Embryonic Subcellular Distribution of 13-cis- and All-trans-Retinoic Acid Indicates Differential Cytosolic/Nuclear Localization

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Isotretinoin (13-cis-retinoic acid [13CRA], Accutane®) is used for the treatment of dermatological diseases. Isotretinoin is, however, teratogenic in animals and humans. The mechanism of action of its teratogenicity is still not clearly identified. It has little or no binding properties to cytosolic retinoid-binding proteins or nuclear retinoid receptors (RAR, RXR). One hypothesis is that the teratogenicity of 2 approximately equipotent teratogenic doses of 13CRA and all-trans-retinoic acids (ATRA) could mainly be correlated to ATRA in the nuclei, where the retinoid acid receptors (RARs) are located. To test this hypothesis, female mice at gestational day 11 were treated with approximately equipotent teratogenic doses of 13-cis-retinoic acid (100 mg/kg orally) or all-trans-retinoic acid (10 mg/kg orally) and sacrificed 1 h and 4 h after administration. Embryos were homogenized and centrifuged into 4 fractions, and the purity of the fractions was tested by quantification of marker constituents for various cell compartments. We analyzed, by RP-HPLC, nuclear, mitochondrial, microsomal, and cytosolic fractions, as well as embryo homogenate and maternal plasma. After treatment with 13-cis-retinoic acid, this substance was mainly located in the nuclear fraction of the embryo (−82%), whereas all-trans-retinoic acid, after ATRA treatment, was mainly located in the cytosolic supernatant (−64%). The binding to cellular retinoid-binding protein (CRABP) may limit the access of ATRA to the nucleus, in contrast to 13CRA, which does not bind to CRABP. The concentration of ATRA in the nuclear fraction was similar after administration of either 13CRA or ATRA. The teratogenic activity of 13-cis-retinoic acid could therefore be explained by its access to the nucleus and its possible conversion to all-trans-retinoic acids, which will interact with the nuclear retinoid receptors.

Key Words: retinoid toxicity; retinoic acid; subcellular localization; mouse embryo; teratogenicity.

Isotretinoin (13-cis-retinoic acid, 13CRA, Accutane®) and its isomer, all-trans-retinoic acid (ATRA, tretinoin) are well known teratogens (Lammer et al., 1985; Nau et al., 1994; Willhite et al., 1989) and are very potent drugs in the treatment of several dermatological diseases. 13CRA is used in the oral therapy of acne and all-trans-retinoic acid is used in the topical treatment of several dermatological diseases (Vahlquist, 1994). After topical application of ATRA, endogenous ATRA levels were not significantly elevated in the plasma of humans (Buchan et al., 1994; Nau, 1993). Significant concentrations of ATRA in the plasma and embryo were reached after oral treatment in mice, rats, rabbits, and monkeys, and this finding correlated with the teratogenic outcome (for a review, see Nau et al., 1994).

ATRA has been known to bind to the cellular retinoic acid-binding proteins (CRABPs) in the cytoplasm and to activate the nuclear retinoid receptors (RARs). These RARs are mainly thought to transmit retinoid embryotoxicity via formation of RAR-RXR (retinoid-X-receptor) heterodimers, which bind to DNA and initiate transactivation of retinoid-responsive elements (RARE; for reviews, see Mangelsdorf et al., 1994; Ong et al., 1994). 13-cis-retinoic acid, however, has little or no binding properties to either CRABPs or RARs (Astrom et al., 1990).

Isomerization from all-trans-retinoic acid to retinoic acid isomers has been reported to mainly take place in microsomal fractions, as has been reported by Roberts et al. (1980) and Urbach and Rando (1994). Novel studies have shown that sulfhydryl groups (Shih et al., 1997) and glutathion S-transferases act as isomerases in isomerization of 13-cis-retinoic acid to all-trans-retinoic acid (Chen and Juchau, 1997). 13CRA-induced teratogenicity in mice was associated with isomerization to ATRA, and a significant embryonic exposure to ATRA, following exposure of teratogenic doses of 13CRA, was observed (Creech-Kraft et al., 1987, 1991). The teratogenicity of 13CRA in mice was therefore attributed to the all-trans-isomer (Creech-Kraft et al., 1987). Both retinoic acids obtain different quantitative, but similar qualitative embryotoxic effects (Creech-Kraft et al., 1989, 1991). On the other hand, levels of the isomerization product, ATRA, were very low in rat and rabbit embryos (Tzimas et al., 1995, 1996).
Pharmacokinetic and metabolic studies of 13CRA and ATRA have, therefore, provided important insight into the mechanism of retinoid embryotoxicity and have been reported intensively in different species at comparable dose levels (Creech-Kraft et al., 1987, 1989, 1991; Klug et al., 1989; Tzimas et al., 1995) showing considerable concentrations of ATRA in the embryo after treatment of the mothers with 13CRA.

