Pharmacokinetics of All-trans Retinoyl β-Glucuronide in Rats following Intraperitoneal and Oral Administration

David A. Romans[1], Arun B. Barua[2] and James Allen Olson[3]

Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 50011 USA

Received for publication: July 3, 2002

Abstract: The purpose of this study was to examine the pharmacokinetics of a single dose (6.3 μmol, 3 mg) of all-trans retinoyl β-glucuronide (RAG), when given either orally in corn oil or by intraperitoneal (IP) injection in dimethylsulfoxide (DMSO) to adult Sprague-Dawley rats. Following dosing, serial blood samples were collected at various times up to 48 hours from each rat via saphenous vein puncture. Retinoids were extracted from plasma samples and analyzed by high-performance liquid chromatography. In the plasma of IP-dosed rats (n = 6), a derivative of RAG, tentatively identified as the lactone of RAG (RAGL), was the major product found. RAGL persisted in the plasma for up to 48 hours. Much smaller concentrations of RAG and of retinoic acid (RA) were also present in the plasma at two to four hours, but generally not thereafter. In orally dosed rats (n = 6), neither RAG nor its products, except for occasional traces of the lactone, were detected. Plasma retinol levels decreased in both IP-injected and orally treated rats, the decrease being significant in orally dosed rats.

Key words: Retinoyl β-glucuronide, retinoic acid, retinol, pharmacokinetics, rats

Introduction

Retinoyl β-glucuronide (RAG) is a naturally occurring, biologically active, water-soluble metabolite of vitamin A. Formation of RAG from retinoic acid (RA) was considered a mechanism for removal of excess RA that may otherwise be toxic to the body. However, it is now known that RA retains its biological activity after conjugation with glucuronic acid, but becomes much less toxic [1, 2].

Following a dose of RA, RAG was found as a significant metabolite in the serum and small intestine [3, 4]. RAG is formed from RA by microsomal UDP-glucuronosyl transferases [5]. RAG shows properties both similar to and different from RA. Like RA, RAG stimulates the growth of vitamin A-deficient animals [2], induces the differentiation of a variety of cell lines [6–9], and is effective in the treatment of acne in humans [10, 11]. Unlike RA however, RAG is nontoxic when applied topically to human [10, 11] or pig skin [12] and nonteratogenic when given orally to pregnant Sprague-Dawley

[1] Present address: Des Moines University, Des Moines, IA.
[2] Corresponding author.
[3] Deceased (September 22, 2000).

Abbreviations: RAG, all-trans retinoyl β-glucuronide; RA, all-trans retinoic acid; RAGL, retinoyl β-glucuronolactone; HPLC, high-performance liquid chromatography.

rats [13]. RAG is currently being tested as a treatment for neuroblastoma [14].

Despite the known effectiveness of RAG as a therapeutic agent, its metabolism is not well defined *in vivo*. It has been shown that orally administered RAG was absorbed well and converted to RA in vitamin A-deficient rats, whereas neither absorption nor conversion to RA was observed in vitamin A-sufficient (normal) rats [15]. It was concluded that the absorption and metabolism of RAG depends on the vitamin A status of the animal [15]. Following subcutaneous (sc) injection of RAG to pregnant National Medical Research Institute, U.K. (NMRI) mice, extensive hydrolysis of RAG to RA resulting in teratogenicity was reported [16]. A recent study carried out in a similar way by injecting RAG sc to Sprague-Dawley rats, however, did not result in extensive hydrolysis of RAG to RA [17]. In two other recent studies, RAG administered by either sc injection to Harlan mice [18] or by intravenous injection to Sprague-Dawley rats [19] did not result in extensive hydrolysis or toxicity. Thus, it is possible that the mode of administration of RAG, the species and vitamin A status of the animal, and other factors might play a significant role in its absorption and metabolism. In order to understand better the pharmacokinetics of RAG, we have investigated the differences in the metabolism of RAG after oral and intraperitoneal (IP) administration to vitamin A-sufficient rats.

