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PHARMACOKINETICS RELEVANT TO THE ANTI-CARCINOGENIC AND ANTI-TUMOR ACTIVITIES OF GLUCARATE AND THE SYNERGISTIC COMBINATION OF GLUCARATE:RETINOID IN THE RAT

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Abstract—Alone and in synergistic combination with retinoids, dietary glucarate inhibits both the chemical induction and growth of rat mammary tumors. To investigate the pharmacokinetics of glucarate, [^{14}C]glucarate was synthesized, converted to the calcium salt, and administered to rats bearing primary mammary tumors. When given by gavage, [^{14}C]glucarate, as the calcium salt, showed a biphasic response in the blood. After peaking within 1 hr of administration at a level of 0.4 μmol/mL (normal endogenous level is approximately 0.04 μmol/mL), its plasma concentration dropped to 0.1 μmol/mL at 3 hr. In the second phase, there was a semilog increase to 0.6 μmol/mL at 15 hr, followed by a slow rise to 0.75 μmol/mL at 24 hr. Of the 38% of the administered glucarate that was recovered, 38% was excreted in the urine, and 30% remained in the gastrointestinal tract at 24 hr. Glucarate was concentrated 3- to 4-fold in the liver and intestinal mucosa, compared to the level in serum. With minor exception, the pharmacokinetics of [^{14}C]13-*cis*-retinoic acid administered by gavage to rats was similar whether or not the semipurified diets were supplemented with 64 mmol/kg of calcium glucarate. During the interval between 5 and 10 hr post-administration of [^{14}C]13-*cis*-retinoid, there was a transient 35–50% rise in the plasma level in rats on the glucarate-supplemented diet. This rise had no observable effect on the level of retinoid in major organs or in the tumor. A glucarate-binding protein was detected in the tumor cytosol. This potential receptor had a K_a of 1.49×10^7 M^{-1}.

Key words: glucarate; retinoids; synergistic interaction; pharmacokinetics; anti-tumor activity; chemopreventive activity

D-Glucaric acid was shown previously to have both anti-tumor [1] and cancer chemopreventive or anti-carcinogenic [2] activity when fed as a single agent to rats at a dose of 128 mmol/kg diet. Most studies to date focused on the DMBA§-induced rat mammary tumor system [1, 2], although activity has also been demonstrated against carcinogenesis in the skin, liver and intestinal mucosa [3–5]. The anti-tumor activities of glucarate have been demonstrated in cell [6] and organ [7] culture. Glucarate is of particular interest as a potential anti-cancer or cancer chemopreventive agent since it has significant activity, yet appears to be non-toxic [8]. That retinoids similarly exhibit anti-tumor and cancer chemopreventive activity (usually in the range of 1.5 to 2.0 mmol/kg diet) against a wide spectrum of organs is well documented [9]. However, long-term treatment with effective concentration of retinoids in both humans and experimental animals is often complicated by cumulative toxicity at or near the effective dose [10]. The finding that the dosage of retinoid could be reduced at least 50% through combination with glucarate [1, 2] offers a potential means of circumventing the toxicity. The combination was effective against DMBA-induced rat mammary tumors *in vivo* [1, 2] and the human MCF-7 breast adenocarcinoma cell line *in vitro* [6]. In all *in vivo* studies, glucarate was administered as the calcium salt and equimolar calcium glucarate was shown to be inactive [1–5].

Mechanistically, retinoids interact initially with specific nuclear receptors [9] and later cause changes in signal transduction pathways [10]. However, little is known concerning either the pharmacokinetics or mechanism of action of glucarate. The ability of the 1,4-lactone of this 6-carbon polyhydroxydicarboxylic acid to inhibit β-glucuronidase [11] cannot account for most of its anti-cancer activity in feeding protocols [12]. Rather, like retinoids, glucarate appears to act through signal transduction pathways involving cAMP, tumor growth factor-β and protein kinase C [13].

