Influence of selenised dairy proteins on biomarkers of colon cancer risk

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Abstract
Selenium is an essential trace element for mammals, and a component of at least 25 selenoproteins which incorporate the amino acid selenocysteine (Sec). These proteins include a number with oxidoreductase functions. An examination of the selenoproteins and their influence on carcinogenesis in an animal model may assist in determining their relevance in chemoprevention.

Food sources offer a number of organic forms of selenium, with selenomethionine a common component, as in selenised yeasts. A selenium-rich dairy protein product has been developed (TaturaBioSe, Tatura Milk Industries, Tatura, Victoria) which could improve selenium status in populations considered marginal or deficient. It could also provide higher intakes (e.g. several fold above recommended dietary intake recommendations) of potential benefit as chemopreventive to those at greater risk of some selenium-responsive diseases, such as some sporadic colorectal cancers. Clinical studies are showing it to be a safe, palatable product for consumption in the form provided.

Dairy protein selenium at 1 ppm in diet had a significant chemopreventive effect compared with control (0.05 ppm Se) in an azoxymethane model of colon cancer. Colon tumour incidence in rats was down by 29%, and tumour burden (colon tumours/rat) was halved, effects not observed when an equivalent Se concentration was provided as yeast selenium. When assessed by plasma Se concentration, this dairy protein form of selenium showed greater bioavailability (as assessed by plasma selenium) as well as efficacy in chemoprevention. Programmed cell death (apoptosis) was increased in colonic crypts and crypt height significantly diminished. The influence on early changes in carcinogenesis provides an insight into possible mechanism(s) of action. Histological and biochemical assays (e.g. monitoring oxidoreductase enzymes) could potentially provide early biomarkers with clinical relevance.

Key words: biomarkers, colorectal cancer, selenium.

INTRODUCTION
Selenium is an essential trace element for mammals, and a component of at least 25 selenoproteins which incorporate the amino acid selenomethionine or selenocysteine (Sec). Selenomethionine is a common organic form in plants, can substitute for methionine in proteins or can be metabolized to selenocysteine within mammalian metabolism, but is unable to be synthesised in vivo. As selenocysteine in mammals, it is inserted into specific selenoproteins. Common selenoproteins include P, S and W forms, iodothyronine deiodinases, glutathione peroxidases (GPXs 1–4), thioredoxin reductases and selenophosphate synthetases, the latter having oxidoreductase functions. Mapping the selenoproteomes across the active terminal domains of molecules incorporating Sec have paved the way for the functional characterisation of these proteins and are useful in assessing their involvement in the aetiology of disease.1

Evidence has been accumulating that selenium supplementation may significantly inhibit some forms of cancers, both in circumstances where selenium was shown to be deficient, and in circumstances where selenium levels might have been considered adequate on the basis of recommended dietary intakes (RDI), and is provided at intakes exceeding such nutritional requirements, that is, supranutritional levels.2–6

The potential of selenium in differing food sources to provide chemoprevention may be influenced by the molecular form, and both protein and non-protein organic forms are found in plants, and are recognised as differing in their efficacy as well as their in vivo metabolism.5–7 High intakes of four to five times RDI have also been proposed as offering significant protection.3,4,6,8 This hypothesis has led to further investigation of existing epidemiological evidence, as well as the initiation of intervention studies aimed at clarifying the influence of such high intakes, often with organic sources of selenium, such as the amino acid L-selenomethionine, or selenised yeast products.5 It is interesting in this regard to note that the evidence in epidemiological, as well as animal cancer models points to reductions in cancer along a
continuum of increasing supplementation to levels of intake several fold above requirements for nutritional adequacy. This toxicity is recorded in animal experimentation when long-term dietary Se levels as selenite or selenomethionine of 5 ppm or above were used. 

In the first experiment, three diets were provided to rats (n = 10/treatment) over five weeks and the rats were killed six hours after a single injection of AOM. Colons were fixed (formol saline) for histological analysis after sectioning and staining, for apoptosis (haematoxylin) and proliferating cells (Proliferating Cell Nuclear Antigen antibody). Twenty complete crypts per rat were assessed.

TaturaBio Se was produced by Tatura Milk Industries (Tatura, Australia), in which selenium-rich proteins (caseins and whey) were separated and concentrated to provide a guaranteed concentration of 5 ppm selenium. The requirements for its production in dairy herds have been reported. It is being used in studies including a preliminary human trial, with a view to obtaining an assessment of its potential benefits in human health and disease prevention.