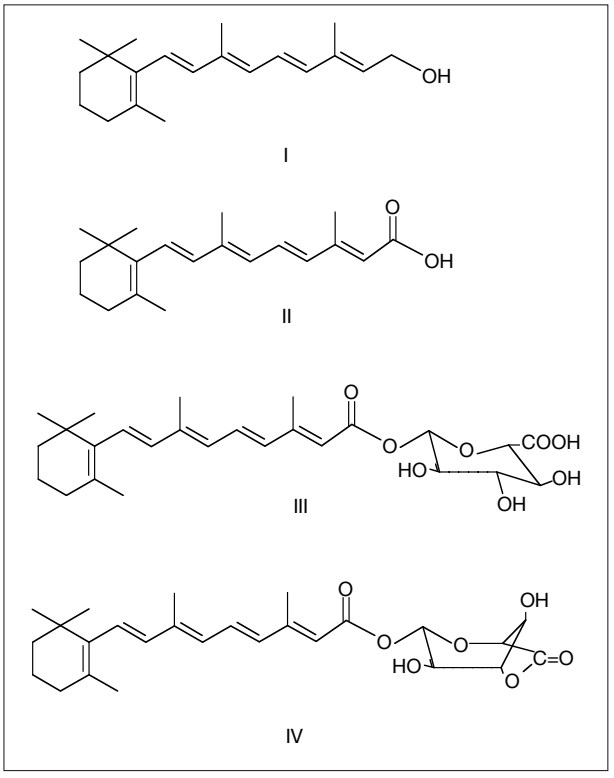

Figure 1: Chemical structures of retinoids. I) all-trans retinol, II) all-trans retinoic acid, III) all-trans retinoyl β-glucuronide, and IV) all-trans retinoyl β-glucurono-γ-lactone.

Materials and Methods

Chemicals: Methanol, dichloromethane, ethanol, ethyl acetate, acetic acid, hexane, and ammonium acetate were supplied by Fisher Scientific Co. (Fair Lawn, NJ, USA). For high-performance liquid chromatography (HPLC), HPLC-grade solvents were used. RA was obtained from BASF (Parsippany, NJ, USA). Retinol (ROL), retinyl acetate (RAC), and β-glucuronidase from *E. coli* (activity 570 000 units/g) were purchased from Sigma Chemical Co. (St. Louis, MO).

Reference compounds: RAG was synthesized in the laboratory according to published procedures [20]. Reference compounds (Fig. 1) were checked for purity by HPLC and spectrophotometry. Concentrations of RA, RAG, ROL, and RAC in standard solutions were determined from their published extinction coefficients (E, 1%, 1 cm) [21]. Standard curves were prepared from peak areas of the retinoids during HPLC analysis, and used to calculate the concentrations of ROL, RA, and RAG found in the plasma of rats following an IP or oral dose of RAG. The concentration of retinoyl β-glucuronolactone (RAGL) was determined from the standard curve of RAG.

Rats: Sprague-Dawley male rats (n = 12) weighing approximately 350 g were obtained through Laboratory Animal Resources, Iowa State University. All experiments with animals were in accord with NIH Guidelines for the Use of Animals and were approved by the University Committee on the Use of Animals in Research. The rats were kept in individual cages and fed normal rat chow throughout the study.

Preparation of RAG for IP and oral dose: The IP dose of RAG was prepared by dissolving pure RAG in dimethylsulfoxide (DMSO). The concentration of RAG in DMSO was determined spectrophotometrically by diluting aliquots in methanol [21]. The final concentration of RAG in the stock solution was 3671 µg/100 µL (7.68 µmol/L, 3.67 mg). Eighty-two µL of the stock solution was injected to give 3000 µg (6.3 µmol/L, 3 mg) of RAG/rat. For the oral dose, RAG was prepared by grinding solid RAG with corn oil in a mortar with pestle until a clear solution was obtained. An aliquot of the oily solution was diluted with methanol, and the concentration of RAG was determined as described above. The final concentration of RAG in oil was 3000 µg/111 µL (6.3 µmol/L, 3 mg) of oil.

Dosing: Each rat was weighed, and a baseline blood sample (t = 0) was drawn via saphenous vein puncture. By means of a Microman positive-displacement pipette, each rat in the oral group (n = 6) was fed 111 µL (6.3 µmol/L, 3 mg) of RAG dissolved in corn oil. Each rat in the IP group (n = 6) was administered 82 µL (6.3 µmol/L, 3 mg) of RAG in DMSO via IP injection. Following the dose, serial blood was collected from the saphenous vein at 0.5, 1, 2, 4, 6, 8, 24, and 48 hours.