In view of the unknown basis of its anti-cancer activity, it was important to obtain information on the uptake, the tissue distribution and the excretion of glucarate and the effect of co-administration of this compound on similar parameters for the retinoids. Furthermore, it was important to evaluate the possibility that glucarate is acting via a receptor. Although anti-tumor synergism has been

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§ Abbreviations: DMBA, 7,12-dimethylbenzanthracene; and PEG, polyethylene glycol.

demonstrated between glucarate and either *N*-(4-hydroxyphenyl)-retinamide or 13-*cis*-retinoic acid, the latter was selected for these studies due to the availability of the labeled compound. The agents are evaluated in the mammary tumor-bearing rat.

MATERIALS AND METHODS

In vivo protocols. Primary mammary tumors were induced [1, 2] by dosing 50-day-old female Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) by gavage with 15 mg of DMBA in 1.0 mL of sesame oil. Mammary tumors developed within 8–10 weeks at which time the rats weighed approximately 200 g. Rats were selected from a pool of DMBA-treated rats so that each had one tumor of approximately 2.5 g, that was of similar volume as determined with micrometer calipers [13]. The rats were maintained on an AIN-76A semipurified diet (Dyets, Bethlehem, PA) in which the carbohydrate source was 52% corn starch and 13% dextrose. Where indicated, glucarate was incorporated into the diet as the calcium salt (Gallard Schlessinger, New York, NY). Additives were mixed into the diet with a commercial food mixer, and the final nutrient density was the same for all diets. [^{14}C]13-*cis*-Retinoic acid and unlabeled 13-*cis*-retinoic acid were obtained from Hoffman–LaRoche, Nutley, NJ. The [^{14}C]retinoic acid was adjusted to a specific activity of 1.5 μCi/μmol before being administering by gavage in ethanol : tricaprylin (1 : 4). [^{14}C]Glucarate, synthesized as described below, then mixed with unlabeled calcium glucarate to give a specific activity of 0.03 μCi/μmol, was also administered by gavage as a uniform suspension in 1.0 mL of 10% gum acacia as previously described [3]. Control rats received an equivalent volume of the vehicle.

[^{14}C]*Glucarate synthesis.* [^{14}C]Glucarate was prepared by us (M.D., A.T.H.) from [^{14}C]glucose (American Radiochemistry Corp., St. Louis, MO) by nitric acid oxidation, and then recrystallization from hot water [14]. The D-[U-^{14}C]glucose (2.0 mCi, 340 mCi/mmol) was first mixed with 0.126 mmol of unlabeled glucose in water. The solution was taken to dryness under argon in a 1.0-mL conical reaction flask. Water (20 mL), concentrated nitric acid (64 μL), and one crystal of sodium nitrate were added to the residue, and the reaction was heated with stirring at 60° for 4 hr, during which time brown gas evolved. The reaction mixture was taken to dryness under a stream of argon at 60°. A few drops of water and two drops of a saturated solution of potassium carbonate were added to bring the solution to pH 8.0. The reaction mixture was heated for an additional 30 min; then it was transferred to a Craig tube and acidified with acetic acid to pH 2.0. Monopotassium glucarate crystallized out to yield 7.0 mg (22.5%) of product with m.p. 185° (dec.) [lit. 188°]. The final activity was 502.4 μCi with a specific activity of 17.69 mCi/mmol. The potassium salt was converted to the calcium salt for *in vivo* studies and adjusted to a final specific activity of 0.03 μCi/mol. For the receptor studies, the glucarate was used as prepared without dilution with cold glucarate.

In vivo distribution of [^{14}C]*glucarate and* [^{14}C]-*retinoid.* In any one experiment, two rats were housed individually in two plastic metabolic cages, and the experiment was repeated to achieve the numbers of rats specified below. In one series of experiments, 500 μL of blood was collected from the tail vein of each of two rats at the specified intervals. In a second series of experiments using four rats, the major organs were removed, weighed, and homogenized in 5.0 mM EDTA for recovery of [^{14}C]glucarate. The blood, urine, gastrointestinal contents and feces were also obtained at 24 hr. The homogenate was first boiled and then centrifuged, and both the supernatant and the pellet, dissolved in tissue solubilizer (Unisol, Isolab Inc., Akron, OH), were radioassayed in liquid scintillant (Unisol Complement, Isolab Inc.). An attempt was made to recover all of the [^{14}C]glucarate administered by repeatedly (three times) boiling the carcass in 5.0 mM EDTA and counting aliquots of the extracts. The feces and gastrointestinal contents were treated as for the tissues. Aliquots of the urine and plasma were counted directly in liquid scintillant. To determine the effect of dietary glucarate on the pharmacokinetics of [^{14}C]13-*cis*-retinoic acid at 24 hr, the tissues, feces and gastrointestinal contents were similarly extracted, but with absolute ethanol; then the extract and solubilized pellet were counted as before. The plasma and urine were counted directly. No attempt was made to recover all of the [^{14}C]retinoid. For the temporal change in blood [^{14}C]retinoid, one control rat and one rat on the glucarate-supplemented diet were monitored. The 24-hr assay of organ, blood and excreted [^{14}C]retinoid was based on three control and three experimental rats. The rats were followed in pairs, individually housed in metabolic cages.

