Inhibition of Mammary Tumor Growth by a Novel Nontoxic Retinoid: Chemotherapeutic Evaluation of a C-Linked Analog of 4-HPR-Glucuronide

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Abstract. Previous studies from our laboratory suggest that 4-HPROG, the O-glucuronide derivative of 4-HPR, has improved mammary cancer chemopreventive/antitumor activities as well as reduced toxicity, as compared to 4-HPR. This O-linked glucuronide derivative is a substrate to the β-glucuronidase enzyme and may also undergo hydrolysis in vivo to the vitamin A metabolite, retinoic acid, that is toxic at high concentrations. In an effort to improve analog potency relative to its toxicity, the 4-HPROG’s phenolic oxygen was replaced with a methylene group, thus preventing biological cleavage of the glucuronide moiety. The resulting C-linked analog, 4-HPR-C-glucuronide (4-HPRCG), cannot be hydrolyzed to 4-HPR. The results of this study show that 4-HPRCG is an effective chemotherapeutic agent that caused 49% regression of DMBA-induced mammary tumors in rats, while showing almost no side-effects that are often observed with other natural or synthetic retinoids, such as a reduction in blood retinol level, elevation in blood triglyceride (TG) level, and decrease in bone mineral content (BMC). These results suggest that 4-HPRCG should be considered as a better candidate for breast cancer treatment.

Recent studies suggest that retinoid glucuronides may have improved cancer chemopreventive/chemotherapeutic activities and/or reduced toxicity relative to the parent compounds (1-5). Retinoyl-β-glucuronide has been shown to retain potency equal to all-trans retinoic acid (atRA) in maintaining normal animal growth and promoting cellular differentiation (6-8). This glucuronide has also been found to inhibit proliferation and induce differentiation of the human promyelocytic HL-60 leukemia cells (6, 8). Additionally, retinoid glucuronides have been shown to inhibit the growth of premalignant and malignant cells in vitro and in vivo (9, 10). While serving as effective inducers of cellular differentiation, retinoid glucuronides show much less cytotoxicity (6, 8) and teratogenicity (11) than RA. Both retinoyl-β-glucuronide and retinyl-β-glucuronide, the glucuronide conjugates of RA and retinol respectively, were also found to be highly active in inhibiting the induction of neoplastic changes in mammary gland cultures (5).

More recently, studies from our laboratory suggest that 4-HPR-O-glucuronide (4-HPROG), in which the phenolic hydroxyl group is linked to the sugar, demonstrated improved chemopreventive/antitumor activity as well as reduced toxicity in the DMBA-induced rat mammary tumor model (1, 2). This analog also showed anti-proliferative activity in cultures of MCF-7 breast cancer cells (9). However, this O-linked glucuronide derivative is a substrate to the β-glucuronidase enzyme and, thus, may be hydrolyzed in vivo to 4-HPR and further to RA (12). In addition, it has not been determined if the O-glucuronide analog was advantageous due to improved bioavailability of the hydrolyzed intermediates, or whether the intact glucuronide had activity in its own right. To study this issue, a stable analog of the retinoid glucuronide was synthesized by replacing the phenolic oxygen with a methylene group (13). The C-linked analog, 4-HPR-C-glucuronide (4-HPRCG)
was tested in our laboratory in the rat mammary tumor model and was found to have more potent chemopreventive activity than an equimolar concentration of 4-HPROG (3). Although promising as a chemopreventive agent, the antitumor activity of 4-HPRCG has not been tested in vivo against mammary cancer. The purpose of the present study was, therefore, to evaluate the chemotherapeutic effect of this stable non-hydrolysable analog against the growth and progression of DMBA-induced rat mammary tumors. We found that this C-linked analog, in addition to its potent antitumor effect, has markedly less toxic side-effects than either atRA or 4-HPR, as it did not significantly decrease the in vivo plasma retinol levels, nor raise the triglyceride (TG) levels, or cause a reduction in bone mineral content (BMC). These results, therefore, suggest that the analog, 4-HPRCG, with its demonstrated chemopreventive and antitumor potency, as well as markedly lower toxic side-effects, represents an improved candidate for breast cancer treatment.

Materials and Methods

Reagents and chemicals. 7,12-Dimethylbenz(a)anthracene (DMBA) and all other reagents with the highest purity available were purchased from Sigma Chemical Co., St. Louis, MO, USA. 4-HPR was provided by McNeil Pharmaceuticals, Springhouse, PA, USA and all-trans retinoic acid (atRA) was purchased from Eastman Kodak. Starting materials and reagents used for the synthesis of 4-HPRCG were supplied from Sigma-Aldrich Co., Milwaukee, WI, USA.