Saphenous vein puncture and blood collection: Each rat was placed in a conical plastic restraint. A single hind leg was displaced through a slit in the plastic restraint and held extended between the thumb and forefinger. The leg was then shaved, and a thin coat of Vaseline (petroleum jelly) was applied. Slight pressure was applied to the leg along with palpation to determine the location of the saphenous vein. An 18-gauge needle was used to puncture the saphenous vein, and blood (0.6 mL) was collected into two Microvette CB300 collection tubes. Pressure was then applied to the puncture wound to stop the bleeding. Alternate legs were used to allow maximum time for the wound to close between bleeds. Each rat was administered 3.0 mL saline by subcutaneous injection after the 0.5-, 2- and 6-hour blood collections. Blood samples were centrifuged immediately following collection, and plasma samples were kept frozen at –20°C until analysis time.

Extraction of retinoids: All extractions were made at ice temperature in laboratories illuminated with yellow lights (gold fluorescent lamp) to minimize light-induced isomerization. Retinoids were extracted from plasma by published procedure [20] as follows: Plasma (200 µL) was transferred to a fresh glass tube on ice, and 500 µL of ethanol containing 0.01% butylated hydroxytoluene, 500 µL of ethyl acetate, and 50 µL of acetic acid (10%, v/v) were then added. The mixture was vortexed for 30 seconds and centrifuged at 2000 rpm for 1 minute in a clinical centrifuge. The supernatant was removed and transferred to a fresh glass tube, and the pellet was vortexed with 1 mL of hexane for 30 seconds and centrifuged at 2000 rpm for 1 min. The two supernatant solutions were pooled and 250 µL of water was added. The solution was again vortexed for 30 seconds and centrifuged at 2000 rpm for 1 min. The upper organic phase was then transferred to a fresh glass tube and evaporated under a slow stream of argon. The residue was dissolved in 100 µL of dichloromethane/isopropanol (1:1). An aliquot of 75 µL was analyzed by HPLC as described below. In representative samples an internal standard, retinyl acetate (RAC), was added during the extraction procedure. The percentage recovery was determined to be 90 to 95%.

HPLC analysis: For analysis of reference compounds and plasma retinoids, a reverse-phase gradient HPLC procedure [22] was used with the following Waters (Milford, MA, USA) components; namely, a refrigerated autosampler (WISP Model 717 plus), plus an automated gradient controller, two pumps (model 510), a photodiode array detector (PDA) (model 996), a Millennium 2010 Chromatography Manager, a NEC Powermate (model 486/33I), and a Hewlett-Packard Laser Jet III printer. A 3-µm Microsorb-MV column (100 × 3.6 mm) (Rainin, Woburn, MA, USA) was used, which was preceded by a guard column of C18 material (Upchurch Scientific, Oak Harbor, WA, USA). The solvent system consisted of methanol/water (70:30) containing 10 mM ammonium acetate (solvent A) and methanol/dichloromethane (4:1) (solvent B). A 15-minute linear gradient from solvent A (100%) to solvent B (100%) was followed by isocratic elution with solvent B for 10 minutes. The flow rate was 0.8 mL/minute. The chromatograms were monitored at 350 nm. At the end of the run, the gradient was reversed to initial conditions by applying a linear gradient from solvent B to solvent A for 5 minutes. The column was then allowed to equilibrate for 10 minutes with solvent A before the next injection.

Treatment with β-glucuronidase: Standard RAG solution in methanol or HPLC fractions containing RAG or RAGL were evaporated to dryness under a stream of argon. The residue was dissolved in ~50 µL of methanol and diluted with phosphate-buffered saline (0.15 M sodium phosphate/0.15 M NaCl, pH 7.5; 0.5 mL) and incubated with β-glucuronidase (5 mg) at 37°C for 1 hour. Retinoids were extracted and analyzed as described above.

Statistical analysis: Statistical analysis was carried out using Student's t-test [23].