The low sensitivity of 13CRA in the mouse is related to its low degree of placental transfer and its rapid elimination from the maternal circulation, mainly due to glucuronidation. In contrast, the high teratogenic potency of all-trans-retinoic acid in mice correlates with its efficient transplacental transfer (Creech-Kraft et al., 1989).

Studies in NMRI mice, after administration of 13CRA (100 mg/kg orally) or ATRA (10 mg/kg orally) at gestation day 11 (GD 11), showed a comparable teratogenic outcome (Creech-Kraft et al., 1989, 1991). These two approximately equipotent teratogenic doses of different retinoic acid derivatives led to comparable concentrations of ATRA in the embryo after administration of 13CRA or ATRA (Tzimas et al., 1995). Therefore, these dose levels were used in the present experiment to elucidate the intracellular distribution of ATRA and 13CRA after administration of approximately equipotent teratogenic doses to pregnant mice on GD 11.

Our hypothesis is that the teratogenicity of two approximately equipotent teratogenic doses of 13CRA and ATRA could be correlated to ATRA in the nuclei, where the RARs are located. Subcellular fractions from embryos of 13CRA- and ATRA-treated mice were analyzed with HPLC to clarify whether a different distribution of both retinoic acid isomers, especially of the RAR-binding ATRA, could be correlated to the induced teratogenic effects.

MATERIALS AND METHODS

Animals and treatment. NMRI mice (Han: NMRI; Fa. Harlan-Winkelmann, Germany) were kept under specific pathogen-free conditions at a constant day/night cycle (light from 9:00 A.M. to 9:00 P.M.). They received a standard pellet diet (Altromin 1324; Altromin, Lage, Germany) and tap water ad libitum. One male was caged with 3 female animals for a mating period from 6:00 to 8:00 A.M., and the following 24 h were designated day 0 of pregnancy if a vaginal plug was detected.

NMRI mice of GD 11 were administered 13CRA (100 mg/kg) or ATRA (10 mg/kg) by oral intubation. Each agent was dissolved in 25% Cremophor EL in distilled water and given as 5 ml/kg. Treated animals of each group were sacrificed 1 h and 4 h after administration under dim amber light, and the embryos were removed, weighed, and frozen prior to centrifugation and analysis of the marker constituents.

Preparation of the subcellular fractions. The embryos of each time point were prepared and fractionated by differential centrifugation, according to the methods of Harrison et al. (1987). All work concerning retinoid-containing samples was carried out under dim amber light. Blood samples were withdrawn from the retro-orbital sinus into heparinized hematocrit glass capillaries under light ether anesthesia. Maternal plasma was prepared by centrifugation (10 min at 3000 rpm and 4° C) immediately before storage. After killing the animals by cervical dislocation, plasma and embryo samples were taken and stored at −20° C before analysis or fractionation procedures.

The embryos were diluted with a 3-fold volume of 0.25 M sucrose and carefully homogenized with a glass/telfon Potter homogenizer. The resulting homogenate was centrifuged with a Sorvall SS-34 rotor in a Sorvall RC-5B refrigerated centrifuge at 1000 rpm (600 × g) for 10 min at 4° C, to yield the nuclear (NUC) fraction. The pellet was washed 3 times, and the pooled supernatant was centrifuged at 12,000 rpm (15,000 × g) for 13 min to yield the mitochondrial (MIT) fraction; this pellet was washed once and the resulting supernatants were transferred to plastic centrifuge tubes. The last centrifugation step (50,000 rpm, 100,000 × g, at 4° C, 30 min) was performed with a T647.5 rotor in a Sorvall Combi Plus centrifuge, to obtain the microsomal (MIC) fraction (pellet) and the cytosolic (CYT) fraction (supernatant). All fractions were immediately weighed and used for the determination of marker constituents as well as retinoids.

