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A single dose pharmacokinetic study of 13-cis retinoic acid (Isotretinoin, Ro 04-3780) in the pregnant New Zealand rabbit using intravenous infusion

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Abstract

The pharmacokinetics of 13-*cis* retinoic acid in the pregnant New Zealand rabbit were determined after intravenous administration. This drug displayed linear pharmacokinetics in this species. Noncompartmental pharmacokinetic analyses were performed on individual data sets and an average value determined for each parameter (n = 3/dose group). The mean V_{dss}, CL and $t_{1/2}$ for the 0.5 mg/kg dose group were 534.0 ± 144.8 ml/kg, 139.2 ± 41.0 ml/h/kg and 3.8 ± 0.8 h, whereas for the 5.0 mg/kg dose group these parameters were 676.3 ± 145.5 ml/kg, 169.8 ± 18.9 ml/h/kg and 4.2 ± 1.3 h, respectively. The mean data from the 5.0 mg/kg dose group was modeled to a three-compartment model with elimination occurring from the first compartment only. The pharmacokinetic parameters of AUC, CL, V_{dss}, MRT and the effective half-life calculated from compartmental analysis were similar to those obtained from noncompartmental analysis. The noncompartmental pharmacokinetic parameters determined for the rabbit were found to compare favorably with those reported for the monkey. The monkey is considered to be an appropriate specie for modelling the pharmacokinetics of 13-*cis* retinoic acid in humans. We propose that since the pharmacokinetics of 13-*cis* retinoic acid are similar between the two species and the rabbit displays great sensitivity to the teratogenic effects of 13-*cis* retinoic acid that it may be a good animal model to use to assess the risk/benefit of 13-*cis* retinoic acid therapy in humans.

Keywords: 13-*cis* retinoic acid; Intravenous infusion; Pregnant New Zealand rabbit; Pharmacokinetics

1. Introduction

Retinoids are among the most potent animal and human teratogens. Alterations in levels of retinol and other retinoids have long been known to effectively produce malformations in animal model systems (Kochhar, 1967; Creech Kraft et al., 1987; Nolan, 1989; Creech Kraft et al., 1989; Hummler et al., 1990). Inadvertent exposure during the first trimester of human pregnancy to 13-*cis* retinoic acid (isotretinoin, Accutane®, Ro 04-3780), a compound used for the treatment of severe cystic acne, produces characteristic malformations, involving craniofacial, cardiac, thymic and central nervous system in infants born

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(Lammer et al., 1985). This phenomenon, termed retinoic acid embryopathy has rekindled interest in research on retinoid teratogenicity.

The teratogenicity of 13-*cis* retinoic acid appears to be dose and species dependent (Miller et al., 1987). It is marginally teratogenic in mice at doses which produce terata in humans (Kochhar et al., 1984; Kochhar et al., 1987; Kamm et al., 1984). However, elevated multiple dosing with 13-*cis* retinoic acid in mice produces significant terata (Kochhar and Penner, 1987; Creech Kraft et al., 1991a). Transplacental pharmacokinetic studies using all-*trans* and 13-*cis* retinoic acids in mice and rats, suggest that the proximate teratogen is all-*trans* retinoic acid (Creech Kraft et al., 1987; Creech Kraft et al., 1989; Creech Kraft et al., 1991a; Gunning et al., 1993). It readily crosses the placenta and reaches the embryo at higher concentrations in comparison to 13-*cis* retinoic acid (Creech Kraft et al., 1989; Creech Kraft et al., 1991b). All-*trans* and 13-*cis* retinoic acids are interconvertible. Therefore, the teratogenicity of 13-*cis* retinoic acid is postulated to be due to its isomerization to the all-*trans* isomer (Creech Kraft et al., 1991a; Klug et al., 1989; Gunning et al., 1993). Indeed, there appears to be a direct correlation of fetal concentrations of all-*trans* retinoic acid produced upon dosing with 13-*cis* retinoic acid and teratogenicity, in rodents (Creech Kraft et al., 1991b; Klug et al., 1989; Gunning et al., 1993).