Results

Retinoids in serum after IP administration of RAG: HPLC analysis of plasma of rats dosed by IP injection with RAG showed that endogenous retinol (ROL) was the major retinoid, but its concentration changed with time (Fig. 2A). Baseline value of 1.07 ± 0.21 µmol/L of retinol decreased to a minimum of 0.98 ± 0.16 µmol/L in 8 h. The decrease of plasma retinol values following IP administration was, however, not statistically significant from baseline values.

HPLC analysis of plasma of rats that received RAG by IP injection showed the presence of very small amounts of RAG and RA. The concentrations of both RAG (0.092 ± 0.06 µmol/L) (Fig. 3A) and RA (0.093 ± 0.05 µmol/L) (Fig. 3B) peaked at 2 hours after the dose, and then fell

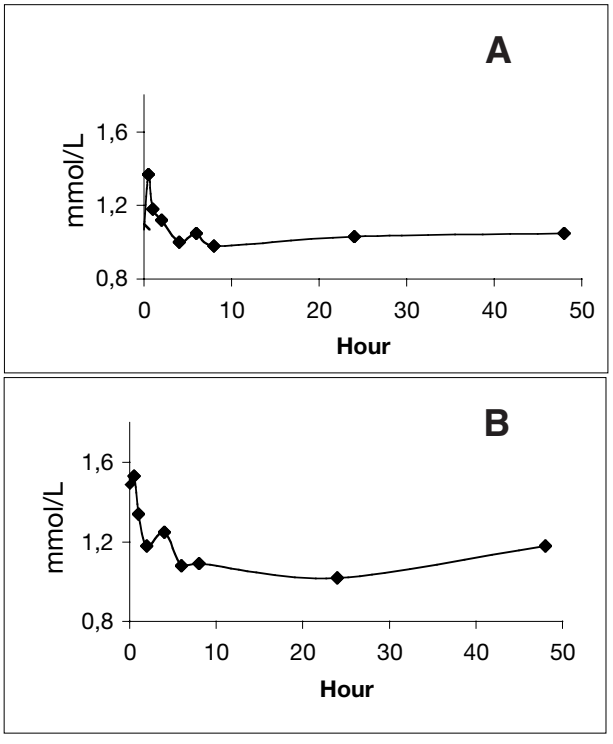

Figure 2: The average change in plasma retinol values over time in rats dosed A) by IP injection, and B) orally with all-trans retinoyl β-glucuronide. Error bars are represented as standard errors.

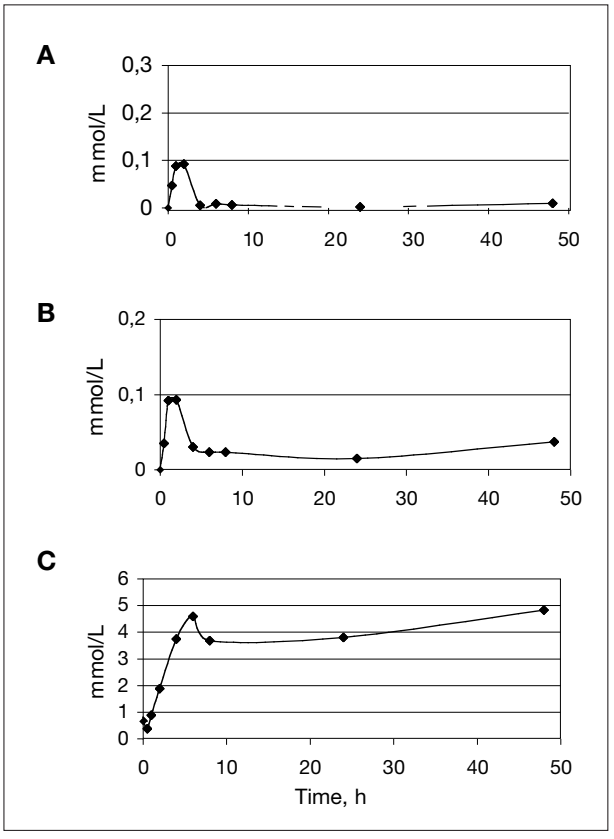

Figure 3: The change in plasma A) retinoyl β-glucuronide values, B) retinoic acid, and C) retinoyl β-glucuronolactone values over time in rats dosed with all-trans retinoyl β-glucuronide by IP injection. Error bars are represented as standard errors.