Marker constituent analyses. To test the purity of each fraction, protein and specific marker constituents were determined in each. These marker constituents are molecules that occur exclusively or mainly in the appropriate organelles. Protein was assayed by the method of Lowry et al. (1951). DNA was determined, using a method of LaBarca and Paigen (1980), by a fluorometric determination of a reaction product from diphenylamin and deoxyribose. Lactate dehydrogenase was determined with a BM/Hitachi 747 kit of Boehringer Mannheim, using the Hitachi 747 analyzer. Alkaline phosphatase was assayed with a standard test kit from Boehringer Diagnostics. NADH-cytochrome oxidase was assayed according to Gluamann and Daliner (1970). Cytochrome oxidase was determined according to Beaufay et al. (1974).

Retinoid analyses. Due to the photosensitivity of retinoids, the experimental and analytical procedures were also performed under dim amber light. Tissue samples were homogenized in a 3-fold volume of isopropanol; the supernatant obtained after centrifugation of the homogenate was submitted to solid phase extraction as described (Collins et al., 1992). If the weight of a tissue sample was below 100 mg, it was adjusted to 100 mg by addition of distilled water before isopropanol was added. The solid phase cartridges carrying the extracted retinoids were submitted to reversed phase HPLC, with detection at 340 and 356 nm. Retinoids were identified by comparison with retention times and ratios of absorbance units (340/356 nm) of reference compounds. Multilinear calibration was performed by analysis of solutions of 5% bovine serum albumin that had been spiked with known amounts of reference retinoids.

RESULTS

Distribution of the marker constituents of the several cell organelles in the fractions. A complete subcellular fractionation of 4 pooled embryo samples was performed. The distribution of protein and several marker constituents in the various cell compartments is shown in Table 1. The distribution agreed well with data previously reported by several groups (Amar-Costeesec et al., 1974; Harrison et al., 1979) using the same fractionation technique. These data show that the subcellular organelles were fairly well resolved from one another and recovered in high yield in the appropriate fraction (Table 1).

For example, the mitochondrial fraction contained more than 89% of mitochondria, as assessed by the distribution of the mitochondrial marker enzyme, cytochrome oxidase. Contamination of the mitochondrial fraction (MIT) with endoplasmatic reticulum (NADH-cytochrome-C reductase), plasma membrane (alkaline phosphatase), as well as small amounts of cytosol (lactate dehydrogenase) and DNA were present.

The most important fractions for our experiment are the nuclear (NUC) and the cytosolic (CYT) fractions. The marker
constituent for the nuclei is DNA, which occurs mainly in the nuclei, although smaller amounts are found in the mitochondrial fraction. The pattern of our fractionation shows 82% of the DNA in the nuclear fraction and 10% in the cytosolic fraction. Major contaminations of the nuclear fraction are the endoplasmatic reticulum (NADH-cytochrome-C reductase), the plasma membrane (alkaline phosphatase), and the mitochondrial (cytochrome-C-oxidase).

Eighty-eight percent of the marker constituent of the cytosol lactate dehydrogenase was located in the cytosolic fraction, with small amounts also found in the nuclear, mitochondrial, and microsomal fractions. The cytosolic fraction is slightly contaminated with endoplasmatic reticulum and DNA.

Retinoids in the plasma after administration of ATRA and 13CRA. Plasma concentrations of naturally occurring retinoids were determined by HPLC at 1 h and 4 h after treatment with either ATRA or 13CRA. One h after administration of ATRA, the plasma concentration of ATRA was 429 ng/ml, while the concentrations of the metabolites 13CRA, ATRAG (all-trans-retinoyl-glucuronide), and T4OXORA (all-trans-4-oxo-retinoic acid) were increased. Four h after the treatment with ATRA, the concentrations were: ATRA, 7.6 ng/ml; 13CRA, 6.4 ng/ml; and ATRAG, 12.6 ng/ml (Table 2).

In the plasma, the concentration of 13CRA at 1 h after 13CRA treatment was (11,297 ng/ml). The concentrations of the metabolites also found were 13-cis-retinoic acid (13CRAG; 11987 ng/ml) and ATRA (860 ng/ml). After 4 h the concentrations of 13CRA were decreased to 1378 ng/ml and the concentrations of the metabolites were 13CRAG (7574 ng/ml) and ATRA (211 ng/ml; Table 2). The concentration of ATRA (860 ng/ml) 1 h after 13CRA treatment was higher than the concentration of ATRA after ATRA treatment (429 ng/ml). Levels of the oxo-metabolite T4OXORA were much higher after ATRA treatment than after 13CRA treatment. Retinol (ROL) and retinyl palmitate (RETPAL) were increased over endogenous levels at 1 h after 13CRA treatment and 4 h after ATRA treatment. High concentrations of ATRAG after 13CRA treatment may occur more from isomerization of 13CRAG than from glucuronidation of the ATRA.

