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Pharmacokinetic Profile of (±)-Gossypol in Male Sprague–Dawley Rats following Single Intravenous and Oral and Subchronic Oral Administration (42700)

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Abstract. The pharmacokinetic profile of (±)-gossypol was determined in male Sprague–Dawley rats following a single intravenous or oral 10 mg/kg dose and after receiving a daily oral 10 mg/kg dose for 14 days. The intravenous plasma (±)-gossypol level data were fitted with a three-compartment, open-model system. The apparent half-life of elimination of (±)-gossypol following intravenous administration was 11.44 hr, corresponding to an elimination rate constant of 0.05 hr^{-1}. The total plasma clearance (Cl), volume of distribution (V_d), and AUC_{plasma} following a single intravenous administration were 0.16 liter/hr/kg, 0.05 liter/kg, and 63.09 mg · hr/liter, respectively. The bioavailability of a single oral dose of (±)-gossypol in rats was 60%. The change in plasma (±)-gossypol concentration after a single or after multiple doses showed a biphasic pattern. A single oral dose of (±)-gossypol, however, was eliminated five times faster than the daily administered chemical. Thus, a single oral dose of (±)-gossypol was eliminated at a rate constant of 0.01 hr^{-1}, corresponding to a half-life of 64.76 hr. Subchronic oral administration of (±)-gossypol showed an apparent half-life of 101.91 hr^{-1}, corresponding to a rate constant of 0.007 hr^{-1}. The results indicate that multiple oral dosing of (±)-gossypol resulted in its longer retention in body tissue than a single oral dose. This study suggests that pharmacokinetics of (±)-gossypol may play, at least in part, a role in the reproductive toxicity of subchronic but not single oral dosing.

Gossypol [1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl (2,2′-binaphthalene)-8,8′-dicarboxaldehyde] is a yellow substance occurring in cotton plants (1). Its toxic effects have limited the use of cottonseed meal for human and animal consumption. It has been reported to reduce the oxygen-carrying capacity of blood and to cause hemolytic effects in erythrocytes (2), dyspnea (3), progressive weakness, and emaciation (4). It uncouples the respiratory chain and oxidative phosphorylation of rat liver mitochondria (5). Gossypol has been found to be an effective male contraceptive agent in humans and rats (6, 7).

Several studies have been carried out to determine ^{14}C distribution and excretion following the administration of a single oral dose of [^{14}C]gossypol in various species (7–10). The distribution pattern of bound and unbound gossypol was studied in rats (11). Ingested gossypol is deposited in all tissues, with high concentrations found in the liver, heart, kidneys, spleen, and lungs (7–12). Gossypol is a weak organic acid with a pK_a of 7.2, and it has been postulated to be secreted by the liver and kidneys via an organic anion transport system (1). A recent study has confirmed that gossypol is transported by the renal tubule through the classic organic anion system (13).

Although the rat has been used for many studies on the infertility action of gossypol, no studies are available on the pharmacokinetics of gossypol in rats. This study was carried out, using a specific high-performance liquid chromatographic method (14–16), to determine the pharmacokinetics, clearance, and volume of distribution of gossypol following a single or daily oral dose of 10 mg/kg body weight for 14 days and single intravenous injection of 10 mg/kg body weight in male Sprague–Dawley rats.

Materials and Methods. *Chemicals.* Analytical grade (99.9%) racemic gossypol (in the form of gossypol acetic acid, Batch No. CDB-1371 G) was obtained from WHO Gossypol Research Program, World Health Organization (Geneva, Switzerland), certified A.C.S. Gossypol was stored in a freezer at −20C. Polyethylene glycol 400 and Cremophor EL were obtained from Sigma Chemical Co. (St. Louis, MO). Disodium ethylenediamine-tetraacetate (Na_2-EDTA) was obtained from Fisher Scientific Co. (Raleigh, NC). All solvents used were HPLC

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grade and were purchased from Fisher Scientific (Raleigh, NC). Other chemicals used in this study were obtained in the highest purity available.