rapidly. The plasma of rats dosed with RAG by IP injection showed the presence of another less polar metabolite that eluted after ROL, RAG, and RA. The retention time of this metabolite was 21.5 minutes (peak 4, Fig. 4A). The concentration of this metabolite peaked at 6 hours (4.58 ± 1.87 μmol/L) after the dose (Fig. 3C). Unlike RAG or RA, the concentration of this metabolite remained at that level throughout the study period of 48 hours. The ultraviolet-to-visible (UV-visible) absorption spectrum (λmax 358 nm in HPLC solvent) (Fig. 4B) of the metabolite, however, was identical to that of RAG. Upon treatment of the compound with β-glucuronidase, RA was generated, pointing towards the presence of glucuronic acid moiety in the compound. Because the metabolite was less polar than RAG or RA, it was tentatively identified as a lactone derivative of RAG.

Retinoids in plasma after oral administration of RAG: As in the case of retinoids in plasma of rats dosed IP with RAG, endogenous ROL was the major retinoid in plasma of rats dosed orally with RAG, but the initial concentration (1.5 ± 0.2 μmol/L) changed with time (Fig. 2B). The concentration fell to a minimum of 1.06 ± 0.09 μmol/L (29% of base line value) 6 hours after the dose, and then rose (1.16 ± 0.17 μmol/L) after 48 hours. Plasma retinol values (6–24 hours) were significantly different ($p < 0.05$) from the baseline value. Analysis of plasma of rats dosed orally with RAG showed that neither RAG nor RA was detectable at any time after the dose. Retinoyl β-glucuronolactone was also not detected in the plasma of any rats, except in one who had a barely detectable level of the metabolite 4 hours after the dose.

Cleavage of RAG and RAGL by β-glucuronidase: When extracts of plasma or HPLC fractions containing either RAG or RAGL were treated with the enzyme β-glucuronidase for 1 hour at 37°C, approximately 95% of RAG and RAGL were converted to RA as determined by HPLC.

Discussion

It was found in the present study that administration of RAG by IP injection or by oral administration to rats re-

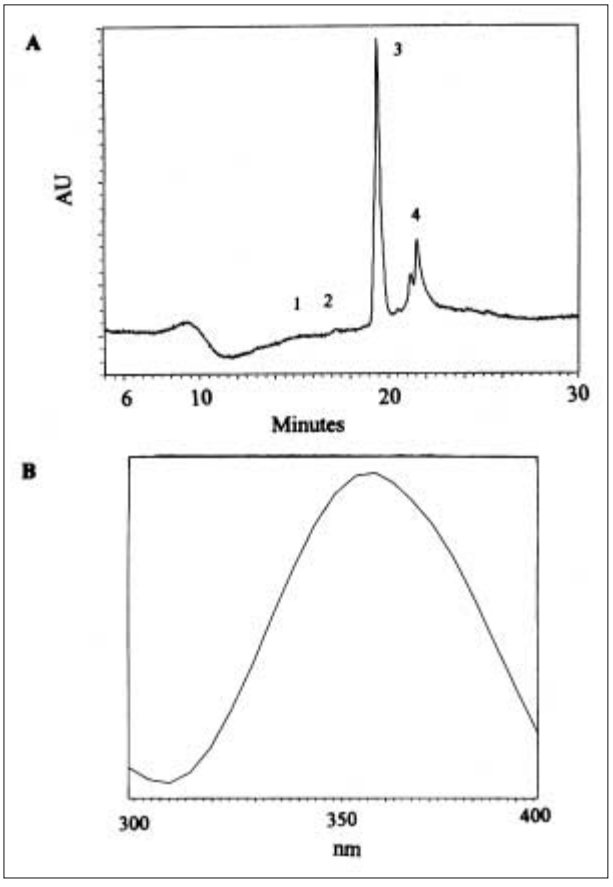

Figure 4: A) HPLC chromatogram of plasma extract of a rat dosed with all-trans retinoyl β-glucuronide by IP injection. Blood was collected 6 hours after the dose. Peak identification: 3:retinol, 4: retinoyl β-glucuronolactone. The elution position of 1) all-trans retinoyl β-glucuronide, and 2) all-trans retinoic acid are shown. B) UV-visible spectrum of retinoyl β-glucuronolactone in peak 4.

duced plasma ROL levels. While the decrease of plasma ROL values was significant following oral dosing, it was not significant following IP administration. The reason for this difference is not clear. The reduction of serum ROL level following administration of RA, RAG or retinyl β-glucuronide has been previously reported from this laboratory [24, 25]. This was confirmed in the present study. The reduction of serum ROL values may be a consequence of *in vivo* specific binding of retinoids to ROL-binding protein (RBP) [26].

