

*Gut Metabolism of Glucaric Acid*

Calcium D-Glucarate

(Stomach)

Glucaric acid

D-glucaro-6,3-lactone

\*D-glucaro-1,4-lactone

\* *inhibits beta-glucuronidase***Calcium-D-Glucarate****Introduction**

Calcium-D-glucarate is the calcium salt of D-glucaric acid, a substance produced naturally in small amounts by mammals, including humans. Glucaric acid is also found in many fruits and vegetables with the highest concentrations to be found in oranges, apples, grapefruit, and cruciferous vegetables.<sup>1</sup> Oral supplementation of calcium-D-glucarate has been shown to inhibit beta-glucuronidase, an enzyme produced by colonic microflora and involved in Phase II liver detoxification. Elevated beta-glucuronidase activity is associated

with an increased risk for various cancers, particularly hormone-dependent cancers such as breast, prostate, and colon cancers.<sup>2</sup> Other potential clinical applications of oral calcium-D-glucarate include regulation of estrogen metabolism and as a lipid-lowering agent.

**Pharmacokinetics**

Upon ingestion and exposure to the acidic environment of the stomach, calcium-D-glucarate is metabolized to form D-glucaric acid. D-glucaric acid is further metabolized in the gastrointestinal tract into three compounds existing in equilibrium and comprised of approximately 40-percent D-glucaric acid, 30-percent D-glucaro-1,4-lactone, and 30-percent D-glucaro-6,3-lactone. These compounds are then transported to the blood and various internal organs, and are subsequently excreted in the urine and bile. Although D-glucaro-1,4-lactone seems to be the most pharmacologically active of the three, it is not commercially available. Also, calcium-D-glucarate administration results in longer inhibition of beta-glucuronidase (five hours versus one hour) than does D-glucaro-1,4-lactone, so it is the compound used.<sup>3</sup>

**Mechanism of Action**

Calcium-D-glucarate's detoxifying and anticarcinogenic properties are attributed to its ability to increase glucuronidation and excretion of potentially toxic compounds. During Phase II detoxification, chemical carcinogens, steroid hormones, and other lipid-soluble toxins are conjugated with glucuronic acid in the liver (glucuronidation), and excreted through the biliary tract. Beta-glucuronidase is capable of deconjugating these potential toxins, making it possible for them to be reabsorbed rather than excreted. D-glucaro-1,4-lactone is the metabolite that has been shown to inhibit beta-glucuronidase activity, increasing excretion of conjugated xenobiotic compounds and decreasing activity of harmful substances that are most

active in their deconjugated state.<sup>4,5</sup> Inhibition of beta-glucuronidase ultimately results in potentially decreasing the risk of carcinogenesis.<sup>6</sup> In addition, by reducing the beta-glucuronidase viability and activity of intestinal bacteria, salts of D-glucaric acid have been shown to enhance enterohepatic circulation and reduce steady state levels of cholesterol synthesis, resulting in decreased serum lipid levels.<sup>7</sup>

## Deficiency States

Calcium-D-glucarate is not an essential nutrient so, technically, no deficiency state exists. However, since it is only produced in small amounts by humans, it is important that dietary intake be adequate. Diets low in fruits (particularly oranges, apples, and grapefruit) and cruciferous vegetables (broccoli, cabbage, and brussel sprouts) may result in a relative deficiency of calcium-D-glucarate and its metabolites. Research has shown a low level of D-glucaric acid correlates with a higher level of beta-glucuronidase, which in turn is associated with an increased risk for various cancers.<sup>2</sup>

## Clinical Indications

### Cancer

The anticarcinogenic properties of D-glucaric acid and its salts have been studied in various animal tumor models, including colon,<sup>8,9</sup> prostate,<sup>2</sup> lung,<sup>10</sup> liver,<sup>11,12</sup> skin,<sup>13</sup> and breast<sup>14-18</sup> cancer, with the mechanism of action for tumor inhibition being very similar in each. These studies demonstrated decreases in beta-glucuronidase activity, carcinogen levels, and tumorigenesis. The preponderance of research, however, has been conducted on mammary tumors in the rat, the animal model most frequently used for breast cancer research.

### Breast Cancer

A number of studies have shown calcium-D-glucarate alone, and in combination with retinoids, inhibits mammary carcinogenesis in rats by as much as 70 percent.<sup>3</sup> Natural retinoids have been shown to be effective chemopreventive

agents at high doses, but unfortunately the cumulative toxic effects of high doses have restricted their prolonged use. Several studies have demonstrated low-dose retinoids in combination with calcium glucarate interact synergistically to inhibit mammary tumor growth in both animal models and human cell lines.<sup>14-18</sup> The mechanisms responsible for the chemopreventive effects of these two agents may be similar. Both retinoids and calcium-D-glucarate inhibit carcinogenesis during the promotion and initiation phases. Calcium-D-glucarate inhibits protein tyrosine kinase-C activity and induces transformation growth factor beta, possibly resulting in an increase in cellular differentiation and slower progression through the cell cycle.<sup>15</sup> Retinoids induce many of these same biochemical effects.<sup>19</sup> Additionally, calcium-D-glucarate enhances glucuronidation and subsequent excretion of carcinogens and other cancer-promoting agents.