Retinoids in the embryo after maternal administration of ATRA and 13CRA. ATRA exhibits relatively high concentrations in the embryo (531 ng/ml) in comparison to the plasma (429 ng/ml), whereas 13CRA yields up to 11297 ng/ml in the plasma and just 600 ng/ml in the embryo (Table 3). The embryo-maternal plasma concentration ratio after 1 h is 1.24 for ATRA and 0.05 for 13CRA. The 13CRA concentrations were 58.0 ng/ml 1 h after 13CRA treatment and 154 ng/ml after 4 h. ATRAG could not be detected after either 13CRA or ATRA treatment. 13CRA, after ATRA treatment, was not detectable. Isomerization of 13CRA yielded 235 ng/ml ATRA.
1 h and 48.3 ng/ml 4 h after treatment with 13CRA. At both time points, T4OXORA was higher in the ATRA-treated embryos than in the 13CRA-treated embryos. ROL and RETPAL concentrations were in the same range at 1 h and 4 h after treatment with 13CRA or ATRA (Table 3).

The concentrations of ATRA in the embryo 4 h after treatment with 13CRA or ATRA yielded a comparable concentration level of 64.0 ± 7.7 ng/g after ATRA treatment or 48.3 ± 9.9 ng/g after 13CRA treatment, respectively.

Intracellular distribution of several retinoids in the subcellular fractions of mouse embryo after treatment with ATRA or 13CRA. The following values are not presented as concentrations in ng/ml but as masses in ng. The various fractions were obtained at different degrees of dilution with the added homogenization media; therefore, the concentrations of each fraction could not be compared. To obtain comparable values, the total content of the retinoids in each fraction was determined by multiplication of the amounts of each fraction with the concentration of the contained retinoids. The obtained amounts of each retinoid were presented in a compound bar chart (Fig. 1). Amounts of retinoids in the various fractions led to conclusions about the intracellular distribution of each retinoid in the various fractions of the embryo.

13CRA was mainly located in the nuclear fractions (~82%), whereas ATRA was mainly present within the cytosolic fraction. Retinol (ROL) shows an intracellular distribution pattern in the embryo comparable to ATRA; 74–83% of the retinol occurs in the cytosolic fraction and ~20% in the nuclear fraction. Retinyl palmitate (RETPAL), as an example of a retinyl-ester, was found predominantly in the nuclear fraction. Both glucuronides, all-trans-retinoyl-glucuronide (ATRAG) and 13-cis-retinoyl-glucuronide (13CRAG), were also found mainly in the nuclear fraction of the embryo and only in smaller amounts in the cytosolic fraction. All-trans-4-oxo-retinoic acid (T4OXORA) was mainly present in the cytosolic fraction and was low in the nuclear fraction.

After approximately equipotent teratogenic administration with either 10 mg/kg ATRA or 100 mg/kg 13CRA, similar amounts of ATRA were found in the nuclear fraction 4 h after

### TABLE 3

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<th>Retinoids (in ng/g) in Mouse Embryo after Treatment with 13CRA (100 mg/kg) and after Treatment with ATRA (10 mg/kg)</th>
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Note. Treated mice were killed after 1 h and 4 h; n > 3. All values in ng/g. UD, under the detection limit; NQ, non quantifiable peak; T4OXORA, all-trans-4-oxo-retinoic acid; 13CRAG, 13-cis-retinoyl-glucuronide; ATRAG, all-trans-retinoyl-glucuronide; 13CRA, 13-cis-retinoic acid; ATRA, all-trans-retinoic acid; ROL, retinol; RETPAL, retinyl palmitate.

![Subcellular distribution of retinoids in mouse embryo. The data are presented as amounts of retinoids (in ng) in each of the fractions originating from 500 mg embryos: in the NUC, nuclear fraction; CYT, cytosolic fraction; MIT, mitochondrial fraction; and MIC, microsomal fraction after treatment with 10 mg/kg ATRA (A) and (B) and after treatment with 100 mg/kg 13CRA (C) and (D). Treated mice were killed after 1 h (A) and (C) or after 4 h (B) and (D). T4OXORA, all-trans-4-oxo-retinoic acid; 13CRAG, 13-cis-retinoyl-glucuronide; ATRAG, all-trans-retinoyl-glucuronide; 13CRA, 13-cis-retinoic acid; ATRA, all-trans-retinoic acid; ROL, retinol; and RETPAL, retinyl palmitate.](http://toxsci.oxfordjournals.org/)
treatment (4.63 ng ATRA and 4.72 ng ATRA, respectively; Fig. 1).