Assay of cytosol for glucarate-binding proteins. The rat mammary tumor cytosol was assayed for proteins that bound [^{14}C]glucarate as follows. Mammary tumors induced in rats as described above were homogenized in 4 vol. (w/v) of phosphate-buffered saline (PBS). The cytosolic fraction was obtained by recentrifuging the supernatant, obtained after removal of nuclei and mitochondria at 10,000 *g* for 10 min, for an additional 90 min at 100,000 *g*. The cytosolic fraction, containing approximately 2.5 mg protein/mL as determined by the biuret assay, was aliquoted and stored at −70° until used. The "total binding" assay consisted of 100 μL of cytosol, 100 μL of glycerol (50%)–bovine serum albumin (5%), 100 μL [^{14}C]glucarate (sp. act. 21.45 μCi/mmol; concentration range 20–500 nM) and 1.7 mL of binding buffer (50 mM Tris, pH 7.6, at 25° 2.0 mM $CaCl_2$; 1.0 μM $MgCl_2$; 21.0 mM dithiothreitol). The "competitive assays" contained in addition 10.0 μM unlabeled glucarate, used as the moderately soluble monopotassium salt. A "total recovery" assay, used to correct for small losses, consisted of the binding buffer, the glycerol + bovine serum albumin, and [^{14}C]glucarate.

Following a 30-min incubation at 35°, 1.0 mL of 10% polyethylene glycol (PEG; mol wt 8000; Sigma Chemical Co., St. Louis, MO) was added to co-precipitate the proteins. After holding for 5 min in an ice bath, the mixtures were poured into all glass filter holders (Millipore Corp., Bedford, MA) with 2.4 cm glass microfiber membranes (GF/C; Whatman

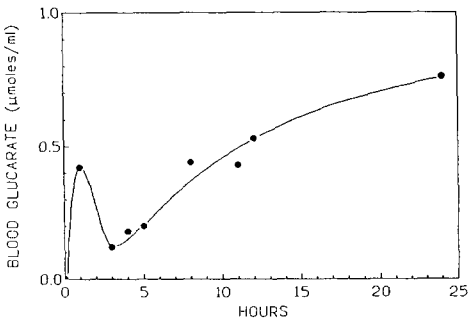

Fig. 1. Blood levels of [^{14}C]glucarate in rats bearing a mammary tumor. The rats were gavaged with 30 μCi of [^{14}C]glucarate at zero time. Values represent averages of two rats (with mammary tumors of approximately 2.5 g).

Table 1. Pharmacokinetics of [^{14}C]glucarate in the female rat

Biological specimen	[^{14}C]Glucarate content at 24 hr*	
	μCi/g or mL	μmol (total)
1. Blood	0.03 ± 0.00	11.30 ± 2.25
2. Urine	0.74 ± 0.500	109.80 ± 9.00
3. Feces	0.75 ± 0.682	45.82 ± 15.81
4. Stomach contents	0.04 ± 0.012	0.60 ± 0.30
5. Intestinal contents	0.87 ± 0.173	82.53 ± 9.60
6. Organs		
Liver	0.10 ± 0.022	18.81 ± 5.60
Intestine	0.10 ± 0.021	15.0±1.80
Lung	0.03 ± 0.001	1.35 ± 0.15
Brain	0.02 ± 0.010	0.92 ± 0.60
Spleen	0.05 ± 0.001	0.93 ± 0.11
Kidneys	0.05 ± 0.005	2.34 ± 0.45
Heart	0.02 ± 0.002	0.66 ± 0.00
Stomach	0.04 ± 0.010	1.55 ± 0.32
7. Mammary tumor	0.04 ± 0.014	3.55 ± 2.33
8. Carcass	0.01 ± 0.001	12.64 ± 0.67

* These data represent the means ± SEM of 24-hr samples from four rats with single mammary tumors of approximately 2.5 g.

