of D-004, we mixed oleic acid with D-004 instead of administering the radiolabeled substrate alone. Treatments were administered orally using gastric gavage (1 mL/200 g) at 8:30 AM. In addition, groups of rats treated with vehicle alone or similar doses of D-004, but not with the radioactive compound (controls), were included to assess whether radioactive cross-contamination was present and to calculate the total radioactivity (TR) for each sample, subtracting any background radioactivity.

Measurement of Radioactivity in Plasma and Tissues

After study drug administration, 3 rats were anesthetized with ether and exsanguinated through the abdominal aorta at 0.25, 0.5, 1, 1.5, 2, 4, 8, 24, 48, 72, 96, and 144 hours after study drug administration. Blood samples were drawn into heparinized tubes, and plasma was obtained by centrifugation at 3000 rpm at 25°C for 20 minutes.

The tissues (liver, lungs, spleen, brain, kidneys, adipose tissue [dorsal subcutaneous], muscle [right leg], stomach, and small and large intestines [the most proximal and distal position, respectively], adrenal glands, and heart, testes, prostate, and seminal vesicles) were quickly removed, washed in cold (10°C) 0.9% sodium chloride, blotted with filter paper, and weighed and homogenized into the counting vials. Aliquots of plasma (100 µL) and tissues (100 mg) were dissolved into the counting vials by adding 200 and 500 µL of Tissue Solubilizer (TS) (BDH Laboratory Supplies, Poole, England), respectively, and were maintained at 40°C for 20 minutes. When required, samples were blanched with hydrogen peroxide (H_2O_2) (30 wt% sol in water) and acidified with acetic acid.

Measurement of Radioactivity in Urine and Feces

After study drug administration, rats euthanized at the last experimental time point were housed individually in metabolism cages. Urine (100 µL) and feces (10 mg) were collected daily. To ensure uniform volumes, urine samples were diluted with distilled water and later shaken and treated as described previously for plasma samples to determine TR. Feces were dried at 60°C overnight and weighed. Once powdered and homogenized with a mortar, 10-mg samples were transferred into scintillation vials and hydrated with distilled water (200 µL) and TS (1 mL). The mixture was stirred at 60°C until it was totally dissolved. Samples were blanched with H_2O_2 30%, acidified with acetic acid (30 µL), and allowed to stand for at least 30 hours before radioactivity was measured.

For TR measurements, scintillation liquid (10 mL) was added to all prepared samples. Radioactivity was measured as TR; whether it corresponded to un-

changed (^{3}H)-oleic acid and/or metabolites was not determined. All samples were assayed in duplicate for radioactivity in a RACKBETA model 1219 liquid scintillation spectrometer (Pharmacia LKB Biotechnology, Uppsala, Sweden), and radioactivity was determined by adding 15 mL of Cocktail T (BDH Laboratory Supplies). Counting efficiencies were determined automatically using the equipment, and quenching corrections were performed using the external standard method.

TR data (dpm/min) were expressed as µgEq of the radiolabeled oleic acid per milliliter of plasma or urine or per gram of tissue or feces. For this calculation, the specific activity of (^{3}H)-oleic acid mixed in D-004 was estimated. Because of the high specific activity of the radiolabeled compound (10.0 Ci/mmol) added to the isotopic mixture diluted in D-004, we assumed the mass contribution of radiolabeled oleic acid to the overall mass of oleic acid present in the mixture to be negligible. The final TR value of each sample was calculated after subtracting the radioactivity determined in similar samples of rats that were not administered the radiolabeled compound.

Pharmacokinetic Analysis

Pharmacokinetic properties were calculated using WinNonlin version 2.1 (Pharsight Corporation, Mountain View, California) based on a 2-compartment and a noncompartmental model for calculating the AUC of the plasma profile and the tissue distribution, respectively. Excretion was expressed as the percentage of the radioactivity excreted via each route with respect to the total radioactive dose administered to each rat.

RESULTS

A total of 50 rats were included in the experiment (mean age, 4 weeks).