Synthesis of 4-HPRCG. 4-HPRCG was synthesized using our recently modified synthesis of the compound (13). Briefly, in this synthesis the key nitrobenezyl sugar component is prepared by Suzuki coupling of 1-bromo-4-nitrobenzene with a protected 1-methylene glucose followed by oxidation to the glucuronic acid derivative and reduction of the nitro group to the aniline. Treatment of the protected aminobenzyl glucuronic acid with retinol chloride, followed by protecting group removal, affords 4-HPRCG in 24% overall yield, which represents a 10-fold improvement over our earlier syntheses.

Dietary and mammary tumor induction protocols. Mammary tumors were induced in 50-day-old female Sprague-Dawley rats (Harlan Industries, Indianapolis, IN, USA) with a single dose of 15 mg DMBA in 1.0 ml of sesame oil per rat. The rats were then maintained on a powdered Teklad 22/5 rodent Chow diet (W): 8640 (Harlan Industries, Indianapolis, IN, USA), and allowed food and water ad libitum. Four months later, rats with palpable tumors were randomly assigned to retinoid-treated groups, each containing 12 tumor-bearing rats. The control (untreated) group received the chow diet only. The other three groups were fed diets supplemented with 2 mmol/kg diet of atRA, 4-HPR and 4-HPRCG, respectively, for 28 days. The retinoids were added to the diet in a vehicle consisting of 25 ml of ethanol: tricaprylin (1:4 v/v) plus 2% (w/v) α-tocopherol, as previously described (1, 2). This vehicle was also added to the control diet. The additives were blended into the chow diets using a Hobart food mixer. The diets were fed in stainless steel feeders designed with food hoppers. The food was replaced weekly with freshly prepared diets. Food consumption was determined once weekly, and from that the average daily consumption/rat was estimated. Animals were also weighed weekly and monitored for general health status and signs of possible toxicity due to treatment. Baseline measurement of initial tumor sizes, numbers and rat body weights were determined immediately before commencement of treatments, and final measurements were recorded just prior to sacrifice of the animals. The animals were palpated for tumors twice weekly and tumor diameters were measured weekly by a micrometer caliper. Tumor volumes were calculated using the formula \[ V = \frac{4}{3} \pi r^3 \] where \( r \) is one-half the mean of the sum of the largest diameter and the axis at right angle to it. All tumors, as well as lung, liver and femur, were excised at the end of the experiment for chemical and histopathological evaluation. Blood samples were also collected for determination of plasma retinol and triglyceride levels.

Plasma retinol assay. To 500 µL of serum was added 150 µL of ethanol containing 0.75 µg of internal standard (N-(4-chlorophenyl) retinamide). After 30 seconds mixing, 500 µL of ethyl acetate was added followed by 1 minute of mixing and centrifugation for 5 minutes at 1000 rpm in an IEC CL centrifuge. The ethyl acetate layer was removed and syringe filtered through a 0.45 µm filter. The ethyl acetate extraction was repeated two more times. The combined extracts were evaporated and the residue reconstituted in 100 µL of methanol. The methanol extract (20 µL) was analyzed by HPLC on a Beckman Instruments model 127, an instrument equipped with a model 166 UV detector. Chromatography was done on a pre-column equipped 250 x 4.6 mm Beckman Ultrasphere ODS column with an 85% methanol/water mobile phase (both containing 10 mM ammonium acetate) flowing at 1 mL/min. Analysis for both internal standard and retinol was conducted at 350 nm and internal standard recoveries and retinol levels were determined by comparison with standard curves, with adjustment of the retinol level based on recovery. Recoveries of internal standard averaged ca. 78%. Previous extraction of serum from vitamin A-deficient rats showed no substances interfering with the elution position of the retinol or internal standard.

Plasma triglyceride assay. Blood was drawn at day ten from a subset (3 per group) of anesthetized animals in the presence of EDTA as an anticoagulant, and the resulting plasma was used for the measurement of plasma true triglyceride levels using a kit from Sigma-Aldrich (St. Louis, MO, USA). Briefly, the total plasma triglyceride and glycerol concentrations were determined, and the glycerol component was subtracted from the total plasma triglyceride measurement to obtain the true serum triglyceride concentration.