It was found in the present study that RAG administered by IP injection was metabolized to a compound that was very similar to RAG. The UV-visible absorption spectrum of both RAG and the metabolite was the same. Both produced RA on treatment with β-glucuronidase, pointing toward the presence of a glucuronic acid moiety in the metabolite. However, the retention time of the derivative during HPLC analysis was different from RAG. The longer retention time of the metabolite indicated that the metabolite in question might be a lactone derivative. This has been concluded for several reasons: during column chromatography of pure RAG (as judged from single peak during HPLC) on silica gel, a small fraction of RAG consistently eluted with a solvent mixture of methanol and dichloromethane much earlier than RAG (unpublished observations in this laboratory). The UV-visible spectra of RAG and this fraction are similar. During HPLC, the retention time of this fraction is much longer (21.5 minutes) than RAG (13.8 minutes), RA (17.2 minutes), and ROL (19.4 minutes). The change in chromatographic behavior of RAG pointed toward chemical modification of RAG. Gamma-lactones do form spontaneously [27] and enzymatically [28]. Because glucuronic acid easily undergoes lactonization to glucurono-3,6-lactone, it is possible that the glucuronic acid moiety in RAG underwent lactonization. The formation and identification of retinoyl β-glucurono-γ-lactone during the isolation procedure was reported following incubation of RAG with an anion-exchange resin in the presence of methanol [28]. The retention time of the metabolite of RAG found in rat serum during the present study is the same as the compound formed from RAG during column chromatography. It was, however, not clear whether RAGL was formed from RAG during the isolation procedure. Based on these observations, the metabolite isolated as a major product in rat serum following IP injection of RAG is tentatively identified as retinoyl β-glucurono-γ-lactone (RAGL). A possible structure of the metabolite is shown in Figure 1. In view of the small serum samples, further characterization of this new metabolite was not possible during this study.

RAGL levels rose markedly in the IP-dosed group, peaking at 6 hours following dosing and remaining elevated over the 48-hour analysis period. The peak RAGL value was roughly 100 times that of the peak values detected for both RA and RAG. The relative steady level of RAGL seen over the 48-hour analysis period may be explained by its relatively slow clearance from the plasma by tissues.

Several years ago, the metabolism of ^{3}H-RAG dissolved in saline and administered by IP injection was studied in rats in this laboratory [29]. In this study, ^{3}H-RAG was the major circulating labeled retinoid in serum, but small amounts of ^{3}H-RA and another labeled metabolite believed to be methyl retinoate were also detected in serum. The identity of methyl retinoate was based on the observation that RA was obtained on alkaline hydrolysis of the metabolite. The action of β-glucuronidase on this metabolite was, however, not studied. RA can also be formed from RAGL on hydrolysis. Therefore, it was very

likely that the metabolite reported in the previous study was indeed RAGL. The reason for the difference in the concentrations of RAGL in serum or plasma in these two studies is not clear. Whether RAGL was formed due to a difference in the vehicle of injection, or due to artifact formation during the isolation step remains unclear.

Contrary to a report that RAG sc injected in NMRI mice resulted in rapid hydrolysis of RAG to RA [16], it was found during the present study that the hydrolysis of RAG to RA, given by IP injection, was very slow. In three recent studies, rapid hydrolysis of RAG to RA was not observed in Sprague-Dawley rats and Harlan mice when RAG was administered by sc or iv injection [17–19]. The reason for such difference is not known, but it is possible that the metabolism of RAG in NMRI mice is different from Sprague-Dawley rats or Harlan mice. As stated earlier, several studies from this and other laboratories have shown that RAG is relatively nontoxic and nonteratogenic as compared with RA. Excess RA is toxic, and glucuronidation occurs to get rid of any excess RA. Therefore, it is possible that during oral administration, RAG is not absorbed by vitamin A-sufficient animals, as there is no need for any additional vitamin A to form RA in the body. Consequently, orally administered RAG is most likely excreted. During vitamin A deficiency, however, the body senses the need for vitamin A, and any orally administered RAG is hydrolyzed to RA and absorbed. A previous study reported that the conversion of RAG to RA was dependent upon the vitamin A status of the animal [15].