Plasma Profile of Total Radioactivity and Absorption

Figure 1 shows the plasma TR-versus-time profile after oral dosing of D-004 radiolabeled with (^{3}H)-oleic acid, and **Table I** shows the experimental and predicted values of the pharmacokinetic parameters measured.

Mean (SD) experimental C_{max} was 195.56 (31.12) µgEq/mL, and mean T_{max} was 2 hours, with a biphasic decrease observed thereafter. Thus, an open, 2-compartment pharmacokinetic model fit this plasma concentration–time pattern well ($r^2 = 0.99$); the first distribution phase was rapid ($t_{1/2\alpha}$, 1.33 hours) and was followed by a slower second elimination phase ($t_{1/2\beta}$, 36.07 hours). The experimental (195.56 µgEq/mL) and predicted (190.45 µgEq/mL) C_{max} values were similar, as were the experimental (2.00 hours) and calculated (2.58 hours) T_{max} values.

Distribution in Tissues

Table II shows the $t_{1/2\lambda}$ and the mean residence time (MRT) of the radioactivity in selected tissues. Fifteen minutes after study drug administration, radioac-
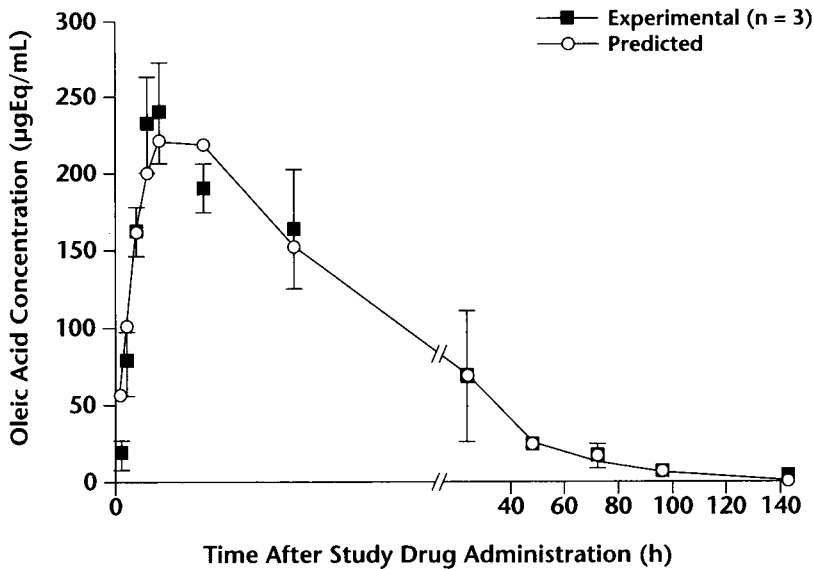
Figure 1. Mean (SD) total radioactivity accumulated in plasma from 0 to 144 hours after single-dose administration of oral D-004 radiolabeled with (^{3}H)-oleic acid in rats.

Table I. Pharmacokinetic properties of radioactivity after single-dose administration of oral D-004 radiolabeled with (^{3}H)-oleic acid to rats. Values are means except where otherwise marked.

Property	Value
Experimental C_{max}, mean (SD), µgEq/mL	195.56 (31.12)
Predicted C_{max}, µgEq/mL	190.45
Experimental T_{max}, h	2.00
Predicted T_{max}, h	2.58
$t_{1/2\alpha}$, h	1.33
$t_{1/2\beta}$, h	36.07
Predicted $AUC_{0-144\ h}$, µgEq/mL · h^{-1}	5368.40
k_a, h	1.31
k_e, h	8.67
r^2	0.99

Table II. Pharmacokinetic parameters of the distribution of radioactivity in tissues. Values are mean hours.