RNA isolation/Northern blotting. RNA was isolated from liver tissue and Northern blots were prepared by the methods previously described (14). Briefly, tissue was collected and flash-frozen in liquid nitrogen until use. The tissue was homogenized, total RNA was isolated according to the method of Chomczynski and Sacchi (15), poly (A)+ RNA was isolated and separated (2 µg) on a 1% formaldehyde-agarose gel followed by transfer to a nylon membrane. A rat CYP26A1 partial cDNA was generated by PCR amplification from E10.5 rat embryo cDNA. The upstream (5'
GCA GAT GAA GCG CAG GAA ATA CG 3') and downstream (5' CCC ACG AGT GCT CAA TCA GGA 3') primers were designed based on the murine full-length cDNA (gi:668110). The 635 bp cDNA was subcloned into pGEM-Teasy (Promega, Madison, WI, USA) and sequenced. The rat CYP26B1 cDNA was generated as described previously (14). Radiolabelled probes for Northern analysis were prepared from the cDNA insert using random hexamers. Hybridization was performed as described previously (16).

Nuclear retinoid receptor binding assay. The ability of 4-HPR and 4-HPRCG to compete with all-trans-[3H]-RA for binding to retinoic acid receptors (RARs) was determined using an in vitro ligand binding assay, as described previously (17). Briefly, recombinant murine RARβ and human RARγ were expressed using the baculovirus insect cell expression system, as previously described (4, 18). RAR-containing nuclear extracts were diluted and incubated at 4°C for 3 hours with ca. 4 nM all-trans-[3H]-RA in the absence and presence of increasing concentrations of unlabelled retinoid analogs. The retinoid-receptor complex was separated from the unbound retinoid compound using a hydroxylapatite assay (17). All-trans-[3H]-RA bound to RAR was detected by liquid scintillation counting. Determination of Ki values was performed using the LIGAND program (19). Similarly, binding to the murine RXRγ (20) was determined by competition for [3H]-9-cis RA binding (ca. 2 nM) in the absence and presence of increasing concentrations of unlabelled retinoid analogs.

Bone mineral content (BMC) determination. The femur was disarticulated from the leg, and the adhering soft tissue was removed by dissection. Femurs were scanned using the Lunar PIXIImus 2 system (Model X2608, General Electric using the LUNAR software version 1.45), and control measurements were made using the small animal quality control phantom. Femurs were scanned 5 times each with re-positioning at each measure. The average value of the bone mineral content (BMC) in grams (g) for each animal is reported as one independent measure.

Table I. Effect of retinoids on mammary tumor volume.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Mean tumor volume (cm³)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Control</td>
<td>0.21±0.05</td>
<td>0.39±0.07</td>
</tr>
<tr>
<td>atRA</td>
<td>0.41±0.10</td>
<td>0.20±0.04</td>
</tr>
<tr>
<td>4-HPR</td>
<td>0.63±0.18</td>
<td>0.32±0.11</td>
</tr>
<tr>
<td>4-HPRCG</td>
<td>0.66±0.23</td>
<td>0.34±0.14</td>
</tr>
</tbody>
</table>

*aThe animals were fed 2 mmol/kg diet of each retinoid.
*bValue = Mean±SEM.
*cChange from baseline.
*dDenote statistical significance, p<0.05
Statistical analysis. Descriptive statistics on tumor volumes, tumor numbers, plasma retinol and triglyceride levels, and bone mineral content were examined and compared among the experimental groups. The statistical significance of the groups’ comparisons were obtained using analysis of variance (ANOVA) and ANOVA with repeated measures tests (21-23). Values were considered significant when \( p \leq 0.05 \).