Lack of hydrolysis of RAG to RA in vitamin A-sufficient rats is evidence for supporting the low toxicity and teratogenicity of RAG. The properties of RAG, both similar to and different from RA, make it a promising therapeutic agent.

Acknowledgments

This work was supported by NIH-DK 39733, Journal paper No. J-19790 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA Project No. 3335, and supported by Hatch Act and State of Iowa funds.

References

1. Formelli, F., Barua, A. B. and Olson, J. A. (1996) Bioactivities of N-(4-hydroxyphenyl) retinamide and retinoyl β-glucuronide. FASEB J. 10, 1014–1024.
2. Barua, A. B. (1997) Retinoyl β-glucuronide: A biologically active form of vitamin A. Nutr. Rev. 55, 259–267.
3. Zile, M. H., Inhorn, R. C. and De Luca, H. F. (1982) Metabolism of all-trans retinoic acid in the bile: Identification of all-trans retinoyl- and 13-cis-retinoyl β-glucuronides. J. Biol. Chem. 257, 3544–3550.
4. Barua, A. B., Gunning, D. B. and Olson, J. A. (1991) Metabolism *in vivo* of all-trans-[11-^{3}H]retinoic acid after an oral dose in rats. Characterization of retinoyl β-glucuronide in the blood and other tissues. Biochem. J. 277, 527–531.
5. Genchi, G., Wang, W., Barua, A., Bidlack, W. R. and Olson, J. A. (1996) Formation of β-glucuronides and β-galacturonides of various retinoids catalyzed by induced and noninduced microsomal UDP-glucuronosyltransferases of rat liver. Biochim. Biophys. Acta 1289, 284–290.
6. Gallup, J. M., Barua, A. B., Furr, H. C. and Olson, J. A. (1987) Effects of retinoid β-glucuronides and N-retinoyl amines on the differentiation of HL-60 cells *in vitro.* Proc. Soc. Exp. Biol. Med. 186 (3), 269–274.
7. Janick-Buckner, D., Barua, A. B. and Olson, J. A. (1991) Induction of HL-60 cell differentiation by water-soluble and nitrogen-containing conjugates of retinoic acid and retinol. FASEB J. 5, 320–325.
8. Biesalski, H. K., Doepner, G. and Gerharz, C. D. (1993) All-trans retinyl- and all-trans retinoyl β-glucuronides: Synthesis, pharmacology, and biological activity. In: Retinoids (Livrea, M. A. and Packer, L., eds.), pp. 329–339, Marcel Dekker, New York, NY, USA.
9. Becker, B., Barua, A. B. and Olson, J. A. (1998) Effects of novel carbohydrate conjugates of retinoic acid on the differentiation of promyelocytic leukemia cells. Med. Biol. Environ. 26, 95–101.
10. Gunning, D. B., Barua, A. B., Lloyd, R. and Olson, J. A. (1994) Retinoyl β-glucuronide: A nontoxic retinoid for the topical treatment of acne. J. Dermatol. Treat. 5, 181–185.
11. Goswami, B. C., Baishya, B., Barua, A. B. and Olson, J. A. (1999) Topical retinoyl β-glucuronide is an effective treatment of mild to moderate acne vulgaris in Asian-Indian patients. Skin Pharmacol. Appl. Skin Physiol. 12, 167–173.
12. Gunning, D. B., Barua, A. B., Myers, R. K., Ueltschy, A., Romans, D. and Olson, J. A. (2002) Comparative histologic effects of daily application of creams containing all-trans retinoic acid or all-trans retinoyl β-glucuronide on pig skin. Skin Pharmacol. Appl. Skin Physiol. 15, 205–212.
13. Gunning, D. B., Barua, A. B. and Olson, J. A. (1993) 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 41, 29–36.
14. Barua, A. B. and Sidell, N. (2003) Retinoyl β-glucuronide: A biologically active interesting retinoid. J. Nutr. (in press).
15. Barua, A. B., Duitsman, P. K. and Olson, J. A. (1998) The role of vitamin A status in the conversion of all-trans retinoyl β-glucuronide to retinoic acid in male Sprague-Dawley rats. Nutr. Biochem. 9, 8–16.
16. Nau, H., Elmazar, R. R., Thiel, R. and Sass, J. O. (1996) All-trans retinoyl glucuronide is a potent teratogen in the mouse because of extensive hydrolysis to all-trans retinoic acid. Teratology 54, 150–156.
17. Ueltschy, A., Gunning, D. B., Barua, A. B. and Olson, J. A. (2002) Effects of subcutaneously injected graded doses of all-trans retinoic acid and all-trans retinoyl β-glucuronide on the outcome of pregnancy in Sprague-Dawley rats. Int. J. Vit. Nutr. Res. 72, 229–235.