Int., Maidstone, UK). The filters were pretreated by immersion for 20 min in 0.5% polyethylenimide (Sigma). The protein–receptor complex trapped on the filters was washed quickly three times with 10% PEG, and the filters were counted in liquid scintillant (Scintiverse BD; Fisher Scientific Co., Fair Lawn, NJ) using an LS8100 counter (Beckman Instruments, Irvine, CA). The total and non-specific binding (nmol) was calculated from the known specific activity of glucarate and was corrected for losses (recovery assay) to give the actual concentration of the free and bound glucarate. The K_d was estimated from a Scatchard analysis [15] of the binding data.

In these experiments, care was taken to minimize conversion of glucarate to the corresponding lactones by preparing fresh neutral solutions just before use. Significant lactone formation occurs only during prolonged exposure in solution at elevated temperature in acid solution.

HPLC analysis of glucarate in sera. For analysis by HPLC, the plasma samples were deproteinized by use of Centricon 3 filters (Amicon Inc., Beverly, MA) to avoid low pH, which might induce some equilibrium formation of the lactones. HPLC analysis was done as previously described [16] on an HPX87-H Aminex column (Bio-Rad Laboratories, Richmond, CA) with isocratic elution using 0.01 N H_2SO_4–10% acetonitrile as the mobile phase and monitoring at 210 nm. The plasma was diluted 1:2 with the mobile phase, and 100 μL was loaded on the column. Authentic glucaric acid and D-glucaro-1,4-lactone (Sigma) were used as standards.

RESULTS

[^{14}C]*glucarate uptake and excretion.* Female rats, each bearing a mammary tumor of approximately 2.5 g were each given 30 μCi of [^{14}C]glucarate in 1.0 mmol of calcium glucarate (sp. act. 0.03 μCi/μmol; 900 μmol), which approximates the daily uptake of rats fed an effective dose of dietary calcium glucarate, i.e. 128 mmol/kg diet. Following treatment, the rats were housed in metabolism cages. In one series 0.5 mL of blood was drawn at intervals up to 24 hr and in the second series the 24-hr urine and feces, tissues and intestinal contents were obtained for analysis. The results of the radiochemical analyses are summarized in Fig. 1 and Table 1.

The [^{14}C]glucarate concentration in the blood showed an initial transient rise with a peak at 1 hr post-[^{14}C]glucarate administration. After falling to a minimum at 3 hr the blood concentration rose until 12 hr, then increased slowly at a rate of 0.01 μmol/hr over the next 12 hr. The peak concentration of glucarate at 1 hr (0.4 μmol/mL) was 10-fold higher than the concentration normally present in the blood [17], while at 15 hr it was more than 15- to 20-fold higher. It is probable that the initial transient peak at 1 hr was the result of glucarate absorption from the stomach and that the subsequent more prolonged absorption phase was due to absorption from the intestine. Since 0.5 mL of blood was withdrawn at each time point, it was not unexpected that the glucarate level at 24 hr was 25% lower than that of rats bled only at 24 hr (see Table 1).

An examination of the data (μCi/g) in Table 1 indicates that glucarate was present in all organs, and was 3-fold higher in the liver and intestinal mucosa than in the blood. The data (total μmol) in Table 1 also indicate that by 24 hr post-administration, 9.2% of the total amount of glucarate administered was still present in the intestinal contents. Only 38% of the total radioactivity administered was recovered. Of this, 38% was excreted in the urine by 24 hr.