Tissue	$t_{1/2\lambda}$	MRT
Prostate	181.93	260.23
Adipose tissue	141.02	225.27
Adrenal glands	92.24	134.36
Testes	79.45	116.14
Seminal vesicles	60.27	90.04
Small intestine	45.92	65.10
Liver	35.60	52.44

MRT = mean residence time.

tivity was broadly distributed in plasma and all the tissues examined. However, the time course varied in the different tissues, as shown by the AUC at the intervals of 0 to 8 hours, 0 to 24 hours, and 0 to 144 hours after study drug administration (**Figure 2**). Within the first 8 hours (**Figure 2A**), the highest TR values were detected in the prostate, followed by the liver, small intestine, and plasma. For the interval 0 to 24 hours (**Figure 2B**), the prostate, adipose tissue, liver, plasma, and small intestine still had the highest TR values, but radioactivity had increased in the small intestine and decreased in the liver with respect to the first hours (**Figure 2A**). The peak radioactivity occurred later in the small intestine (at 4 hours) than in the liver (at 2 hours). In the small intestine and the liver, $t_{1/2\lambda}$ values were 45.92 and 35.60 hours, respectively, and MRT values were 65.10 and 52.44 hours, respectively (**Table II**).

During the period of 0 to 144 hours after study drug administration, a large change occurred in the AUC of radioactivity of the adipose tissue (**Figure 2C**). While radioactivity decreased in the tissues and plasma, a gradual and sustained increase in radioactivity was found in adipose tissue. After 24 and 144 hours, AUC in the adipose tissue was second only to that in the prostate (**Figures 2B and 2C**).