Animals. Male Sprague–Dawley rats weighing 250–300 g were obtained from Harlan–Sprague–Dawley (Indianapolis, IN). They were housed in temperature-controlled (21–23°C) rooms with a 12-hr light/dark cycle before and during the study. The animals were supplied with feed (Rodent Laboratory Chow, Ralston Purina Co., St. Louis, MO) and water *ad lib.*

Treatment of animals: Intravenous injection. (±)-Gossypol acetic acid (500 mg) was dissolved in 20 ml polyethylene glycol 400, and 3 ml cremophor-El was added as described previously (17). Then, the solution was filtered and distilled water was added to a volume of 100 ml. The final solution was checked for concentration by HPLC. The intravenous injection was given in the tail vein using a plastic syringe attached to a butterfly (size 25 × 3/4) with 12 in. tubing. Groups of 4 rats were injected with a single 10 mg dose of (±)-gossypol (2.0 ml)/kg body weight. Blood samples were withdrawn from the tail vein at time intervals of 5, 10, 15, 20, 25, and 30 min, 1, 2, 4, 8, 12, and 24 hr after injection.

Oral administration. (±)-Gossypol acetic acid was dissolved in corn oil (10 mg gossypol/ml corn oil). Oral dosage was carried out using a plastic syringe attached to a curved, stainless steel animal intubation needle with a spherical ball tip, size 20 g × $1\frac{1}{2}$ in. (Popper and Sons, Inc., New Hyde Park, NJ). For the single oral dose, groups of four rats were treated with a 10 mg of (±)-gossypol (1.0 ml)/kg dose. Blood was collected from tail vein at 1, 2, 4, 6, 8, 12, 16, 24, and 48 hr after treatment. For the repeated doses, groups of four rats were treated daily with 10 mg/(±)-gossypol (1.0 ml)/kg for 14 days, and blood was withdrawn from tail vein at 4, 8, 12, 24, 72, 96, 168, and 240 hr after the last dose.

Separation of blood plasma. At each time interval, the collected blood samples were centrifuged at 2000*g* for 4 min at 5°C to separate red blood cells from plasma.

Extraction of gossypol. To a test tube containing 150 μl plasma, 150 μl of ethanol and 1 ml of saturated Na_2-EDTA were added. The mixture was vortexed at a speed setting of 6 for 1 min. The mixture was then transferred to a 10-ml MIXXOR separatory cylinder for liquid–liquid extraction (Xydex Corporation, Bedford, MA) and extracted with 2 ml ether. The ether layer was separated and evaporated to dryness by a gentle blowing of nitrogen. The residue was redissolved in 100 μl acetonitrile, and 10 μl was injected in the HPLC. Retention time and peak area were measured for each component. Recovery was determined by adding 60 μg of pure gossypol to 1 ml of plasma; 150 μl was sampled, mixed, extracted, and analyzed similar to that described for plasma from gossypol-treated animals. A minimum of four replications was performed for each sample analysis and recovery determination.

HPLC. The HPLC system consisted of a Model 660 solvent programmer equipped with two Model 6000A pumps, a Model 440 ultraviolet detector, a U-6K injector, and an RCM-100 radial compression separation system with C_{18} cartridge (Waters Associates, Inc., Milford, MA), a guard column filled with C_{18} μBondapak (Merck, Darmstadt, Germany), a recorder (Fisher Recordall, Series 5000), and a chromatopac EIA data processor (Shimadzu Seisakusho, Ltd., Kyoto, Japan). The retention time, peak areas, and percentage of each peak in the chromatogram were measured by the data processor. Millipore membrane filters, type HA or FH, pore size 0.45 μm (Millipore, Bedford, MA), were used to filter the solvents. Plasma extracts were injected in 10 μl vol and eluted from the column isocratically by acetonitrile:water:acetic acid (7:2:1) at a solvent flow rate of 1.5 ml/min. The uv absorbance of the column elutes monitored at 254 nm was used to detect and quantify gossypol concentration. A standard curve was obtained by injecting known amounts of the standard gossypol and measuring the peak area (15, 16).

Kinetic analysis. The kinetic analysis of gossypol concentration in plasma was performed using a VAX-11/785 computer (Digital Equipment Co., Maynard, MA). Pharmacokinetic analysis of plasma concentration as a function of time was carried out by fitting the curve using nonlinear least-squares regression analysis by means of a

CONSAM modeling package (18). The terminal half-life of gossypol was calculated from the elimination rate constant, β, which was obtained by linear regression of the terminal linear exponential decline in gossypol concentration using the formula:

$$t_{1/2} = \frac{0.693}{\beta}. \quad [1]$$

The total area under the gossypol concentration vs time curves for plasma (AUC_{plasma}) was calculated by the trapezoidal rule and extrapolated to infinity by using the last data point and the respective terminal linear exponential decline (19). The pharmacokinetic parameters, total clearance (Cl), and apparent volume of distribution (V_d) area, for intravenous administration, were determined using the following equations where D is the dose (mg/rat):

$$Cl = \frac{D}{AUC_{plasma}} \quad [2]$$

$$V_d \text{ area} = \frac{D}{\beta(AUC)_{plasma}} = \frac{Cl}{\beta}. \quad [3]$$

The oral bioavailability was calculated as follows (17):

$$\text{Bioavailability} = \frac{AUCpo}{AUCiv} \times \frac{Div}{Dpo}. \quad [4]$$

Statistics. The values obtained were a means ± SEM of four animals at each time point. Comparison of pharmacokinetic models was carried out using analysis of variance to determine the best fit model. Significant differences between groups were determined using Fisher's least significant difference test. A P value of less than 0.05 was considered significant.