DISCUSSION

The present study describes the intracellular distribution of 13-cis-retinoic acid and all-trans-retinoic acid and their metabolites in mouse embryo at GD 11 after approximately equiteratogenic doses of either retinoid. Marker constituents of several cell organelles in the 4 fractions studied show a fairly good resolution from one another and recovery in high yield in the appropriate fractions. The separation of the cellular compartments of the embryo was improved when compared to that of liver samples (Harrison et al., 1987).

Intracellular distribution has been calculated as amounts in each fraction, and from these data (Fig. 1), concentrations have also been estimated (Fig. 2). The percentage of intracellular composition (especially nuclei and cytosol) were estimated by area determination and volume calculation of the intra- and intercellular matrix, as well as nuclei and cytosol, from 3 major cell types of the embryo: mesenchyme, neuroepithelium, and liver cells from histology photographs of mouse embryos at GD 11. Calculation of average embryonic intercellular matrix was 44%. The 56% intracellular matrix consists of 51% nuclei and 49% cytosol (including organelles).

Accumulation of retinyl palmitate and retinoyl glucuronides in the embryo are a result of retinoid treatment of the mice as well as of the down-regulation of retinol concentrations 4 h after administration (Tzimas et al., 1995). Endogenous retinol in the mouse embryo of GD 11 has been reported with 142 ng/ml and retinoic acid with 15.7 ng/ml (Tzimas et al., 1995). The endogenous concentrations of the retinoic acids in each fraction could not be quantified due to dilution of the embryos for fractional centrifugation (data not shown). Pharmacokinetic data of 13-cis- and all-trans-retinoic acid in the plasma and the embryo of the mice have been comparable to the results of Tzimas et al. (1995), indicating a low placental transfer of 13-cis-retinoic acid and a high transfer rate for all-trans-retinoic acid. The concentrations of ATRA, after administration of 13CRA or ATRA, were always lower in the embryonic nuclei than that of the whole embryo, indicating an important role of CRABP-binding properties for the intracellular distribution, metabolism, and access to the nuclei of this retinoic acid. In contrast, 13CRA accumulated higher concentrations in the embryonic nuclei than in the whole embryo.

The presence of high concentrations of ATRA in the embryo and comparable low concentrations in the plasma, as well as high concentrations of 13CRA in the plasma to comparable low concentrations in the embryo, could also be explained by the occurrence of high CRABP concentrations in the embryo (Dencker et al., 1990; Gustafson et al., 1993; Maden et al., 1992; Ruberte et al., 1991, 1992; Scott et al., 1994; Vaessen et al., 1990). The distribution of the synthetic non-CRABP binding retinoid CD394 is analogous to that of 13CRA, indicated by high concentrations in the plasma in comparison to the embryo (Sass et al., 1995). The transfer of ATRA to the retinoic acid receptors in the nuclei, which have been thought to transmit the main retinoid activity for embryotoxicity (Elmazar et al., 1997) as well as delivery for the catabolism is mediated by these CRABPs (reviewed by Donovan et al., 1995 and Noy, 2000). The binding properties of ATRA to CRABP could be correlated to the attraction of ATRA to the CRABP-containing embryo (Dencker et al., 1990; Gustafson et al.,

FIG. 2. Concentrations of ATRA and 13CRA in plasma and embryo as well as estimated concentrations of ATRA and 13CRA in the embryonic nuclei, after treatment with either 10 mg/kg ATRA or 100 mg/kg 13CRA to mice at GD 11. (A) Concentration of ATRA 1 h after administration of ATRA or 13CRA. (B) Concentration of ATRA 4 h after administration of ATRA or 13CRA. (C) Concentration of 13CRA 1 h and 4 h after administration of 13CRA (13CRA, 13-cis-retinoic acid; ATRA, all-trans-retinoic acid).
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1993; Scott et al., 1994) and vulnerability of these tissues to RA excess (Dencker et al., 1990; Gustafson et al., 1993; Maden et al., 1992; Ruberte et al., 1991; Vaessen et al., 1990, 1992).