Figure 3 shows the distribution of total radioactivity in the prostate over time, with mean C_{max} being 562.41 µgEq/g and mean T_{max} being 4 hours and decreasing slowly thereafter. The mean value measured at 8 hours (522.87 µgEq/g) was similar to that found at 2 hours; the values remained elevated after 24 and 72 hours (498.43 and 416.41 µgEq/g, respectively). From 0 to 144 hours after study drug administration, the prostate had the longest mean $t_{1/2\lambda}$ (181.93 hours) and the greatest MRT (260.23 hours) and cumulative AUC values compared with plasma and the other tissues studied (**Table II**). Thus, the AUC value was higher in the prostate compared with all of the other sites studied, including the testes and seminal vesicles (**Figure 4**).
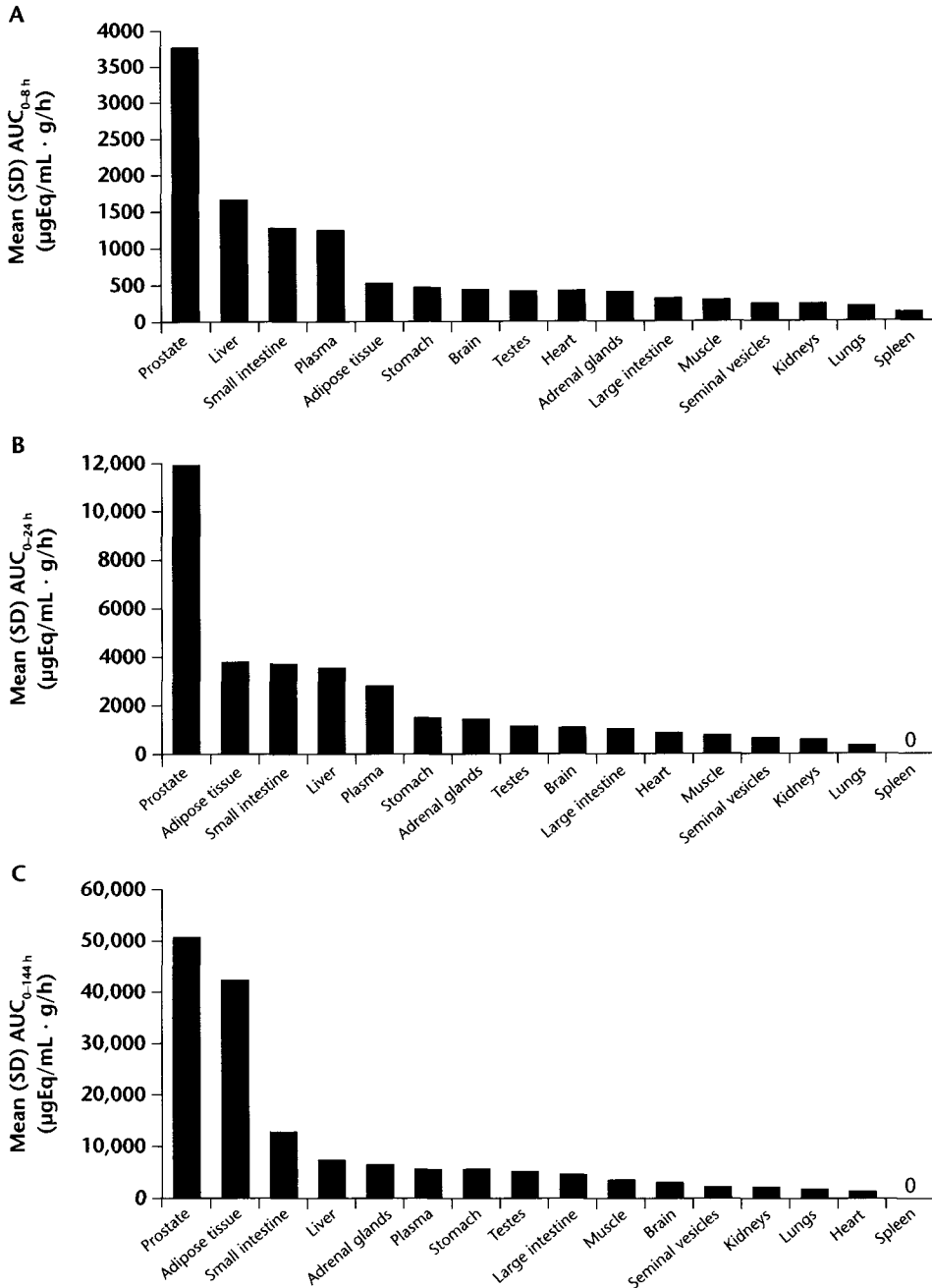
Figure 2. AUC of radioactivity in tissue (μgEq/g) and plasma (μgEq/mL) after oral administration of a dose of D-004 radiolabeled with (^{3}H)-oleic acid in rats for the intervals (A) 0 to 8 hours, (B) 0 to 24 hours, and (C) 0 to 144 hours.
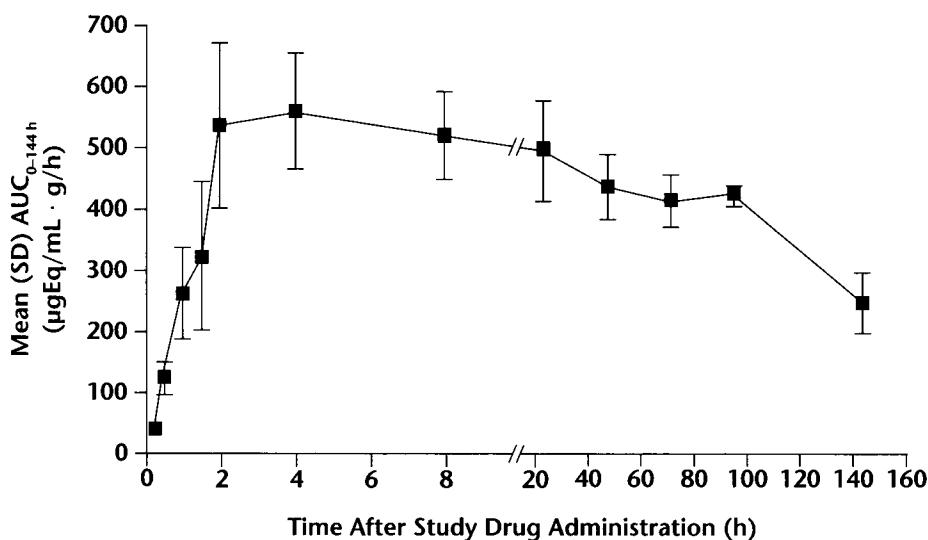
Figure 3. Total radioactivity in the prostate 0 to 144 hours after oral administration of a dose of D-004 radiolabeled with (^{3}H)-oleic acid in rats.