Despite their teratogenic effects, the retinoids are vitally important in human development and have therapeutic value as both dermatological and chemotherapeutic agents. Therefore, it would be advantageous to develop good animal models which can serve as a basis to evaluate human retinoid risk. The animal model demonstrating the teratogenicity of 13-*cis* retinoic acid at oral doses closest to those in humans is the cynomolgus monkey (Hummler et al., 1990). However, there are some notable differences. The main metabolites observed in the plasma of the nonpregnant cynomolgus monkey after oral dosing with 13-*cis* retinoic acid are 4-oxo-13-*cis* retinoic acid and the β-glucuronide conjugate of 13-*cis* retinoic acid (Creech Kraft et al., 1991b). Whereas, in humans, the most predominant metabolite during 13-*cis* retinoic acid therapy is 4-oxo-13-*cis* retinoic acid (Vane and Bugge, 1981). Also, although qualitatively similar, the elimination of 13-*cis* retinoic acid in the nonpregnant cynomolgus monkey is more rapid than in human, and approximately a 10-fold greater dose of 13-*cis* retinoic acid is required in the monkey to produce the AUC values comparable to the human (Creech Kraft et al., 1991b).

Even though nonhuman primates may be the best animal model to evaluate human retinoid risk, they are expensive to maintain and it is difficult to administer drug intravenously and continuously to them. Also it is difficult to perform pharmacokinetic experiments using a significant number of animals. Therefore, we chose the pregnant New Zealand rabbit as our model species. Its sensitivity to the teratogenic effects of retinoids is between that of rodents and nonhuman primates and a significant number of animals can be routinely used for pharmacokinetic studies. We chose continuous intravenous infusion as the route of administration because it is the most appropriate to maintain constant maternal plasma concentration of compound. Thus a pharmacodynamic effect, retinoid induced terata, can be ultimately related to maternal plasma concentration. Also this particular animal model system has been in use at BioResearch Laboratories in Montreal, Canada, to investigate the teratogenic and toxic potential of other compounds.

The objective of this particular study was to determine the intravenous pharmacokinetics of 13-*cis* retinoic acid and its metabolites, all-*trans*, 4-oxo-13-*cis* and 4-oxo-all-*trans* retinoic acids in the pregnant New Zealand rabbit using infusion for 1 h at two dosage levels and to determine if there was a vehicle effect on pregnancy outcome.

2. Materials and methods

2.1. Chemicals

The retinoids, 13-*cis* retinoic acid (isotretinoin, Ro 04-3780), all-*trans* retinoic acid (tretinoin, Ro 01-5488) and acitretin (Ro 10-1670) were obtained from the Quality Control Department, Hoffmann-

LaRoche Inc., Nutley, NJ. The 4-oxo metabolites of 13-*cis* and all-*trans* retinoic acids, Ro 22-6595 and Ro 12-4824, respectively, were obtained from Dr P. Sorter, Preclinical R and D Staff, Hoffmann-LaRoche, Inc., Nutley, NJ. HPLC grade acetonitrile, hexane, glacial acetic acid and 85% phosphoric acid were obtained from Fisher Scientific, Fair Lawn, NJ. Certified ACS potassium phosphate, monobasic and ammonium acetate were also obtained from Fisher Scientific. Ethanol (200 proof) was obtained from Florida Distillers Co., Lake Alfred, FL. Dulbecco's phosphate buffered salt solution (1 ×, sterile, without calcium, without magnesium) was obtained from Mediatech and distributed through Fisher Scientific, Fair Lawn, NJ. Distilled water was purified with a Milli-Q UF Plus water purification unit, Millipore Corp., Bedford, MA. Control female rabbit plasma was obtained from Pel-Freeze Clinical System, Brown-Deer, WI.

2.2. Animals

Adult female white New Zealand rabbits (*Oryctolagus cuniculus*) were obtained from HRP, Inc., Denver PA. The females used for this study were 5 months of age and weighed between 2.7 and 3.7 kg. All animals were housed individually in stainless-steel cages in a controlled environment (targeted conditions: temperature 18 ± 3°C, humidity 50 ± 20%, 12 h light, 12 h dark). The rabbits were fed PMI Laboratory Chow (No. 5322) and allowed free access to water.

Nineteen days prior to insemination all female rabbits were luteinized with an intravenous injection of 50 I.U. of human gonadotrophin. Two to 4 h prior to insemination, a second 50 I.U. dose of human chorionic gonadotrophin was administered intravenously to those females assigned to be inseminated.

Proven bucks (untreated) of the same strain and source were used to provide semen samples for artificial insemination. Sperm samples were collected and a diluted pooled sample was prepared using an isotonic saline solution. Each female was inseminated with at least 0.5 ml of the pooled sample which contained at least 12×10^7 spermatozoa/ml. The day of insemination was designed as Day 0 of gestation. Implantation of catheters into the femoral vein was performed 7 or 9 days prior to insemination. The catheter was contained in a tether system which allowed the animal freedom of movement. Where required, additional repair surgery was performed on animals prior to insemination.