ATRA from ATRA-treated mice was absorbed quickly, and high concentrations of ATRA could be found in the plasma, whereas ATRA from 13CRA-treated mice increased slowly but steadily (Tables 2 and 3, comparable to Tzimas et al., 1995). This could be explained by the fact that 13CRA is just weakly metabolized by CYP26 to hydroxyl- and oxo-retinoic acids, in contrast to the preferred substrate ATRA (White et al., 1996, 2000), indicating a possible interaction of CRABP (subtype I) and RA-metabolizing enzymes (Dong et al., 1999). Different distribution and a higher persistence of 13CRA compared to ATRA in the organism would lead to a steady release of ATRA (Table 2); this could be interpreted as high embryotoxic potency of 13CRA. On the intracellular level, high concentrations of 13CRA are reached in the nuclei and could possibly isomerize there or, by catalyzed isomerization, in extra nuclear compartments (Chen and Juchau, 1997; Shih et al., 1997). ATRA is buffered by cellular retinoic acid-binding proteins in the cytosol and metabolized to oxo- and hydroxyl-retinoic acids by RA-metabolizing enzymes (Table 3). The catabolism of ATRA is mainly mediated by CRABP I, whereas in contrast, the CRABP subtype II has caused enhancing effects on RAR-RA-induced transcriptional activity (Delva et al., 1999; Dong et al., 1999).

These differences in the intracellular distribution could not be correlated with different physical and chemical characteristics of these retinoid acid isomers, but rather with different binding properties to the CRABP and the RAR. The low amounts of 13CRA in the cytosolic fraction could be explained by lack of binding to cytosolic proteins, whereas ATRA occurred in high amounts in the cytosolic fraction due to binding properties for the CRABP.

Intracellular distribution of several other retinoids like all-trans-4-oxo-retinoic acid (T4OXORA), retinoyl-glucuronides (RAG), retinol (ROL), and retinyl palmitate (RETPAL) have also been determined in these experiments. Retinoids with binding affinities to specific cytosolic retinoid-binding proteins like CRBP and CRABP (for reviews, see Napoli, 1994 and Ong et al., 1994) are mainly located in the cytosolic fraction, whereas retinoids with no CRABP- or CRBP-binding affinity are mainly located in the nuclear fraction. In rat liver centrifugal fractions, retinol was also mainly located in the cytosolic fraction and, to a lower percentage, in the nuclear as well as in the microsomal fractions. Surprisingly those two retinoids with opposite physical and chemical characteristics could mainly or exclusively be found in the nuclear fraction. These data indicate that retinoid subcellular distribution is mainly determined by their binding properties to binding proteins in the cytosol and not by their physical and chemical characteristics. The data also lead to the conclusion that retinoids try to avoid the hydrophilic environment of the cytosol.

The exact mechanism of 13CRA-induced teratogenicity is still not clear. In the plasma of orally 13CRA-treated mice, ATRA was also found after 1 h and 4 h, similar to what has been described earlier. The low concentration of 13CRA and high concentration of ATRA in the embryo could be related to the relative binding properties of the CRABPs (Creech-Kraft et al., 1987). The question of whether ATRA is the proximate teratogenic metabolite for 13CRA in mice could be postulated. Concentrations of ATRA in embryonic nuclear fractions, 1 h after 100 mg/kg 13CRA treatment, were lower than those after 10 mg/kg ATRA treatment (Fig. 2), but the estimated concentrations after 4 h were similar (Fig. 2).

Comparable results about 13CRA-mediated activity via ATRA have been observed in sebocyte culture in vitro. ATRA is present in the cells after ATRA and 13CRA exposure, indicating that antiproliferative effects of 13CRA on sebocytes are mainly mediated by ATRA (Zouboulis et al., 1998).

The mechanism of 13CRA-induced embryotoxic effects could be (a) accumulation of 13CRA in the embryonic nuclei and isomerization to ATRA; (b) isomerization of 13CRA in the maternal tissue to ATRA, then the plasma ATRA transferred to the embryo, and finally into the nuclei, where ATRA could interact with the RARs; or (c) a combination of both. Other metabolites of 13CRA such as retinoyl-glucuronides lack binding and transactivation of RARs (for a review, see Kastner et al., 1994). Thus, the hypothesis that 13CRA-mediated teratogenicity in mice at the dose levels used is dependent on 13CRA isomerization to ATRA is supported by the present study.

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REFERENCES


