

Taper-2005-1

IF 0.811 PMNT

PDF

DOI

IF 0.811

PMID 15796175

Possible Adjuvant Cancer Therapy by Two Prebiotics - Inulin or Oligofructose

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Abstract. Dietary treatment with inulin or oligofructose incorporated in the basal diet for experimental animals: (I) reduced the incidence of mammary tumors induced in Sprague-Dawley rats by methylnitrosourea; (II) inhibited the growth of transplantable malignant tumors in mice; (III) decreased the incidence of lung metastases of a malignant tumor implanted intramuscularly in mice. (IV) Moreover, besides such cancer risk reduction effects, dietary treatment with inulin or oligofructose significantly potentiated the effects of subtherapeutic doses of six cytotoxic drugs commonly utilized in human cancer treatment. (V) The same prebiotics potentiated the effects of radiotherapy on solid form of TLT tumors to a statistically very high level. Such dietary treatment, with the inulin or oligofructose potentiating the effects of cancer therapy, might be introduced into classic protocols of human cancer treatment as a new, non-toxic and easily applicable adjuvant cancer therapy without any additional risk to patients.

The protective and inhibitory influence of dietary components on cancer development and growth is a topic of major interest (1-3). The identification of such components, the understanding of their mechanisms of action, as well as their use in the human diet is one of the objectives of functional food science, which is a part of modern nutrition.

A functional food is a food which contains one or a combination of components that interact with physiological functions in the body so as to improve them (4). When such functional food acts directly on some physiological function it is called probiotic, when its action is indirect *e.g.* by promoting some functionally active bacteria, it is called prebiotic.

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Key Words: Prebiotics, inulin, oligofructose.

The development of such functional foods starts with the identification of an interaction between a food ingredient and a particular function in the body, followed by a proper understanding of the mechanism of such a positive interaction leading to the demonstration of a beneficial effect in humans (4).

Among the many cited physiological functions of non-digestible carbohydrates, one of the most important is probably the capacity to prevent carcinogenesis in its early stages. Immunomodulation by carbohydrates or their bacterial metabolites, or by the intestinal bacteria that they selectively promote, has been reported as a possible mechanism of cancer prevention (5, 6).

Much work has been done to identify components (*e.g.*, carotenoids, allylic sulfides, dietary fibers) in the diet with the capacity to prevent the initiation and, possibly, promotion of carcinogenesis. These products are classified as anti-carcinogens (7).

However, few experiments have been performed to identify dietary components that could help the organism slow down or stop the growth and development of an already existing population of neoplastic cells. It has been reported that live bacteria, which are used as probiotics (8), as well as fractions of their membrane preparations, may inhibit the growth of various types of tumors in experimental models involving *i.p.*, *s.c.* or *i.m.* implantation of tumor cells (9-13) or chemically-induced carcinogenesis (10).

Fructans, such as chicory inulin and oligofructose, belong to a new class of functional food ingredients (4). Being non-digestible in the upper part of the gastrointestinal tract, they flow into the colon where they selectively promote Bifidobacteria (14). Since they selectively promote the growth of certain types of bacteria, they are classified as "prebiotics" (15). Moreover, recent work in our group with rats has demonstrated that supplementing the diet with chicory fructans modulates hepatic lipogenesis, (16, 17). Like the other non-digestible carbohydrates, in particular the pectins, chicory fructans behave as "soluble dietary fiber" (18).

The objective of this article is to present a summary of the results of experiments performed in our laboratory

concerning the effects on cancer growth and treatment of two prebiotics, inulin and oligofructose.

Materials and Methods

All experiments were performed on mice (except one on rats) fed *ad libitum* with 15 g inulin or oligofructose per 85 g of basal diet for experimental animals. Inulin (Raftiline®) is a linear b(2-1) fructan (degree of polymerization ranging between 10 and 60) and is a natural food ingredient of many vegetables and cereals such as chicory, onion, garlic, asparagus, wheat, leek, artichoke *etc.* Oligofructose (Raftilose®) is a shorter chain fructan (degree of polymerization < 8) and is obtained by partial enzymatic hydrolysis of chicory inulin (19). Both fructans are products of ORAFIT, Tienen, Belgium. The caloric density of the control and experimental diets containing fructans were similar (331 and 313 Kcal, respectively).