The inability to account for 65% of the [^{14}C]glucarate administered was surprising. However, enzyme systems can be induced in intestinal tract bacteria which convert glucarate to CO_2 and water [17], although glucarate is the normal end product of glucarate metabolism in the body [18]. It is proposed, therefore, that in excess of one-

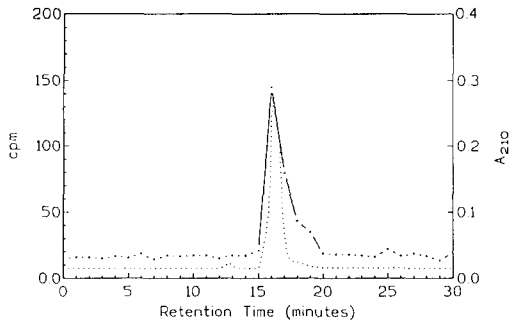

Fig. 2. HPLC analysis (profile) of deproteinized serum from a rat 24 hr after oral administration of [^{14}C]glucarate. Shown is the absorption profile at 210 nm of authentic glucarate (. . . .) and the profile of radioactivity in the serum of a rat that received [^{14}C]glucarate (–■–). Authentic D-glucaro-1,4-lactone eluted with a retention time of 25 min (not shown).

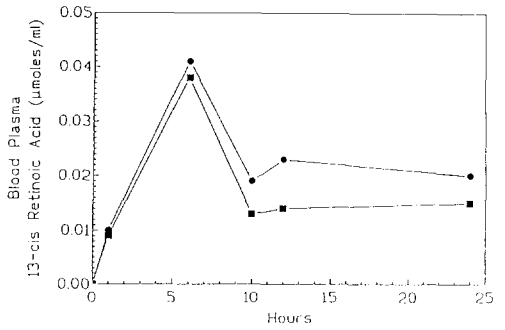

Fig. 3. Blood levels of [^{14}C]13-*cis*-retinoic acid in rats bearing a mammary tumor. The control rats (■) were maintained on standard AIN-76A diet, while the experimental rats (●) received AIN-76A diet fortified with calcium glucarate. The rats were gavaged with [^{14}C]13-*cis*-retinoic acid at zero time. Each profile represents the data from one rat.

half of the administered glucarate was metabolized by intestinal bacteria.

HPLC analysis on an HPX87-H column (Bio-Rad Laboratories) of extracts of sera from rats receiving [^{14}C]glucarate was carried out as previously described [16]. As shown in Fig. 2, essentially all radioactivity eluted with a retention time (16 min) typical of glucarate, with less than 3% eluting with a retention time typical of D-glucarolactone (25 min). No significant amounts of any other components were detected. The profile shown represents the 24-hr blood specimen but was typical of specimens collected at 1, 5 and 10 hr. This analysis does not completely rule out the possibility that minimal amounts of [^{14}C]glucarate metabolites were absorbed from the intestine, and that these are rapidly taken up by tissues, thereby keeping the blood level very low.

Effect of glucarate on [^{14}C]*retinoid uptake and excretion.* This experiment was designed to determine whether dietary calcium glucarate affected the pharmacokinetics of [^{14}C]13-*cis*-retinoic acid. As noted earlier, glucarate and retinoid interact synergistically to inhibit tumor cell growth. Rats bearing mammary tumors of approximately 2–5 g were maintained on control AIN-76A diet with or without fortification with 64 mmol/kg calcium glucarate for 1 week, and then were administered 30 μCi of [^{14}C]13-*cis*-retinoic acid (sp. act. 1.5 μCi/μmol) in 1.0 mL of ethanol:tricaprylin (1:4) by gavage. Results of a radiochemical analysis of the blood plasma at indicated time points, or of the urine and feces, intestinal contents and major organs in addition to the blood at 24 hr post-administration, are summarized in Fig. 3 and Table 2.

As shown in Fig. 3, blood [^{14}C]retinoid transiently increased to peak at 0.04 μmol/mL around 6 hr post-administration, then fell to a constant level of about 0.025 μmol/mL at 10 hr. The concentration of retinoid was 9% higher at 6 hr and 24% higher at 24 hr in the blood of rats fed the calcium glucarate. It seems unlikely that the small difference could account for the synergistic interaction between glucarate and retinoid. In fact, other studies have shown that synergism with glucarate is seen over the range of 0.25 to 1.5 mmol/kg diet of the retinoid (Webb, TE, unpublished data), whereas tumor inhibition by the retinoid as a single agent is not achieved until 2.0 mmol/kg diet is reached [19]. Furthermore, the small differential in the concentration of 13-*cis*-retinoic acid in the blood plasma of rats fed diets with and without glucarate supplementation was not apparent in the tissues. The data in Table 2 further indicate that the concentration (in μCi/g) of the retinoid in the liver, intestinal mucosa, and tumor was slightly higher in the rat on the experimental diet. About 94 and 73% of the retinoid administered was recovered in the fractions (including three organs) that were analyzed (Table 2) for the rats on the control diet and the experimental diet, respectively. In summary, it does not appear that glucarate changes the uptake or excretion of retinoid to a degree that could account for the synergistic interaction.