Excretion of Radioactivity

Table III summarizes the urinary and fecal excretion of radioactivity. At 144 hours after study drug administration, total mean (SD) excretion was 62.40% (5.90%) of the administered dose—feces (33.48% [4.90%]) and urine (28.96% [5.32%]). While almost half of the radioactivity recovered in the urine was excreted within the first 24 hours (13.30% [2.25%]), 13.90% (4.62%) and 24.08% (5.10%) of the administered doses were excreted in the feces after 24 and 48 hours, respectively.

DISCUSSION

After single-dose administration of oral D-004 radiolabeled with (^{3}H)-oleic acid, absorption was rapid, as indicated by the high plasma radioactivity levels reached after 15 minutes and the T_{max} (2 hours). The plasma concentration–time pattern fit an open, 2-compartment pharmacokinetic model, accounting for a rapid first distribution phase, in which plasma levels fell sharply, and a slower second elimination phase. The consistency between the experimental and predicted values of C_{max} and T_{max} suggests that the experimental values matched a 2-phase elimination process.

The tissue distribution of radioactivity was rapid and broad. Concentrations of radioactivity in tested tissues were high beginning with the first samples, which were obtained at 15 minutes after study drug administration. Tissue-specific distributions of radioactivity were observed, with a different time course for each source. Thus, for the first interval (0–8 hours), the prostate,
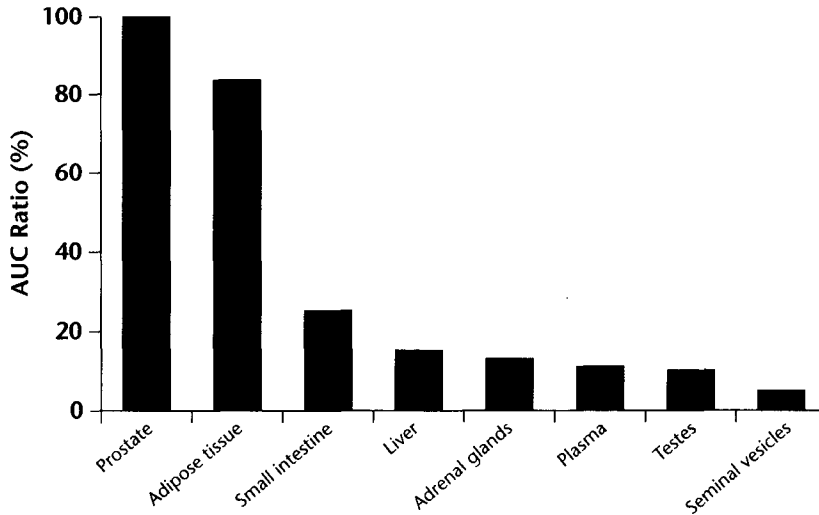
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Figure 4. Ratio of the AUC of radioactivity in selected tissues and plasma.

Table III. Cumulative excretion of radioactivity (feces and urine) after single-dose administration of oral (^{3}H)-oleic acid in rats. Values are mean (SD) %.

Collection Period, h	Excretion
Feces	
0–24	13.90 (4.62)
0–48	24.08 (5.10)
0–72	25.97 (5.39)
0–96	29.13 (4.49)
0–120	30.53 (4.40)
0–144	33.48 (4.90)
Urine	
0–24	13.03 (2.25)
0–48	16.76 (2.05)
0–72	21.03 (2.01)
0–96	23.68 (3.63)
0–120	26.01 (4.37)
0–144	28.96 (5.32)
Total (feces + urine)	62.40 (5.90)

followed by the liver, small intestine, and plasma, was found to have the highest TR value. The high TR values in the liver and small intestine suggest a first-pass effect in these organs. However, the clinical relevance of this finding is believed to be limited because the cumulative AUC values in liver, small intestine, and plasma were similar, allowing substantial systemic bioavailability of radioactivity after oral administration of the radiolabeled compound. This result is consistent with the fact that most (>70%) of the radioactivity administered as radiolabeled oleic acid[16] and other free fatty acids of similar chain length were recovered in plasma in the first hours after administration.