Results

The structures of the retinoids used in these studies are shown in Figure 1. Rats with established DMBA-induced mammary tumors were fed the retinoids (atRA, 4-HPR and 4-HPRCG) for 28 days at a concentration of 2 mmol/kg diet each, and the effects on tumor volumes and numbers \( \text{vis-à-vis} \) cancer load (CL) were measured and compared to those at the baseline and also to control rats. Figure 2 shows the percent change in tumor volumes in each group as a function of control or retinoid-supplemented diets. Mammary tumors continued to grow actively in rats fed the control diet, while the average size of tumors regressed with time in the animals fed 2 mmol/kg diet of each of the retinoids. In the control animals, over the 4-week treatment period, the average tumor volume increased by 86% compared with almost 51%, 49% and 49% reduction in tumor volumes in the atRA, 4-HPR and 4-HPRCG-treated rats, respectively (\( p < 0.05 \), as compared to their baseline volumes at the beginning of treatment (Table I). As shown in Table II, there was no complete regression of mammary tumors in control rats, whereas regression occurred in almost 80% of tumors in rats that received the retinoid treatments. In the control animals, over the 4-week treatment period, the average tumor volume increased by 86% compared with almost 51%, 49% and 49% reduction in tumor volumes in the atRA, 4-HPR and 4-HPRCG-treated rats, respectively (\( p < 0.05 \), as compared to their baseline volumes at the beginning of treatment (Table I). As shown in Table II, there was no complete regression of mammary tumors in control rats, whereas regression occurred in almost 80% of tumors in rats that received the retinoid treatments. Also, during the treatment period, 9 new mammary tumors appeared in the control group or an approximately 27% increase of total number. In contrast, only one new tumor appeared in each of 4-HPR and 4-HPRCG, treated groups.

Table III. Effect of retinoids on plasma retinol and triglyceride (TG) levels and bone mineralization.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Mean Retinol (µg/ml)( ^a )</th>
<th>%Change(^b ) (Retinol)</th>
<th>Mean Total TG (mg/dl)( ^a )</th>
<th>%Change(^b ) (TG)</th>
<th>BMC (gm)( ^a )</th>
<th>%Change(^b ) (BMC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.49±0.07</td>
<td>–</td>
<td>38.0±1.9</td>
<td>–</td>
<td>0.41±0.01</td>
<td>–</td>
</tr>
<tr>
<td>atRA</td>
<td>0.15±0.01</td>
<td>–69%(^c )</td>
<td>245.0±135.0</td>
<td>+545%(^c )</td>
<td>0.37±0.01</td>
<td>–10%(^c )</td>
</tr>
<tr>
<td>4-HPR</td>
<td>0.18±0.06</td>
<td>–63%(^c )</td>
<td>175.7±50.9</td>
<td>+362%(^c )</td>
<td>0.42±0.01</td>
<td>+2%</td>
</tr>
<tr>
<td>4-HPRCG</td>
<td>0.37±0.01</td>
<td>–24%</td>
<td>39.9±2.2</td>
<td>+5%</td>
<td>0.42±0.01</td>
<td>+2%</td>
</tr>
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</table>

\(^a\)Value = Mean±SEM.\n
\(^b\)Relative to control group.\n
\(^c\)Denote statistical significance, \( p < 0.05 \).

One of the major difficulties and limitations in clinical use of natural and synthetic retinoids is their undesirable toxicity and teratogenicity. Particularly notable toxic side-effects include a reduction in blood retinol levels that can lead to night blindness, as well as lipid abnormalities, liver enlargement and bone demineralization. In order to assess whether 4-HPRCG treatment causes such effects, its toxicity profile was evaluated. As shown in Table III, treatment with atRA or 4-HPR daily for 4 weeks caused an almost 69% and 63% decrease in plasma retinol levels, respectively. In contrast, treatment with an equivalent dose of 4-HPRCG caused no significant reduction in plasma retinol levels, suggestive of a reduced tendency to cause the night blindness that 4-HPR induces.

Moreover, treatment with atRA or 4-HPR, perhaps due to amide hydrolysis, increased the average plasma triglyceride (TG) levels, while 4-HPRCG did not cause this undesirable effect. Significant hypertriglyceridemia occurred in all the rats treated orally with atRA or 4-HPR. Administration of natural or synthetic retinoids for an extended period of time has been associated with significant skeletal abnormalities including osteoporosis (24, 25). The daily treatment with atRA caused 10% reduction in the bone mineral content (BMC) relative to the control group; whereas 4-HPR and 4-HPRCG-treated groups did not show this effect (Table III). In addition, during the 4-week period, only atRA caused a significant reduction in normal weight-gain rate, a common sign of retinoid toxicity (data not shown).

