Potential Mutagenic Activity of Some Vitamin Preparations in the Human Gut

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Rutin is a nonmutagenic flavonol glycoside, whereas its aglycone quercetin is mutagenic. Cell-free preparations from fecal cultures (fecal preparations) contain a β-glucosidase that, when incubated with rutin, hydrolyzes it to quercetin. This activity can be further induced when rutin is added to the fecal culture from which the cell-free preparation is made. When vitamin pills that contain rutin are added to the cultures, this induction is equally effective. The vitamin extracts by themselves, like rutin, were nonmutagenic; however, when the vitamin extracts were incubated with fecal preparations containing induced β-glucosidase, a great increase in mutagenicity was observed.

Humans consume in their daily diet a variety of flavonols, most of which occur in nature as glycosides (8). However, when such flavonol glycosides, e.g., rutin (quercetin-3-O-rutinoside), are exposed to human fecal flora (19), rat cecal flora (5), or bacterial isolates of human saliva (17), they can be hydrolyzed to the aglycone, e.g., quercetin (3,3',4',5,7-pentahydroxyflavone). Quercetin is mutagenic in the Ames assay (5, 13), damages DNA and chromosomes (14), and transforms hamster embryo cells in vitro (20). Although little is known about the metabolic fate of flavonols, it is thought that about 50% of ingested quercetin is degraded to other products in the human gut, but less than 1% is absorbed (7).

Rutin is the most common glycosidic form of quercetin and can be found in many foods, food supplements, tobacco, and beverages (4). It can be hydrolyzed to quercetin by a β-glucosidase found in cell-free preparations of fecal cultures (fecal preparations) (11). This activity is inducible by adding rutin to the growth medium of the fecal cultures from which the fecal preparation is made (12). The activity is measured by increased mutagenicity of the rutin incubated with induced fecal preparation in the Ames assay.

It had not been shown previously that food or drug products containing substantial amounts of rutin could induce this β-glucosidase activity and subsequent mutagenesis of rutin. Therefore three vitamin preparations containing rutin sold over the counter at a local health food store were investigated.

The vitamin pills tested were: (i) Trophic (Toronto, Ontario), 100 mg of vitamin C with 5 mg of rutin per tablet, (ii) Trophic Multivitamins and Minerals with 10 mg of rutin per tablet, and (iii) Jamieson (Windsor, Ontario), 500 mg of vitamin C with 50 mg of rutin per table.

Fecal preparations were made as described before (11, 12). Briefly, 100-mL fecal cultures were grown overnight with and without the addition of 2 × 10^{-4} M rutin (Aldrich Chemical Co.), various amounts of crushed vitamin pills containing rutin, or methanol extracts of the crushed vitamin pills. The cultures were centrifuged at 11,500 × g for 20 min at 4°C, and the bacterial pellets were suspended in 5 ml of 10 mM sodium phosphate buffer (pH 7.4)-1.0 mM dithioerythritol. After a second centrifugation, the pellets were again suspended in 5 ml of buffer and lysed in a French pressure cell at 14,000 lb/in². The supernatant fluid from a final centrifugation was dialyzed, filter sterilized, and stored at −70°C.

The Ames Salmonella mutagenicity test (1, 2) was used, with one variation. A preincubation mixture was set up consisting of 8 × 10^{-3} M rutin (5 μl of 100 mM rutin in dimethyl sulfoxide) or an equivalent amount of vitamin pill extract (see below), 0.4 ml of fecal preparation, and 0.2 ml of 10 mM sodium phosphate buffer (pH 7.4). After 1 h of preincubation, 200 μl was added to 2 ml of sloppy agar containing biotin-histidine along with 100 μl of Salmonella sp. strain TA98.

Rutin was extracted from a Jamieson vitamin C pill by adding 10 ml of methanol to a crushed pill in an extraction tube and vortexing. The methanol layer was removed, and the extraction was repeated. The extract was evaporated down to 1.0 ml under nitrogen gas, with periodic filtering through a cotton-plugged Pasteur pipette to remove precipitating ascorbic acid. Six microfilters was added to the preincubation mixture. Assuming the extraction was 100% efficient, this gives a rutin concentration of 8 × 10^{-3} M.

Several cell-free preparations were tested with Trophic C, Trophic multivitamin, Jamieson C, and rutin itself as inducers as well as a cell-free preparation made without inducer. All three brands of vitamins containing rutin were capable of inducing rutin β-glucosidase to give cell-free preparations more active than the noninduced preparation (Fig. 1A). Optimal induction occurred with two Trophic C tablets (10 mg of rutin), 1 Trophic multivitamin (10 mg of rutin), and 1/5 of a Jamieson C tablet (10 mg of rutin). This amounted to a rutin concentration of 1.64 × 10^{-4} M in the medium compared with 2 × 10^{-4} M rutin, which is optimally used to induce the culture (12). When larger amounts of Trophic C and Jamieson C tablets were added to the cultures, a suppression of rutin β-glucosidase activity was seen (Fig. 1A). This was probably due to a drop in pH that occurred in these cultures, most likely due to the high ascorbic acid content. When methanol extracts of the rutin-containing pills were added to the cultures instead of the pills themselves, the suppression in activity was much less pronounced (Fig. 2), and the pH change in the culture was very slight.

Additionally, a methanol extract of a Jamieson C tablet served as the substrate in the preincubation mixture instead of rutin and delivered results comparable to those with 8 × 10^{-3} M rutin as the substrate (Fig. 1B). The extract itself was very weakly mutagenic without preincubation with fecal

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preparation, whereas rutin alone was nonmutagenic (Fig. 1A and B).

Vitamin C and multivitamins that did not contain rutin were also tested. They did not act as inducers or substrates in the system and were not mutagenic themselves when extracted (data not shown).

Although rutin has some therapeutic use (4), the potential carcinogenicity of either rutin or quercetin to humans is not known. However, most mutagens are carcinogens (1, 3), and quercetin has been shown to be carcinogenic to Norwegian rats (16), although not to some other animal models (9, 15, 18). It also reduces the life-span of mice (10).

It has been suggested by the National Research Council’s Committee on Diet, Nutrition and Cancer that the consumption of high-dose supplements of nutrients be avoided (6).

Vitamin preparations other than Jamieson and Trophic brands that contain rutin are also available in health food outlets in the United States and Canada (J. P. Brown, personal communication). We conclude that such vitamin preparations may possibly contribute to the mutagenic burden in the human colon.

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LITERATURE CITED