The high level of radioactivity found in the small intestine was expected because oils are metabolized and absorbed in the small intestine. However, during the first interval (0–8 hours), more radioactivity accumulated in the liver than in the small intestine. This finding was unexpected because drugs must cross the intestinal mucosa before entering the portal circulation and passing through the liver. A possible explanation for this apparent contradiction is related to the distinct mechanisms underlying the uptake of free fatty acids in both organs. In the liver, free fatty acids can enter tissues by simple diffusion or by a carrier protein system,[24–28] which occurs mainly via the chylomicron-remnant receptor.[29] However, the intestinal mucosa can internalize free fatty acids in vivo from the bloodstream,[30] thereby allowing greater accumulation of radioactivity.

The time course of the retention of radioactivity in adipose tissue appears to be as expected. In contrast to the other tissues studied, radioactivity in adipose tissue increased throughout the study, suggesting that some of the radioactivity that was not excreted finally accumulated in adipose tissue. This finding is consistent with the fact that triglycerides, as well as chylomicrons (from intestinal absorption) and lipoproteins (from hepatic synthesis), are hydrolyzed by lipase to glycerol and free fatty acids, which are then absorbed by adipocytes.

Overall, the broad retention of radioactivity throughout the tissues was consistent with its slow decay over time and with findings that 25% to 40% of orally administered (^{14}C)-oleic, -palmitic, and -stearic acids accumulated in organs and tissues.[30,31]

After single-dose administration of oral D-004 radiolabeled with (^{3}H)-oleic acid, the preponderance of radioactivity accumulated in the rat prostate, suggesting a preferential accumulation of radiolabeled oleic acid and/or its metabolites in this organ. This result supports the data regarding accumulation of TR after an oral dose of saw palmetto LE radiolabeled with (^{14}C)-oleic and (^{14}C)-lauric acids.[16] In addition, since oral D-004 therapy prevented PH in rats given T,[19–22] our findings indicate that a main component of D-004 and/or its metabolites reached the prostate, a target organ for the preventive effect of this extract in T-induced PH in the rat. Because TR in prostate decreased slowly throughout the study period, it seems likely that higher concentrations could be achieved after repeated dosing.

Recovery of radioactivity 144 hours after oral administration was incomplete (62.40%), indicating slow elimination of the radioactivity, which is consistent

with retention of radioactivity in the different organs. Excretion of radioactivity via the fecal and urinary routes was similar—33.48% in feces and 28.96% in urine.

In the present study, absolute availability and absorption data were not determined because we did not measure plasma radioactivity or TR excretion immediately after administration.

Pharmacokinetic studies of substances consisting of mixtures of compounds, as are normally found in plant extracts, are particularly difficult, especially when these compounds and/or metabolites are endogenous, as occurs with mixtures of free fatty acids.

In this study, we found that the radiolabeled compound and/or metabolites were broadly distributed across organs, including the prostate, supporting a pharmacokinetic rationale for the preventive effects of D-004 on PH induced with T in rats. Further studies, however, are needed to explore the pharmacokinetic characteristics of other free fatty acids abundant in D-004, such as lauric and myristic acids, which also inhibit prostate 5α-reductase activity,[23] to obtain a more representative picture of the pharmacokinetic behavior of this substance.

CONCLUSIONS

This study found that in rats, after single-dose administration of oral D-004 radiolabeled with (^{3}H)-oleic acid, absorption of radioactivity was rapid and widely distributed across organs and tissues, with the prostate showing the greatest retention of radioactivity throughout the study. Total excretion was limited (62.40% of the administered dose), consistent with slow elimination and high retention of radioactivity in the tissues. A similar amount of radioactivity was excreted via the fecal and urinary routes. These results support the use of D-004 in preventing PH induced with T in rats.

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