The molecular mechanisms of action of 4-HPRCG and other retinoids for the inhibition of mammary tumor growth have not been clarified yet. It is generally believed that many of the actions of retinoids are mediated by binding the nuclear retinoid receptors which regulate the transcription of critical genes (26, 27). Therefore, the ability of 4-HPR and 4-HPRCG to bind to a variety of nuclear retinoid receptors, relative to atRA was assessed. As shown in Figure 3, 4-HPR and 4-HPRCG exhibited only very weak competition for \([3H]\)-atRA binding to RAR\( \beta \) and \( \gamma \) relative to atRA (\( K_i \) value of 0.7 and 1.0 nM, respectively). Similar weak binding of 4-HPR and 4-HPRCG to the bacterially-expressed RARs \( \alpha \) and \( \beta \) was described earlier by our group (3, 32), whereas the binding of 4-HPRCG to RAR\( \gamma \) has not been previously investigated.
analyzed. In addition, neither 4-HPR nor 4-HPRCG showed any significant competition for \([^{3}H]\) 9-cis-RA binding to RXR up to 10^{-5} M (data not shown). These results indicate that 4-HPRCG, like 4-HPR, is a poor ligand for the nuclear retinoid receptors.

The possibility that limited hydrolysis or cleavage of the amide bond could yield atRA in sufficient amounts to activate the RARs was investigated. We studied the ability of 4-HPRCG to induce the expression of atRA-responsive CYP26 genes in the rat liver and lung. The atRA induces its own catabolism by up-regulating, in an RAR-dependent manner, the expression of both the liver CYP26A1 and lung CYP26B1 genes, that are members of the cytochrome P-450 enzyme system (14, 29-31). Therefore, the expression of CYP26 mRNA in rat liver and lung was used as a marker of endogenous atRA activity. As shown in Figure 4, a marked increase of CYP26A1 mRNA was detected in the livers of animals treated with atRA, but not in the control animals that received the vehicle only. In similar fashion, 4-HPR that has been shown to undergo limited hydrolysis to atRA in vivo (14), was able to increase the expression of CYP26A1 mRNA (Figure 4). Remarkably, CYP26A1 mRNA was not induced in the livers of 4-HPRCG-treated animals. A lack of CYP26B1 induction by 4-HPRCG in the lung was also noted, whereas both atRA and 4-HPR produced an increase in this mRNA (data not shown). Collectively, these data suggest that 4-HPRCG resists hydrolysis or enzyme-catalyzed cleavage in vivo, or alternatively, shows a different tissue distribution or pharmacokinetics compared to atRA and 4-HPR.

**Discussion**

Previous results from our laboratory (3, 32) have shown that 4-HPRCG, the stable C-linked analog of 4-HPR-O-glucuronide possesses a potent chemopreventive effect against mammary carcinogenesis, in terms of causing significant reductions in tumor incidence and tumor burden (multiplicity). The present study shows that 4-HPRCG also has chemotherapeutic effects, and causes a significant regression of pre-established DMBA-induced rat mammary tumors. The chemotherapeutic effects of 4-HPRCG are comparable to the effects of 4-HPR, a potent well-established chemopreventive and antitumor retinoid, in terms of inhibition of tumor burden as well as reduction in tumor volume. 4-HPRCG not only significantly inhibited the growth of established tumors, but also caused nearly 80% of the tumors to regress. These data may be relevant to humans in whom a drug is considered active in any particular tumor histotype if it gives positive results in at least 70% of the patients (33).

One of the major advantages of 4-HPRCG is the relative low toxicity of this analog as compared with other retinoids. Intervention studies in humans or experimental animals by retinoids have been hindered by their significant toxicity at the relatively high and effective concentrations. Efforts have been made to modify atRA to retain its therapeutic potential while reducing its inherent toxicity. 4-HPR represents one such modified retinoid, with significantly reduced toxicity compared to atRA. However, in a clinical study of 4-HPR for the prevention of secondary breast cancer, abnormal retinal functions and reversible night blindness were
reported (34). This side-effect results from displacement of vitamin A (retinol) from its serum retinoid binding protein (RBP), thereby reducing retinol delivery to the eye (34-36). A similar reduction in serum retinol levels in patients receiving 4-HPR has indeed been previously reported (37), together with a proportional decrease in retinol binding protein (38). The treatment of rats with either 4-HPR or 4-HPROG (the O-linked analog of 4-HPR glucuronide) in our studies caused a significant decrease in the rats’ plasma retinol levels (unpublished data). The results of the present study, however, show that, in contrast to 4-HPR, an equimolar concentration of 4-HPRCG did not cause any significant reduction in rat plasma retinol levels, suggesting a reduced tendency to induce night blindness as compared to other retinoids.