2.3. Laboratory precautions

All handling of retinoids, dosing solutions and plasma samples was performed under dim yellow light.

2.4. Preparation of dosing and placebo solutions

The formulations for vehicle and 13-*cis* retinoic acid for intravenous injection were prepared by Dr S. Dixit, Pharmaceutical R and D, Hoffmann-LaRoche Inc., Nutley, NJ. The drug (6.25 mg/ml) was solubilized into a solution containing alcohol, Tween 80, propylene glycol, sodium hydroxide and sodium metabisulfite. The vehicle consisted of sodium metabisulfite, dextrose, Tween 80 and water. All formulations met standards for sterility and pyrogen content before shipment to BioResearch Laboratories, Montreal, Canada. Dosing solutions of the drug, 13-*cis* retinoic acid, were formulated immediately prior to the experiment by dilution of the drug solution with the appropriate amount of vehicle.

2.5. Groups and treatment

Artificially inseminated white New Zealand rabbits were divided into the following groups: one saline control group of six females (treated from days 7 to 18 of gestation), a vehicle control group of nine females (three treated for 24 h and six from days 7 to 18 of gestation) and two parallel treatment groups of three females each (treated on gestation day 10). All animals in the treatment groups were continuously infused for 1 h at dosage levels of either 0.50 or 5.0 mg/kg. Drug, saline or vehicle was continuously delivered via a catheter which had been surgically inserted into the femoral vein.

2.6. Pharmacokinetic sampling

Blood samples (1 ml) were obtained from the artery of the ear via an indwelling heparinized Abbocath®. For all treated animals, samples were obtained at: 0, 15, 30, 60, 65, 70, 75, 90 and 105 min and 2, 2.5, 4, 6, 8, 12, 16 and 24 h after the start of infusion. Several blood samples were also obtained from vehicle control animals which served to provide the background control. The blood samples were centrifuged at 3000 × *g* for 10 min at 8°C and the plasma stored at −70°C until analysis.

2.7. Pathological observations and clinical tests

In order to assess the suitability of the vehicle the following examinations and tests were performed on both saline and vehicle control groups (treated days 7–18 of gestation): routine observation for mortality or abnormal condition; body weights at various times during the experiment; routine hematology and clinical chemistry tests. On day 29 of gestation the animals in both control groups were sacrificed and given a complete gross pathological examination. Also the uterine contents were examined and the number and position of live fetuses, dead fetuses, early, middle and late resorptions and empty implantation sites were recorded. Treated animals were sacrificed at the conclusion of the experiment only to determine pregnancy.

2.8. Calibration standards and sample preparation

Rabbit plasma was not used to generate the calibration curve since all analytes are endogenous in rabbit plasma. Therefore, control female rabbit plasma was diluted to 5% with phosphate buffered saline in order to eliminate the endogenous retinoid peaks in the chromatograms. Calibration standards were prepared by adding various concentrations of the analytes (13-*cis*, all-*trans*, 4-oxo-13-*cis* and 4-oxo-all-*trans* retinoic acids) to 0.5 ml of 5% rabbit plasma in phosphate buffered saline. Internal standard (Ro 10-1670) was added to calibration standards, quality control and experimental samples. The limit of quantitation was 1 ng/ml for each analyte using 0.5 ml of rabbit plasma.

2.9. Extraction

After the addition of ethanol (0.25 ml) to precipitate the proteins, all samples (calibration standards, quality control and experimental) were acidified by the addition of 1 M potasium phosphate, pH 3.5 (0.5 ml). The retinoids were then extracted into hexane (0.5 ml; × 3). The combined hexane extracts were evaporated to dryness under a constant stream of nitrogen and then reconstituted into 100 μl of modified mobile phase for reverse phase HPLC analysis.