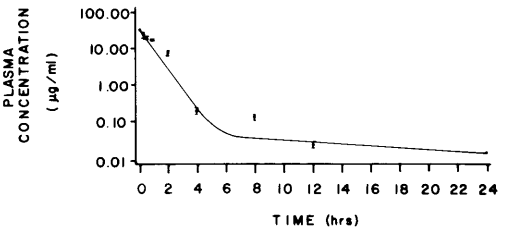

FIG. 1. Plasma concentrations of (±)-gossypol in Sprague–Dawley rats receiving a single intravenous 10 mg/kg dose. The data points are the mean ± SEM of four rats per point.

TABLE I. PHARMACOKINETIC PARAMETERS (MEAN ± SEM) FOR (±)-GOSSYPOL IN MALE SPRAGUE–DAWLEY RATS (n = 4 PER POINT) AFTER A SINGLE INTRAVENOUS DOSE OF 10 mg/kg

Parameter	Value (mean ± SEM)
P, μg/ml	169.31 ± 18.21
A, μg/ml	37.33 ± 4.52
B, μg/ml	0.05 ± 0.007
π, hr^{-1}	4.93 ± 0.41
α, hr^{-1}	1.34 ± 0.149
β, hr^{-1}	0.06 ± 0.007
$t_{1/2\pi}$, hr	0.14 ± 0.016
$t_{1/2\alpha}$, hr	0.52 ± 0.057
$t_{1/2\beta}$, hr	11.44 ± 1.152
k_{12}, hr^{-1}	0.96 ± 0.110
k_{21}, hr^{-1}	1.99 ± 0.201
k_{13}, hr^{-1}	0.04 ± 0.005
k_{31}, hr^{-1}	0.06 ± 0.007
k_{10}, hr^{-1}	3.29 ± 0.351
k_{21}/k_{12}	2.04 ± 0.210
k_{31}/k_{13}	1.50 ± 0.161
α/k_{10}	0.02 ± 0.003
$AUC_{0\to\infty}$, mg·hr/liter	63.09 ± 6.57
Cl, liter/hr/kg	0.16 ± 0.021
V_d, liter/kg	0.05 ± 0.006

Results. *Bioavailability of a single oral dose of (±)-Gossypol.* Bioavailability of (±)-gossypol was determined following oral and intravenous administration of a single 10 mg/kg dose. The oral bioavailability was determined to be 60% according to Eq. [4].

Single intravenous administration of (±)-gossypol. Following the intravenous administration of a 10 mg/kg dose of (±)-gossypol, a triexponential blood level was observed as shown in Fig. 1. The pharmacokinetic profile of (±)-gossypol was determined and is summarized in Table I. Analysis of variance comparing a two-compartment vs a three-compartment model statistically demonstrated that the data best fit a three-compartment, open-model system (20). The time course of (±)-gossypol concentration in plasma (C_p) is described by the following equation:

$$C_p = 169.31e^{-4.93t} + 37.33e^{-1.34t} + 0.05e^{-0.06t}. \quad [5]$$

The rate constants associated with the rapidly (π) and slowly (α) equilibrating sites in

the peripheral compartments were 4.93 and 1.34 hr^{-1}, respectively. These values correspond to apparent half-lives of 0.14 and 0.52 hr, respectively. The overall elimination rate constant, β, was 0.06 hr^{-1}, corresponding to an apparent half-life of 11.44 hr. The total plasma clearance (Cl) and volume of distribution (V_d) of (±)-gossypol in the rat were 0.16 liter/hr/kg and 0.05 liter/kg, respectively. The $AUC_{0\to\infty}$ was 63.01 mg·hr/liter.