It should be noted that 13-*cis*-retinoic acid is known to be absorbed from the intestine into the bloodstream of the intact rat, although 20–25% of the retinoid may be converted to the equally potent *trans*-isomer [20].

Detection of a glucarate-binding protein. Significant binding of glucarate to components in the cytosolic fraction of rat mammary tumors occurred whether the [^{14}C]glucarate was administered to the rat or whether the [^{14}C]glucarate was incubated with the cytosolic fraction *in vitro* (Webb TE, unpublished data).

Initial experiments indicated in the cytosolic fraction of primary DMBA-induced rat mammary tumors the presence of a protein(s) that bound [^{14}C]glucarate in the absence, but not in the presence of a 100-fold excess of unlabeled glucarate. Binding in this fraction was studied further. Shown in Fig. 4 is the saturation curve for specific binding of [^{14}C]glucarate to cytosolic proteins and the Scatchard

Table 2. Effect of dietary glucarate on the pharmacokinetics of [^{14}C]13-*cis*-retinoic acid

Biological specimen (24 hr)	Control (AIN-76A) diet*		AIN-76A + 64 mmol CGT*	
	μCi/g or mL	Total μmol	μCi/g or mL	Total μmol
1. Blood	0.02 ± 0.01	0.34 ± 0.11	0.03 ± 0.00	0.37 ± 0.07
2. Urine	0.23 ± 0.07	1.06 ± 0.33	1.04 ± 0.39	1.79 ± 0.06
3. Feces	2.10 ± 1.55	2.21 ± 1.80	6.32 ± 0.02	1.73 ± 0.65
4. GI tract contents	9.30 ± 1.64	11.85 ± 2.79	8.54 ± 6.08	7.56 ± 2.57
5. Organs				
Liver	0.10 ± 0.02	0.52 ± 0.11	0.11 ± 0.01	0.55 ± 0.02
Intestinal mucosa	0.67 ± 0.06	2.75 ± 0.43	1.11 ± 0.65	2.44 ± 0.68
Tumor	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.21 ± 0.19

* Based on the value obtained 24 hr post-administration of [^{14}C]13-*cis*-retinoic acid. Total μmol refers to the μmol of retinoid in the total blood, urine, feces, intestinal contents, organ or tumor recovered from the rat. Data are means ± SEM of three control and three rats on the experimental (glucarate-supplemented) diet, which had tumors of approximately 2.5 g. CGT = calcium glucarate.

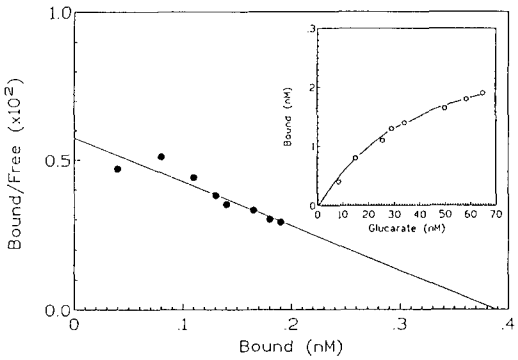

Fig. 4. Scatchard analysis of specific [^{14}C]glucarate binding data from rat mammary tumor cytosol. The number of binding sites relative to volume is indicated by the intercept with the abscissa. The nmol/mg protein and nmol/g tissue (see text) were calculated by extrapolation to zero dilution. The K_a was calculated from the slope of the line obtained by linear regression in the Scatchard plot (B/F vs B). Shown in the inset is the saturation curve of specific binding.

plot of the binding data used to estimate the binding constants. The association constant (K_a) was $1.49 \times 10^7 M^{-1}$ ($K_D = 6.7 \times 10^{-8}$ M) and the receptor concentration was 0.53 nM (0.53 nmol/mg protein).