2.10. HPLC analysis

Sample (45-μl aliquot) was injected onto a 4.6 mm I.D. × 25 cm Zorbax ODS C18 (5-μ particle) column maintained at 45°C. The retinoids were separated using a step gradient consisting of two mobile phases. Mobile phase A consisted of 10% ammonium acetate, water, acetonitrile and glacial acetic acid in a ratio of: 0.39:45.5:51.2:2.9, v/v/v/v. Mobile phase B contained the same components but in a ratio of: 0.4:14.5:84.2:1.0, v/v/v/v. The initial conditions were 80% A and 20% B which were maintained for 6 min. At 13 min, the proportion of B was increased to 100% and maintained for 2 min. Ten minutes later, mobile phase A was increased to 100% and maintained for 15 min before reequilibration prior to the next injection. The total run time was 35 min. The retinoids were detected by UV absorbance at 365 nm. Typical retention times were 12.8, 14.2, 22.4 and 23.8 min. for 4-oxo-all-*trans*, 4-oxo-13-*cis*, 13-*cis* and all-*trans* retinoic acids, respectively. The internal standard, acitretin, had a retention time of 18.9 min. Analysis of the data from the calibration curve and quality control samples showed an overall inter-assay precision of 4.0, 5.0, 4.8 and 3.5% and an overall intra-assay precision of 5.0, 6.0, 7.0 and 6.0% for 13-*cis*, all-*trans*, 4-oxo-13-*cis* and 4-oxo-all-*trans* retinoic acids, respectively. The correlation coefficient across all calibration curves was ≥ 0.98.

2.11. Pharmacokinetic analysis

2.11.1. Noncompartmental pharmacokinetic analysis

Noncompartmental pharmacokinetic analyses were performed with the # PK software. # PK is an RPL program written by PennCorp, Inc. (Ardmore, PA), designed specifically to perform automated noncompartmental pharmacokinetic analysis. All pharmacokinetic analyses were performed on individual data sets and then an average value obtained. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated by the linear trapezoidal rule from time zero to the time of last measurable concentration (C_f) and extrapolated to infinity by adding the quantity of C_f/k, where k is the apparent terminal elimination rate constant. The terminal half-life ($t_{1/2}$) was derived from $\ln(2)/k$. The clearance (CL) was derived from Dose/$AUC_{0-\infty}$. The mean residence time (MRT) was calculated using the formula:

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}} - \frac{\text{Infusion time}}{2}$$

where AUMC is the area under the moment curve from time zero to infinity. The $AUMC_{0-\infty}$ was calculated by log-linear extrapolation from the last measurable drug concentration using $\ln(2)/t_{1/2}$, estimated by un-weighted linear regression of the log-transformed data over the terminal phase of the plasma concentration versus time profile. The volume of distribution at steady state (V_{dss}) was derived from CL·MRT.

2.12. Compartmental pharmacokinetic analysis

Compartmental pharmacokinetic analysis was performed utilizing a nonlinear least squares regression analysis computer program (PCNONLIN, ver.4.2, SGI Software, Lexington, KY) on the mean set of data. We defined the effective half-life ($T_{1/2eff}$) as:

$$T_{1/2eff} = [f_1 \times \alpha_{1/2}] + [f_2 \times \beta_{1/2}] + [f_3 \times \gamma_{1/2}]$$

where f_1, f_2 and f_3 correspond to the fraction of the AUC which is contributed by each compartment and $\alpha_{1/2}$, $\beta_{1/2}$ and $\gamma_{1/2}$ are the half-lives of each compartment. These parameters were obtained from compartmental analysis.

3. Results and discussion

No significant differences were observed in the clinical findings between saline and vehicle control animals. Also, all pathological findings from the vehicle control group, except for an increase in post-implantation loss, were not significantly different from the saline control group. This increase may be unrelated to treatment with the vehicle as the total number of resorptions was not clearly increased over historical values. There was no evidence of fetotoxicity or external malformations in pregnant rabbits infused with vehicle. These observations, taken in total, suggest that the vehicle had no effect on pregnancy or the outcome of pregnancy. Of the six animals administered drug, only one female was not pregnant during treatment. This female was from the low dose group. This animal was included for pharmacokinetic analysis with the other pregnant females after concluding that the kinetic values obtained from the nonpregnant female did not compromise the results of this dose group.