Single oral administration of (±)-gossypol. Mean plasma (±)-gossypol concentrations after a single oral 10 mg/kg dose as a function of time are shown in Fig. 2. The pharmacokinetic profile of (±)-gossypol in the rat is summarized in Table II. Following oral administration of (±)-gossypol, its concentration reached a peak level of 3.6 μg/ml plasma 6 hr after dosing, indicating rapid absorption. (±)-Gossypol concentration in plasma (C_p) then declined biphasically according to the following equation:

$$C_p = 80.53e^{-0.23t} + 0.07e^{-0.01t}. \quad [6]$$

Terminal phase or the "apparent" elimination phase had a rate constant, β, of 0.01 hr^{-1}, corresponding to a half-life ($t_{1/2\beta}$) of 64.76 hr for elimination of (±)-gossypol. The $AUC_{0\to\infty}$ was 37.21 mg·hr/liter.

Daily oral administration of (±)-gossypol. A 10 mg/kg oral dose of (±)-gossypol was administered to rats for 14 consecutive days. Blood was obtained at varying time intervals after the last dose and analyzed for (±)-gossypol. The change in plasma concentration (C_p) with time is presented in Fig. 3. The pharmacokinetic parameters for daily oral

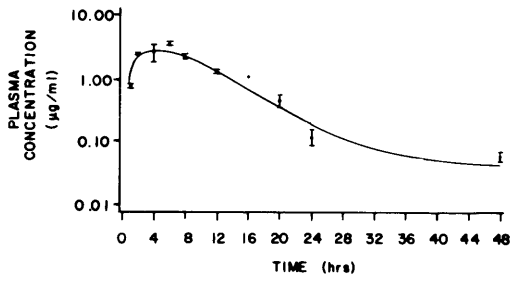

FIG. 2. Plasma concentrations of (±)-gossypol in Sprague–Dawley rats receiving a single 10 μg/kg oral dose. The data points are the mean ± SEM of four rats per point.

TABLE II. PHARMACOKINETIC PARAMETERS (MEAN ± SEM) FOR (±)-GOSSYPOL IN MALE SPRAGUE–DAWLEY RATS (n = 4 PER POINT) AFTER A SINGLE OR 14 DAILY ORAL 10 mg/kg DOSES

Parameter	Single dose (mean ± SEM)	Daily dosing (mean ± SEM)
Peak plasma concentration, μg/ml	3.60 ± 0.40	10.29 ± 1.15
A, μg/ml	80.53 ± 8.16	82.61 ± 8.52
B, μg/ml	0.07 ± 0.008	5.53 ± 0.57
α, hr^{-1}	0.23 ± 0.028	0.35 ± 0.04
β, hr^{-1}	0.01 ± 0.001	0.007 ± 0.001
$t_{1/2\alpha}$, hr	3.02 ± 0.315	1.98 ± 0.20
$t_{1/2\beta}$, hr	63.76 ± 6.41	101.91 ± 11.21
k_{12}, hr^{-1}	0.005 ± 0.001	0.47 ± 0.05
k_{21}, hr^{-1}	0.01 ± 0.001	0.03 ± 0.004
k_{10}, hr^{-1}	0.23 ± 0.031	0.08 ± 0.009
k_{21}/k_{12}	2.00 ± 0.219	0.06 ± 0.007
AUC, mg·hr/liter	37.21 ± 3.81[a]	851.04 ± 87.21[b]

[a] $AUC_{0\to\infty}$.
[b] $AUC_{0\to24}$.

administration of (±)-gossypol are summarized in Table II. Following the oral administration of 14 daily doses of (±)-gossypol, a biexponential plasma level was observed. The concentration of (±)-gossypol in plasma (C_p) reached a peak of 10.29 μg/ml at 8 hr after administration of the last dose, then declined biexponentially according to the following equation:

$$C_p = 82.61e^{-0.35t} + 5.53e^{-0.007t}. \quad [7]$$

The apparent elimination rate constant, β, of the terminal phase was 0.007 hr^{-1}, corresponding to a half-life value of 101.91 hr for elimination of (±)-gossypol. The $AUC_{0\to24}$ was 851.04 mg·hr/liter.

Discussion. The oral bioavailability of (±)-gossypol in rats was determined to be 60%. This value represents the fraction of the administered oral dose which was absorbed and reached the systemic circulation. This high value reflects high absorption from the gastrointestinal tract following ingestion of (±)-gossypol.