Published human studies on calcium-D-glucarate and breast cancer are few but, due to the encouraging results of the animal studies, the National Cancer Institute has initiated a Phase I trial in patients at high risk for breast cancer at Memorial Sloan Kettering Cancer Center. This trial is examining the use of calcium-D-glucarate as an alternative to tamoxifen's blocking of estrogen receptors. Preliminary results are quite encouraging and due to calcium-D-glucarate's excellent safety profile, it may be a more effective option than tamoxifen, which has numerous side effects.<sup>3</sup> Other human trials are being conducted at M.D. Anderson Cancer Center in Houston, Texas and AMC Cancer Research Center in Denver, Colorado.

### Colon Cancer

Studies in rats have shown D-glucarate salts to inhibit colon carcinogenesis alone and in combination with 5-fluorouracil (5-FU). In one study, D-glucarate markedly inhibited azoxymethane-induced colon carcinogenesis as evidenced by a 60-percent reduction in both tumor incidence and multiplicity. It was hypothesized that malignant cell proliferation was suppressed by inhibition of beta-glucuronidase. Another

possible mechanism may involve alterations in cholesterol synthesis or its conversion to bile acids.<sup>8</sup> The second study demonstrated that salts of D-glucarate, in combination with 5-FU in rat colon tumor explants, resulted in a potentiation of 5-FU's antitumor activity. D-glucarate alone also showed antitumor activity.<sup>9</sup>

### Liver Cancer

Hepatocarcinogenesis is thought to be preceded by premalignant hepatic foci that are subsequently transformed to malignant cells. Two separate rat studies by a group of researchers at Ohio State University have demonstrated calcium-D-glucarate delays the appearance of altered hepatic foci and significantly inhibits hepatocarcinogenesis, if given during both the initiation and promotion phases. Maximal inhibition was obtained when calcium-D-glucarate was administered by gavage prior to the carcinogenic agent, diethylnitrosamine.<sup>11,12</sup>

### Lung Cancer

A study conducted on mice demonstrated calcium-D-glucarate inhibits benzo[a]pyrene's ability to bind DNA and induce pulmonary adenomas.<sup>10</sup> Another unpublished phase I clinical trial of 62 patients found D-glucaric acid levels were approximately 29-percent lower in smokers than non-smokers. Regardless of gender, K-ras (an oncogene linked to lung cancer) mutations were found to be present in 38 percent of subjects who smoked, while no K-ras mutations were found in the non-smoking control subjects. It was hypothesized that D-glucaric acid deficiency correlates with K-ras mutations and might be indicative of a higher risk for developing lung cancer.<sup>20</sup>

### Skin Cancer

The efficacy of dietary calcium-D-glucarate as a chemopreventative agent has also been studied in the mouse skin tumorigenesis system. Mice were given 7,12-dimethylbenz[a]anthracene (DMBA) to induce skin tumorigenesis and were fed either a regular chow diet or a chow diet fortified with calcium-D-glucarate. When fed the calcium-D-glucarate

chow through both the initiation and promotion phases, papilloma formation was inhibited by over 30 percent. The data indicate that supplementation of calcium-D-glucarate results in a marked alteration in the retention, activity, and metabolism of carcinogenic substances.<sup>13</sup>

### Estrogen Metabolism

Calcium-D-glucarate's inhibition of beta-glucuronidase activity allows the body to excrete hormones such as estrogen before they can become reabsorbed. Oral administration of large doses of calcium-D-glucarate have been shown to lower serum estrogen levels in rats by 23 percent.<sup>21</sup> Because many breast cancers are estrogen-dependent, calcium-D-glucarate's ability to affect estrogen and other hormone levels has led to Phase I clinical trials at several major cancer centers in the United States. Results of these studies are pending.

### Lipid Lowering

Side effects of currently available hypolipidemic agents present a need for safe and effective lipid-lowering agents. D-glucarates have been shown to significantly reduce total serum cholesterol in rats by as much as 12-15 percent and LDL-cholesterol by 30-35 percent. Preliminary results in humans show D-glucarate reduced total serum cholesterol up to 12 percent, LDL-cholesterol up to 28 percent, and triglycerides up to 43 percent. The lipid-lowering effect of calcium-D-glucarate may be attributed to improved enterohepatic circulation, resulting in increased excretion of bile acids and a reduction in steady state levels of cholesterol biosynthesis.<sup>7</sup>

### Drug/Nutrient Interactions

There are no known drug interactions with calcium-D-glucarate, but many drugs and hormones are metabolized in the liver via glucuronidation. Therefore, taking calcium-D-glucarate may increase elimination of these substances, possibly reducing their effectiveness.