The plasma concentration versus time profiles of 13-*cis* retinoic acid in the pregnant New Zealand rabbit after intravenous infusion for 1 h at two dosages are presented in Fig. 1. The resulting curves are atypical of intravenous infusion in that plasma concentrations of 13-*cis* retinoic acid were observed to increase for approximately 15 min post-infusion. This phenomenon was attributed to the fact that animals received drug via the femoral vein, but samples for pharmacokinetic analyses were obtained from an ear artery. Thus the observed lag in C_{MAX} is most likely due to the time for drug to travel from venous to arterial circulation (Zhi et al., 1994). Therefore, for both noncompartmental and compartmental analysis, the end of the infusion period was taken to be 1.25 h instead of 1 h. Both noncompartmental and compartmental pharmacokinetic parameters for 13-*cis* retinoic acid were derived from the data. These calculations are summarized in Table 1 and Table 2. The AUC values presented in

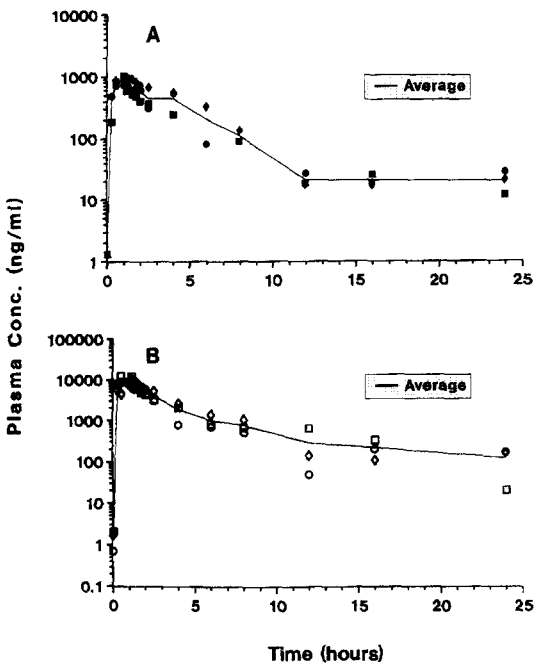

Fig. 1. Plasma concentrations of 13-*cis* retinoic acid (RA) in pregnant New Zealamd rabbits infused for 1 h with 13-*cis* RA at (A) 0.5 mg/kg and (B) 5.0 mg/kg. The solid line represents the average plasma concentration. Each symbol represents the results from an individual rabbit.

Tables 1 and 2 have been extrapolated to infinity. The percent AUC extrapolated for any single animal was less than 5% for noncompartmental analysis.

Table 1
Noncompartmental pharmacokinetic parameters for 13-cis retinoic acid in the pregnant New Zealand rabbit (n = 3/dose group) after intravenous infusion

Parameter	Dose (mg/kg)	
	0.5	5.0
$AUC_{0-\infty}$(ng·h/ml)	3857.4 ± 1350.0	29683.0 ± 3119.3
MRT (h)	3.9 ± 0.8	3.9 ± 0.5
CL (ml/h/kg)	139.2 ± 41.0	169.8 ± 18.9
V_{dss} (ml/kg)	534.0 ± 144.8	676.3 ± 145.5
$t_{1/2}$ (h)	3.8 ± 0.8	4.2 ± 1.3

$AUC_{0-\infty}$, area under the plasma concentration time curve; MRT, mean residence time; CL, clearance; V_{dss}, volume of distribution at steady state; $t_{1/2}$, half-life.

Table 2
Compartmental pharmacokinetic parameters for 13-cis retinoic acid in the pregnant New Zealand rabbit after intravenous infusion

Parameter	Value
Dose (mg/kg)	5.0
$AUC_{0-\infty}$(ng·h/ml)	29470.9
MRT (h)	5.0
CL (ml/h/kg)	171.6
V_{dss} (ml/kg)	857.7
$\alpha_{1/2}$ (h)	0.03
$\beta_{1/2}$ (h)	1.4
$\gamma_{1/2}$ (h)	9.3
$T_{1/2eff}$ (h)	3.7

$AUC_{0-\infty}$, area under the plasma concentration time curve; MRT, mean residence time; CL, clearance; V_{dss}, volume of distribution at steady state; $\alpha_{1/2}$, $\beta_{1/2}$, $\gamma_{1/2}$ are the half-lives of the first, second and third compartments; $T_{1/2eff}$, effective half-life.

The 13-*cis* retinoic acid plasma concentration-time profile during and after infusion of 13-*cis* retinoic acid was described by a three-compartment model with a constant intravenous infusion, for which elimination occurs only from the central compartment. Only the data from the 5.0 mg/kg dose group modelled well. The data from the 0.5 mg/kg dose group could not be modelled to either a two- or three-compartment model. The estimated error of the pharmacokinetic parameters obtained from modelling for the low dose group greatly exceeded the calculated parameter. Consequently, no confidence could be placed on the pharmacokinetic parameters determined after modelling for the 0.5 mg/kg dose group. Also the plasma concentration-time profile for this dose group tended to 'flatten out' at the latter time points. This phenomenon made it difficult to fit this data to any model. However, it does suggest that a longer sampling time of 24 h is necessary to model the 0.5 mg/kg dose group.