18. Sidell, N., Sawatsri, S., Conner, M. J., Barua, A. B. and Olson, J. A. (2000) Pharmacokinetics of long-term administration of all-trans retinoyl β-glucuronide in mice. Biochim. Biophys. Acta 1502, 264–272.
19. Lin, H., Barua, A. B., Olson, J. A., Low, K. S. Y., Chan, S. Y., Shoon, M. L. and Ho, P. C. (2001) Pharmacokinetic study of all-trans retinoyl β-glucuronide in Sprague-Dawley rats after single and multiple intravenous administration(s). J. Pharm. Sci. 90, 2023–2031.
20. Becker, B., Barua, A. B. and Olson, J. A. (1996) All-trans-retinoyl β glucuronide: New procedure for the chemical synthesis and its metabolism in vitamin A-deficient rats. Biochem. J. 314, 249-252.
21. Barua, A. B., Furr, H. C., Olson, J. A. and van Breeman, R. B. (2000) Vitamin A and carotenoids. In: Modern Chromatographic Analysis of Vitamins (De Leenheer, A. P., Lambert, W. E. and Van Bocxlaer, J. F., eds.), vol. 84, pp.1–71, Marcel Dekker, New York, NY, USA.
22. Barua, A. B. and Olson, J. A. (1998) Reversed-phase high performance liquid chromatographic procedure for simultaneous analysis of very polar to nonpolar retinoids, carotenoids and tocopherols in animal and plant samples. J. Chromatogr. B 707, 69–79.
23. Snedecor, G.W. and Cochran, W. G. (1989) Statistical Methods, 8th ed., pp. 83–106, Iowa State University Press, Ames, IA, USA.
24. Barua, A. B., Duitsman, P. K., Kostic, D., Barua, M. and Olson, J. A. (1997) Reduction of serum retinol levels following a single oral dose of all-trans retinoic acid in humans. Int. J. Vitam. Nutr. Res. 67, 423–426.
25. Barua, A. B., Batres, R. O., Gunning, D. B. and Olson, J. A. (1994) Reduction of serum retinol level in the rat after a single IP dose of all-trans retinoyl β-glucuronide or all-trans retinyl β-glucuronide. FASEB J. 8, A444.
26. Berni, R., Clerici, M., Malpeli, G., Cleris, L. and Formelli, F. (1993) Retinoids: In vitro interaction with retinol-binding protein and influence on plasma retinol. FASEB J 7, 1179–1184.
27. Fieser, L. and Fieser, M. (1961) Advanced Organic Chemistry. p. 574 Reinhold, New York, NY, USA.
28. Lippel, K. and Olson, J. A. (1968) Origin of some derivatives of retinoic acid found in rat bile. J. Lipid Res. 9, 580–586.
29. Barua, A. B. and Olson, J. A. (1989) Chemical synthesis of all-trans-[11-^{3}H]retinoyl β-glucuronide and its metabolism in rats *in vivo*. Biochem. J. 263, 403–409.

Arun B. Barua

Department of Biochemistry
Biophysics and Molecular Biology
Iowa State University
Ames, IA 50011 USA