The results suggest that the pharmacokinetic evaluation, following an intravenous injection of (±)-gossypol, would require minimally a three-compartment open-model system (20). The values of the three hybrid rate constants, π, α, and β, reflect a rapid

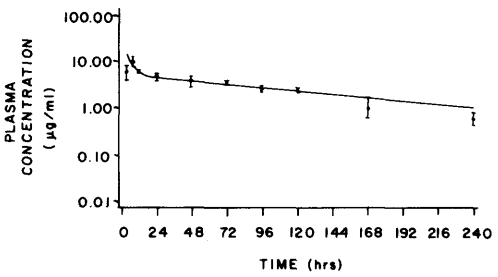

FIG. 3. Plasma concentrations of (±)-gossypol in Sprague–Dawley rats after receiving 14 daily oral doses of 10 mg/kg. The data points are the means ± SEM of four rats per point.

initial distribution of (±)-gossypol and a slower apparent elimination rate of the chemical from the body.

The individual rate constants, k_{12}, k_{21}, and k_{13}, k_{31}, reflect the rate of distribution into and out of the "shallow" and "deep" peripheral compartments (20). The ratio k_{21}/k_{12} of 2.04 suggests rapid equilibrium and relatively free transfer of (±)-gossypol between the central and the shallow compartments. The ratio k_{31}/k_{13} of 1.5 suggests slower equilibration and slower return of the chemical from the deep peripheral compartment to the central compartment when compared to the shallow compartment.

The rate constant k_{13}, which reflects the rate of entry of (±)-gossypol from the central compartment to the deep peripheral compartment, had the smallest magnitude. This result suggests that k_{13} is the rate-controlling step in the elimination of (±)-gossypol from the body. The ratio β/k_{10} of 0.02 indicates that only 2% of the dose in the body localizes in the central compartment and is available for elimination at any time.

Overall, the pharmacokinetic profile of an intravenous injection of gossypol indicates that k_{13} and k_{31}, the rates of entry into and return of (±)-gossypol from the deep peripheral compartment, respectively, and not k_{10}, the true elimination rate constant, are the rate-limiting factors in the elimination of the chemical from the body. Therefore, changes in the binding characteristics of (±)-gossypol to tissues or proteins within this compartment may markedly alter its pharmacokinetic profile. On the other hand, factors that affect the metabolism per se (e.g., in the liver) may only minimally affect this pharmacokinetic profile.

Our previous studies have demonstrated that (±)-gossypol is extensively protein bound in the rat (11). Certain tissues, i.e., heart, liver, kidney, spleen, lung, and digestive tract, showed large and rapid uptake of radioactivity following the oral administration of ^{14}C-labeled (±)-gossypol in rats. These tissues, in turn, showed a slow decline of ^{14}C. The reported extensive protein binding and the slow release of (±)-gossypol from these tissues are consistent with the deep peripheral compartment of the proposed pharmacokinetic model.

Although there was a rapid uptake of radioactivity by the adipose tissues following oral administration of ^{14}C-labeled (±)-gossypol, there was a corresponding rapid decline of ^{14}C in these tissues, suggesting that this compound is not strongly bound to adipose tissues (11). Adipose tissues may represent the shallow compartment depicted in the pharmacokinetic profile.

It is interesting to compare the pharmacokinetic data for a single oral 10 mg/kg dose of (±)-gossypol with those following 14 daily oral doses in male rats. The single oral dose was eliminated at a rate constant approximately five times faster than that of the daily administered chemicals. After reaching maximum concentration, (±)-gossypol level in plasma declined rapidly following a single administration. In contrast, (±)-gossypol plasma concentration decreased at a slower rate constant after daily oral dosing. These results might be attributed to redistribution of (±)-gossypol from blood into tissues, a phenomenon consistent with the protein binding capacity of (±)-gossypol. The slow release of (±)-gossypol from such protein and tissue binding sites probably determines the rate of metabolism and, therefore, elimination of the chemical from the body.

Our conclusion that a single oral 10 mg/kg dose of (±)-gossypol is rapidly eliminated from the rat with a $t_{1/2\beta}$ of 64.76 hr differed from the results that $t_{1/2\beta}$ of a single oral 20 mg/kg (±)-gossypol were 286 hr and 74.5 hr in man and dog, respectively (17). These results might be explained by the inherent high drug-metabolizing ability of the rat com-

pared with that of humans (21) as indicated by the high contents of hepatic microsomal cytochrome P-450 (nmole/mg protein) in rats of 0.84 (22) compared to 0.28 (23) in humans.

It is noteworthy that of the two enantiomers of gossypol the (−)-gossypol, which is the active reproductive toxicant, is nonaccumulative and has a much shorter half-life in humans and dogs than that of the less biologically active (+)-gossypol (17). This information combined with our results suggest that in addition to the pharmacokinetics of gossypol, other factors such as metabolic activation may also contribute to its male reproductive toxicity in humans and animals.

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