There is, in general, good agreement between the calculated compartmental and noncompartmental pharmacokinetic parameters of AUC, CL, V_{dss} and MRT for the 5.0 mg/kg dose group. Also the calculated effective half-life, $T_{1/2eff}$, is in good agreement with the half-life obtained from non-

compartmental analysis. Therefore, the model chosen appears to be appropriate.

It is readily observed from the data that the AUC for 13-*cis* retinoic acid increases with increasing dose after intravenous infusion in the pregnant New Zealand rabbit and this increase is nearly proportional to the dose. A 10-fold increase in dose produced an eight-fold increase in AUC. All other noncompartmental pharmacokinetic parameters calculated for 13-*cis* retinoic acid were dose-independent. Dose-independent pharmacokinetics for 13-*cis* retinoic acid were also observed in the nonpregnant cynomolgus monkey after bolus intravenous administration of drug (Sandberg et al., 1994). However, after bolus intravenous administration of 13-*cis* retinoic acid in the rat, nonlinear pharmacokinetics were observed (Nankervis et al., 1994). The AUC demonstrated a non-proportional increase with increasing dose. The non-proportional increase in AUC resulted from both a decrease in the total body clearance and the volume of distribution at steady state for the higher dose. In humans, linear pharmacokinetics are observed after oral administration of 13-*cis* retinoic acid (Kerr et al., 1982). There is no known data in the literature describing the intravenous pharmacokinetics of 13-*cis* retinoic acid in humans.

The noncompartmental pharmacokinetic parameters of half-life, clearance and volume of distribution at steady state of 13-*cis* retinoic acid in the pregnant New Zealand rabbit were determined to be 4 h, 154.5 ml/h/kg, and 624.5 ml/kg, respectively. These values are quite comparable to those observed for both the monkey and the dog after bolus intravenous administration of drug (Sandberg et al., 1994; Cotler et al., 1984). However, for the rat these values, as noted in the preceding paragraph, were dose dependent. The clearance and volume of distribution at steady state decreased with increasing dose while the half-life increased with increased dose (Nankervis et al., 1994). The half-life, in humans, after oral administration of 13-*cis* retinoic acid averages 13.6 h (Colburn et al., 1983).

The model obtained from compartmental analysis can be used to predict which infusion regimen could be used to obtain a desired steady state plasma concentration of 13-*cis* retinoic acid. A simulation was performed using this model to predict what infusion regimen would be needed to produce steady state concentration of 11 ng/ml of 13-*cis* retinoic acid. (This value is approximately 10 ng/ml above the pre-dose plasma concentrations observed from this study.) The simulation predicts that an infusion regimen of 1.86 μg/h/kg will result in a steady state concentration of 11 ng/ml in 15 half-lives.

After continuous intravenous administration of 13-*cis* retinoic acid to pregnant New Zealand rabbits the metabolites, all-*trans*, 4-oxo-13-*cis* and 4-oxo-all-*trans* retinoic acids were observed in the plasma. The plasma concentration time curves for these metabolites are presented in Fig. 2. The most predominant metabolite observed based upon AUC was 4-oxo-13-*cis* retinoic acid (Table 3). The AUC of 4-oxo-13-*cis* retinoic acid was 20-fold greater than the AUC for both all-*trans* and 4-oxo-all-*trans* retinoic acids. Upon a 10-fold increase in dose of 13-*cis* retinoic acid, the AUC of all metabolites measured also increased 10-fold.

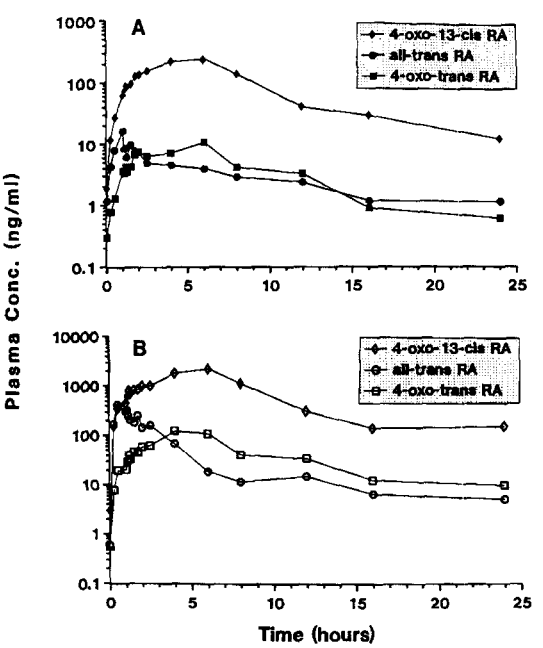

Fig. 2. Average plasma concentrations of the metabolites of 13-*cis* RA in pregnant New Zealand rabbits (n = 3/dose group) after 1 h infusion of 13-*cis* RA at (A) 0.5 mg/kg and (B) 5.0 mg/kg.