## Side Effects and Toxicity

No adverse effects have been observed after prolonged feeding to rats or mice at concentrations of 70, 140, or even 350 mmol/kg.<sup>6</sup> Preliminary results of clinical trials in humans have shown calcium-D-glucarate is without adverse effects.

## Dosage

The recommended oral dosage of calcium-D-glucarate is generally in the range of 1500-3000 mg daily. Until human trials have been completed the optimal dosage remains elusive.

## References

- Dwivedi C, Heck WJ, Downie AA, et al. Effect of calcium glucarate on beta-glucuronidase activity and glucarate content of certain vegetables and fruits. *Biochem Med Metab Biol* 1990;43:83-92.
- Walaszek Z, Szemraj J, Narog M, et al. Metabolism, uptake, and excretion of a D-glucaric acid salt and its potential use in cancer prevention. *Cancer Detect Prev* 1997;21:178-190.
- Heerdt, AS, Young CW, Borgen PI. Calcium glucarate as a chemopreventive agent in breast cancer. *Isr J Med Sci* 1995;31:101-105.
- Horton D, Walaszek Z. Conformations of the D-glucarolactones and D-glucaric acid in solution. *Carbohydr Res* 1982;105:95-109.
- Walaszek Z, Hanausek-Walaszek M. D-glucaro-1,4-lactone: its excretion in the bile and urine and effect on biliary excretion of beta-glucuronidase after oral administration in rats. *Hepatology* 1988;9:552-556.
- Selkirk JK, Cohen GM, MacLeod MC. Glucuronic acid conjugation in the metabolism of chemical carcinogens by rodent cells. *Arch Toxicol* 1980;139:S171-S178.
- Walaszek Z, Hanausek-Walaszek M, Adams AK, Sherman U. Cholesterol lowering effects of dietary D-glucarate. *FASEB* 1991;5:A930.
- Yoshimi N, Walaszek Z, Mori H, et al. Inhibition of azoxymethane-induced rat colon carcinogenesis by potassium hydrogen D-glucarate. *Int J Oncol* 2000;16:43-48.
- Schmittgen TD, Koolemans-Beynen A, Webb TE, et al. Effects of 5-fluorouracil, leucovorin, and glucarate in rat colon-tumor explants. *Cancer Chemother Pharmacol* 1992;30:25-30.
- Walaszek Z, Hanausek-Walaszek M, Webb TE. Dietary glucarate-mediated reduction of sensitivity of murine strains to chemical carcinogenesis. *Cancer Lett* 1986;33:25-32.
- Oredipe OA, Barth RF, Hanausek-Walaszek M, et al. Effects of an inhibitor of beta-glucuronidase on hepatocarcinogenesis. *Proc Am Assoc Cancer Res* 1987;28:156.
- Oredipe OA, Barth RF, Hanausek-Walaszek M, et al. Effects of calcium glucarate on the promotion of diethylnitrosamine-initiated altered hepatic loci in rats. *Cancer Lett* 1987;38:95-99.
- Dwivedi C, Downie AA, Webb TE. Modulation of chemically initiated and promoted skin tumorigenesis in CD-1 mice by dietary glucarate. *J Environ Path Toxicol Oncol* 1989;9:253-259.
- Abou-Issa H, Koolemans-Beynen A, Meredith TA, Webb TE. Antitumour synergism between non-toxic dietary combinations of isotretinoin and glucarate. *Eur J Cancer* 1992;28:784-788.
- Webb TE, Abou-Issa H, Stromberg PC, et al. Mechanism of growth inhibition of mammary carcinomas by glucarate and the glucarate:retinoid combination. *Anticancer Res* 1993;13:2095-2100.
- Bhatnagar R, Abou-Issa H, Curley RW, et al. Growth suppression of human breast carcinoma cells in culture by N-(4-hydroxyphenyl) retinamide and its glucuronide and through synergism with glucarate. *Biochem Pharmacol* 1991;41:1471-1477.
- Curley RW, Humpries KA, Koolemans-Beynen A, et al. Activity of d-glucarate analogues: synergistic antiproliferative effect in cultured human mammary tumor cells appear to specifically require the d-glucarate structure. *Life Sci* 1994;54:1299-1303.
- Abou-Issa H, Moeschberger M, Masry EI, et al. Relative efficacy of glucarate on the initiation and promotion phases of rat mammary carcinogenesis. *Cancer Res* 1995;15:805-810.
- DeLuca LM. Retinoids and their receptors in differentiation, embryogenesis and neoplasia. *FASEB J* 1991;5:2924-2933.
- Walaszek Z, Raich PC, Hanausek M, et al. Role of D-glucaric acid in lung cancer prevention. Unpublished research. AMC Cancer Research Center, Denver, CO.
- Walaszek Z, Hanausek-Walaszek M, Minto JP, Webb TE. Dietary glucarate as anti-promoter of 7,12-dimethylbenz[a]anthracene-induced mammary tumorigenesis. *Carcinogenesis* 1986;7:1463-1466.