In addition, the treatment with atRA or 4-HPR in the present study caused a serious lipid problem, as manifested by a marked increase in plasma triglyceride levels, whereas the 4-HPRCG group did not show such an increase. Rats fed a high dose of 4-HPR (5 mmol/kg diet) for six weeks experienced a significant hepatomegaly and increased liver weights, possibly caused by the accumulation of triglycerides in the liver, whereas rats fed equimolar concentration of 4-HPRCG did not have increased plasma triglyceride levels, showed no such hepatomegaly and their liver weights were similar to those of the control rats (unpublished data).

Retinoids are known to play a significant role in regulating bone development and remodeling, and reduced bone mineral content (BMC) is considered a major risk factor for osteoporotic fractures (39-42). Prevention trials with 4-HPR indicate an improved safety profile regarding its effects on bone mineral density and metabolism (43). In the present study, the treatment with atRA caused a significant decrease in bone mineral content; whereas 4-HPRCG and 4-HPR-treated groups did not show this deleterious effect. Collectively, these results suggest that the stable C-linked analog, with its demonstrated therapeutic activities of potent retinoids such as 4-HPR, deserves continued attention relative to its mechanisms of action and its clinical application, both in cancer chemopreventive and chemotherapeutic activities of potent retinoids such as 4-HPR. However, 4-HPRCG may have a significant advantage over other retinoids since this analog does not reduce blood retinol levels, nor raise blood triglycerides, or produce a decrease in bone mineral content, all of which are possible serious side-effects of retinoids. Therefore, 4-HPRCG deserves continued attention relative to its mechanisms of action and its clinical application, both in cancer chemoprevention and therapy of breast cancer.

The precise mechanism of action of retinamides, including 4-HPR and 4-HPRCG, remains to be elucidated. It is generally believed that retinoid effects occur by the apparent activation of a variety of nuclear retinoid receptors that regulate the transcription of target genes (26, 27, 44, 45). Like other retinoids, either high concentrations of 4-HPR or/atRA that are liberated from 4-HPR hydrolysis in vivo can up-regulate the expression of RARβ and activate transcription of retinoic acid response elements by RARs (44-46). However, the ability of 4-HPR to induce apoptosis and inhibit cell proliferation in cells that are resistant to retinoic acid suggests that 4-HPR may also act in pathways that are independent of the RARs (47-52). The propensity of a retinoid analog to liberate atRA in vivo may be a significant factor in determining its relative toxicity. Although both 4-HPR and 4-HPRCG exhibit poor binding to the RARs, only 4-HPR shows significant induction of the cytochrome P450, CYP26A1 in the liver. Our recent studies show that hydrolysis of 4-HPR to atRA does occur in vivo (14) and, thus, may account for its ability to both induce atRA-responsive CYP26 mRNA expression and to increase blood triglycerides. It has been reported that retinoid-induced hypertriglyceridemia is mediated, at least in part, through the RAR pathway and is significantly inhibited by co-treatment with RAR-selective antagonists (53). The lesser ability of 4-HPRCG to bind to the nuclear retinoid receptors and to induce the atRA-responsive CYP26A1 or CYP26B1 mRNAs shows that activation of the RAR pathway is unlikely to account for the activity of this analog. The 4-HPRCG analog is resistant to the action of the β-glucuronidase enzyme (32) and, in fact, acts as an inhibitor to the β-glucuronidase enzyme (12), and the presence of glucuronide function may further hinder the action of amidases, thus preventing the liberation of atRA. Interestingly, another stable C-linked analog of 4-HPR, 4-hydroxybenzyl retinone (4-HBR), that cannot be hydrolyzed to atRA in vivo, does not bind strongly to retinoid receptors and does not induce the atRA-responsive CYP26B1 in the lungs of vitamin A-deficient (VAD) rats (14), yet also showed chemotherapeutic activity (54). Taken together, the results substantiate the notion that these stable retinoids, that do not hydrolyze in vivo, may act, at least in part, through receptor-independent mechanisms that remain to be elucidated.

In conclusion, the stable C-linked glucurononide analog, 4-HPRCG, shares both the chemopreventive and chemotherapeutic activities of potent retinoids such as 4-HPR. However, 4-HPRCG may have a significant advantage over other retinoids since this analog does not reduce blood retinol levels, nor raise blood triglycerides, or produce a decrease in bone mineral content, all of which are possible serious side-effects of retinoids. Therefore, 4-HPRCG deserves continued attention relative to its mechanisms of action and its clinical application, both in cancer chemoprevention and therapy of breast cancer.

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References