Table 3
AUC (ng·h/ml) of metabolites after intravenous infusion (the values in parentheses are the ratios of $AUC_{(m)}/AUC_{(p)}$)

	Dose (mg/kg)	
	0.5	5.0
All-*trans* retinoic acid	82.6 ± 18.5 (0.02)	1019.6 ± 619.2 (0.03)
4-oxo-13-*cis* retinoic acid	1927.6 ± 827.3 (0.50)	16903.1 ± 4660.8 (0.57)
4-oxo-all-*trans* retinoic acid	82.3 ± 27.4 (0.02)	1020.2 ± 666.7 (0.03)

$AUC_{(m)}/AUC_{(p)}$, ratio of the area under the plasma concentration curve of metabolite to the area under the plasma concentration curve for 13-cis retinoic acid (n = 3/dose group).

A half-life was determined from the terminal phase of the plasma concentration versus time curves for all metabolites measured. The values calculated were comparable to that determined for the parent drug. Therefore, it is concluded that the rate of drug elimination is the rate limiting step for metabolite elimination.

The ratio of $AUC_{(m)}/AUC_{(p)}$ for all metabolites measured was also determined (Table 3). The ratio was less than one in all instances. This data suggests that the clearance of elimination for these metabolites is greater than the formation clearance. This is especially true for all-*trans* and 4-oxo-all-*trans* retinoic acids. For both of these metabolites the elimination clearance is estimated to be 50-fold greater than its corresponding formation clearance.

The pharmacokinetics of 13-*cis* retinoic acid has been determined in the pregnant New Zealand rabbit after intravenous infusion. Preliminary inspection of the data suggests that the pharmacokinetic behavior of 13-*cis* retinoic acid is similar in the rabbit and monkey. The monkey is believed to be the best animal model to predict the pharmacokinetics of 13-*cis* retinoic acid in humans. We conclude here that the rabbit may also be a good model to assess the risk/benefit of 13-*cis* retinoic acid therapy in humans.

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References

Colburn, W.A., Vane, F.M. and Shorter, H.J., Pharmacokinetics of isotretinoin and its major blood metabolite following a single oral dose to man. *Eur. J. Clin. Pharmacol.*, 24 (1983) 689–694.

Cotler, S., Chen, S., Macasieb, T. and Colburn, W.A., Effect of route of administration and biliary excretion on the pharmacokinetics of isotretinoin in the dog. *Drug Metabol. Dispos.*, 12 (1984) 143–147.

Creech Kraft, J., Eckhoff, C., Kochhar, D.M., Bochert, G. and Nau, H., Isotretinoin (13-*cis* retinoic acid) metabolism, cis-*trans* isomerization, glucuronidation and transfer to the mouse embryo: consequences for teratogenicity. *Teratogen. Carcinog. Mutagen.*, 11 (1991a) 21–30.

Creech Kraft, J., Kochhar, D.M., Scott, W.J. and Nau, H., Low teratogenicity of 13-*cis* retinoic acid (isotretinoin) in the mouse corresponds to low embryo concentrations during organogenesis: Comparison to the all-*trans* isomer. *Toxicol. Appl. Pharmacol.*, 87 (1987) 474–482.

Creech Kraft, J., Lofberg, B., Chahoud, I., Bochert, G. and Nau, H., Teratogenicity and placental transfer of all-*trans*, 13-*cis*, 4-oxo-all-*trans* and 4-oxo-13-*cis* retinoic acid after administration of a low oral dose during organogenesis in mice. *Toxicol. Appl. Pharmacol.*, 100 (1989) 162–176.

Creech Kraft, J., Slikker, W. Jr., Bailey, J.R., Boberts, L.G., Fischer, B., Wittfoht, W. and Nau, H., Plasma pharmacokinetics and metabolism of 13-*cis* and all-*trans* retinoic acid in the cynomolgus monkey and the identification of 13-*cis* and all-*trans* retinoyl β-glucuronides. *Drug Metab. Dispos.*, 19 (1991b) 317–324.