RESULTS

The selenised casein diet was effective in reducing colon tumour incidence (29% fewer rats with tumours), and burden (tumours per rat) decreased 52% (P < 0.05) compared with the control. Selenised yeast, when added at similar concentration (1 ppm) and at 4 ppm, despite significantly increasing plasma selenium concentrations, did not change colon tumour expression. Adenocarcinomas as a per cent of total tumours were 22% in selenised yeast treatments compared with 11% in the selenised casein treatment. In a second study with selenium yeast providing selenium at 1, 4 and 8 ppm throughout the study, a significant reduction in tumours was observed only with 8 ppm Se (colon tumour incidence was 15% lower and colon tumour burden was 35% lower than control, P < 0.05). However, this high-Se intake was also associated with a significantly lower bodyweight in this group (down 10.5%, P < 0.05), indicating an influence on energy intake or metabolism, which may have accounted for the reduced tumorigenesis observed. The form of selenium in the diet influenced significantly its bioavailability (plasma level being lower by 9% in the 1 ppm yeast Se rats than the equivalent Se casein rats, P < 0.05), but the GPX levels in liver and colon were not different from each other.

In a smaller study where rats on three treatments were killed six hours after AOM induction and histological quantitative analysis undertaken of crypt cell apoptosis and proliferation, colonic crypt height expressed as cell number/crypt wall was significantly reduced (down 6%, P < 0.01) for the selenised casein-fed rats compared with the other two groups (Se yeast and control, not different from each other). There was an increase in apoptotic index with selenised casein (14% higher than control, P = 0.1), while the Se yeast group was 28% lower than the control (not significant). The directional shift suggests a very different response to these differing Se sources. Proliferation was not significantly different between treatments.

TaturaBio Se has been developed for use in rodent colon cancer studies as well as investigating its potential in humans to supply selenium in a readily utilisable form. In a preliminary trial at Flinders Medical Centre, in which 13 people (eight female/five male) were supplemented with 100 μg of Se in a milk protein (30 g) drink daily over a period of eight weeks, the average increase in plasma selenium was from 103 to 149 μg/L, that is, a 45% increase.
DISCUSSION

Dairy milk proteins can become an excellent source of selenium. While accounting for about 4% of milk, they contained more than half (60%) of the milk selenium content, the remainder being removed by membrane separation. In so far as they are of high nutritional quality and can be absorbed with more than 95% efficiency, they offer an excellent means of increasing selenium status. The comparison with yeast selenium shows them to be significantly more effective in increasing selenium status as assessed by plasma selenium concentration, an effect not supported by liver and colon GPX activities. In this respect, plasma selenium concentration has been useful. It is reported to reflect selenomethionine sources in the dietary supplements, but not as accurately in some other dietary sources, for example, selenite, Se-allyl-selenocysteine, as in garlic. The actual molecular form(s) of selenium in caseins and whey proteins has not been investigated, but need to be.

Selenoprotein P which accounts for most of the selenium in the plasma pool (estimated to contain about 50%) is a Se transporter which offers an alternative and possibly more sensitive measure of Se status. The plasma Se measure has not been so useful when forms such as selenite or methylselenocysteine were used. There is a level of plasma selenium in humans, around 100 µg/L, above which GPX does not respond to further increases in dietary supplementation. Plasma GPX activity generally does not correlate with supplemental levels, and probably finds its main use in establishing deficiency states with respect to nutritional requirements. Murakawa et al. have observed contrasting patterns of selenoproteins under selenium deprivation, and that GPX-2 (gastrointestinal) behaved differently from GPX-1(plasma) and GPX-3 (cellular), being increased under deficiency conditions. If, as appears apparent, cancer inhibitory effects are achieved at supplemental levels several fold above that proposed for RDI, then alternative markers of selenium bioefficacy will be needed, and platelet GPX and tissue TXR measures have been proposed. They respond over a higher selenium range. TXR, a selenium containing enzyme controlling oxidoreductase reactions at cellular level, has been examined for its relevance in an anti-cancer role by Ganther. He doreductase reactions at cellular level, has been examined for its relevance in an anti-cancer role by Ganther. He
CONFLICT OF INTEREST

No conflict of interest has been declared by G.H. McIntosh.

REFERENCES