Essentially identical results were obtained by trapping and washing the cytosolic proteins after incubation with [^{14}C]glucarate on DE-81 filter discs (Whatman Co., Freehold, NJ), which were then counted in liquid scintillant.

The component that binds [^{14}C]glucarate appears to be protein in nature. Approximately 81% of the specific binding was lost upon incubation of the cytosol with 2.0 U/mL proteinase K-acrylic beads (nuclease-free; Sigma) for 15 min at pH 7.5 and 37°. A control was incubated without protease.

DISCUSSION

This study presents for the first time data on the pharmacokinetics of glucarate. The slow prolonged absorption of this compound from the intestine maintained maximal blood levels constant for at least 24 hr. This contrasted with the apparent rapid transient absorption from the stomach. The mammary tumor, in contrast to the liver and intestinal mucosa, did not concentrate glucarate to concentrations above that found in the blood despite the fact that it responded by growth inhibition. It is probable that the steady-state concentration of glucarate in the intestinal mucosa was higher than in the blood and other tissues due to continuous absorption from the intestine and in the liver due to the portal blood supply. Thus, these tissues may be more sensitive to dietary glucarate than, for example, the mammary gland. Calcium glucarate was selected over more soluble salts on the premise that it would act as a sustained release form of glucarate *in vivo*. This expectation appears to have been fulfilled. The bioavailability of glucarate in the calcium salt appeared to be relatively good since less than 20% could be accounted for in the digestive tract and feces, the remainder being metabolized by the intestinal bacteria, taken up into the blood and various tissues and excreted into the urine.

The kinetic data indicate that glucarate did not affect markedly the pharmacokinetics of retinoid in the rat. This suggests that the synergistic interaction between glucarate and retinoids when both are fed in the diet is due to other factors. Similarly, administering high dosages of glucarate by gavage, where significant inhibition of β-glucuronidase is observed [2], does not apply under the condition of chronic feeding in the diet. 13-*cis*-Retinoic acid did not concentrate in the mammary tumor, even in the presence of dietary glucarate.

As shown elsewhere [6, 12, 13], glucarate acts directly on rat mammary tumor cells *in vivo* and *in vitro* to inhibit their growth. Recent evidence [13] suggests that, like retinoids with which it interacts synergistically, the activity of glucarate is mediated via signal transduction pathways involving cAMP and protein kinase C. Since the mechanism by which a simple six-carbon polyhydroxy dicarboxylic acid

could initiate such cellular events is unknown, a search was made for a possible glucarate receptor that could serve as mediator. A high-affinity, low-capacity glucarate-binding protein was identified in the cytosol, which has the expected properties of the putative receptor. Its K_a was 1.49×10^7 M^{-1}, and the concentration was approximately 19.5 pmol/g of tumor. In comparison, the K_a for the interaction of progesterone with its receptor varies from 1×10^9 M^{-1} in the uterus to 3×10^7 M^{-1} in the liver and kidney, with 29–38 pmol of receptor sites per g tissue [21], while the K_a for interaction of dexamethasone with glucocorticoid receptors in Novikoff ascites cells is 1.3×10^8 M^{-1} and in rat liver, 6.9×10^7 M^{-1} [22]. The binding protein thus exhibits a moderate affinity for glucarate. The K_a for glucarate binding to this putative receptor is approximately 10-fold higher than that for the binding of the D-glucaro-1,4-lactone to β-glucuronidase [23].

Within 24 hr post-administration of [^{14}C]glucarate, its concentration in the tumor cytosol was 1.3 μM and at this concentration the putative receptor should be saturated. Since 3.55 μmol of glucarate was bound/5.0 g tumor (Table 1) and 4.0 nmol/mL was bound in the cytosol at saturation, it appears that only approximately 0.004% of the glucarate in the tumor was specifically bound.

In conclusion, the data in this manuscript reaffirm the contention that glucarate, like retinoids with which it interacts synergistically, must have direct effects on normal, preneoplastic and transformed cells. This conclusion is consistent with both *in vitro* chemopreventive and anti-cancer effects of glucarate (cf. review in Ref. 12).

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