Gunning, D.B., Barua, A.B. and Olson, J.A., Comparative teratogenicity and metabolism of all-*trans* retinoic acid, all-*trans* retinoyl β-glucose and all-*trans* retinoyl β-glucuronide in pregnant Sprague-Dawley rats. *Teratology*, 47 (1993) 29–36.

Hummler, H., Korte, R. and Hendrickx, A.G., Induction of malformations in the cynomolgus monkey with 13-*cis* retinoic acid. *Teratology*, 42 (1990) 263–272.

Kamm, J., Achentelter, L. and Ehmann, C., Preclinical and clinical toxicology of selected retinoids. In M. Sporn, A. Roberts and D.S. Goodman (Eds.), *Retinoids*, Vol. 2, Academic Press, NY, 1984, pp. 287–326.

Kerr, I.G., Lippman, M.E., Jenkins, J. and Myers, C.E., Pharmacology of 13-*cis* retinoic acid in humans. *Cancer Res.*, 42 (1982) 2069–2073.

Klug, S., Creech Kraft, J., Wildi, E., Merker, H.J., Persaud, T.V.N., Nau, H. and Neubert, D., Influence of 13-*cis* and

all-*trans* retinoic acid on rat embryonic development in vitro: correlation with isomerization and drug transfer to the embryo. *Arch. Toxicol.*, 63 (1989) 185–192.

Kochhar, D.M., Teratogenic activity of retinoic acid. *Acta. Pathol. Microbiol. Scand.*, 70 (1967) 398–404.

Kochhar, D.M., Creech Kraft, J. and Nau, H., Teratogenicity and disposition of various retinoids in vivo and in vitro. In H. Nau and W.J. Scott (Eds.), *Pharmaceutics and Teratogenesis*, CRC Press, Boca Raton, FL, 1987, pp. 173–186.

Kochhar, D.M. and Penner, J., Developmental effects of isotretinoin and 4-oxo-isotretinoin: the role of metabolism in teratogenicity. *Teratology*, 36 (1987) 67–75.

Kochhar, D.M., Penner, J.D. and Tellone, C.I., Comparative teratogenic activities of two retinoids. Effect on palate and limb development. *Teratogen. Carcinogen. Mutagen.*, 4 (1984) 377–387.

Lammer, E.J., Chen, D.T., Hoar, R.M., Agnish, N.D., Benke, P.J., Braun, J.T., Curry, C.J., Fernhoff, P.M., Grix, A.W., Lott, I.T. Richard, J.M. and Sun, S.C., Retinoic acid embryopathy. *N. Eng. J. Med.*, 313 (1985) 837–841.

Miller, R.K., Brown, K., Cordero, J., Dayton, D., Hardin, B. and Greene, M., Teratology Society Position Paper: Recommendation for vitamin A use during pregnancy. *Teratology*, 35 (1987) 269–275.

Nankervis, R., Davis, S.S., Day, N.H. and Shaw, P.N., Studies on the intravenous pharmacokinetics of three retinoids in the rat. *Int. J. Pharm.*, 101 (1994) 249–256.

Nolan, G.A., Effect of a high systemic background level of vitamin A on the teratogenicity of all-*trans* retinoic acid given either acutely or subacutely. *Teratology*, 39 (1989) 333–339.

Sandberg, J.A., Eckhoff, C., Nau, H. and Slikker, W.Jr., Pharmacokinetics of 13-*cis*, all-*trans*, 13-*cis*-4-oxo and all-*trans*-4-oxo retinoic acid after intravenous administration in the cynomolgus monkey. *Drug Metab. Dispos.*, 22 (1994) 154–160.

Vane, F.M. and Bugge, C.L.J., Identification of 4-oxo-13-*cis* retinoic acid as the major metabolite of 13-*cis* retinoic acid in human blood. *Drug Metab. Dispos.*, 9 (1981) 515–520.

Zhi, J., Massarella, J.W., Melai, A.T., Teller, S.B., Schmitt-Muskus, J., Crews, T., Oldfield, N., Erb, R.J., Leese, P.T. and Patel, I., The pharmacokinetic-pharmacodynamic (digit symbol substitution test) relationship of flumazenil in a midazolam steady-state model in healthy volunteers. *Clin. Pharmacol. Therap.*, 56 (1994) 530–536.