In almost all experiments, the solid or ascitic form of a transplantable liver tumor (TLT) in young male NMRI mice was utilized (20, 21).

Results

Anti-carcinogenic effect of oligofructose. The results of our initial research have demonstrated that oligofructose incorporated (15% w/w) in the basal diet for experimental animals reduced the incidence of mammary tumors induced in Sprague-Dawley female rats by methylnitrosourea. The number of tumor-bearing rats and the total number of mammary tumors were significantly lower in the oligofructose-fed rats than in the rats of the control group fed a basal diet (22).

Tumor growth inhibiting effects of inulin or oligofructose. Similar treatment with an inulin- or oligofructose-containing diet also reduced the growth of intramuscularly transplanted solid mouse tumors originating from two different tumor cells lines, transplantable liver tumor (TLT) and mammary mouse carcinoma (EMT6) (23). These tumor growth inhibitory effects have been confirmed in mice fed inulin or oligofructose and bearing the ascitic form of the intraperitoneally transplanted malignant liver tumor (TLT). The percentage of increase in life span (ILS), when compared with the control group of mice fed a basal diet alone, was 16% and 18% for inulin- and oligofructose-supplemented diets, respectively (24).

Anti-metastatic effects of oligofructose or inulin. The most surprising activity of inulin or oligofructose is the capacity to reduce significantly the number of mice bearing lung metastases as well as the absolute number of lung metastases per group after intramuscular transplantation of the TLT tumor cells in young male C3H mice. The percentage of mice bearing lung metastases in a control

group fed basal diet, in an inulin-fed group and in an oligofructose-fed group were 59, 36 and 35%, respectively. The total number of lung metastases was thirty-seven, eighteen and sixteen respectively, for the three groups (25).

Potentiation of cancer chemotherapy by dietary inulin or oligofructose. Both inulin and oligofructose have also been shown to potentiate the therapeutic effects of all six investigated cytotoxic drugs that are representative of the different groups of cytotoxic drugs classically used in human cancer treatment (5-Fluorouracil, Doxorubicine, Vincristine, Cyclophosphamide, Methotrexate, Cytarabine). These drugs were intraperitoneally injected at single subtherapeutic dose into inulin- or oligofructose-fed mice bearing the ascitic form of the TLT tumor. The therapeutic effects were calculated by comparing ILS in treated and untreated mice groups.

The potentiation of the effects of the chemotherapy induced by the adjuvant treatment with inulin or oligofructose had, in more than 50% of the experiments, a synergistic effect. The results of the remaining experiments were also positive and predisposed to the assumption of an additive effect. No negative result of the adjuvant therapy induced by inulin or oligofructose was ever observed. Distinct differences in the chemotherapy potentiating action between inulin and oligofructose were not observed. Quantitatively the adjuvant therapeutic effect was slightly different for the different drugs. In some experiments, a spectacular effect was observed, for example for cyclophosphamide, a drug for which the therapeutic effect was increased by 47% (increased ILS) by inulin (26, 27).

Potentiation of cancer radiotherapy by dietary inulin or oligofructose. Since dietary treatment with inulin or oligofructose inhibited the growth of tumors and potentiated the effects of cancer chemotherapy, it appeared to be interesting to investigate the possible potentiation of the results of radiotherapy by the same non-digestible carbohydrates. The influence of 15% inulin or oligofructose incorporated in the basal diet for experimental animals was investigated on the 1000 mm³ intramuscularly transplanted TLT tumor in mice. The tumors were locally irradiated with a single dose of 5 to 20 Gy X-rays and the progression of tumor growth was examined by regular, twice weekly measurements. At optimal dose of 10 Gy, the effect of radiotherapy in inulin- or oligofructose- fed mice was potentiated to a statistically highly significant level ($p < 0.0001$) when compared with the tumors of the control group irradiated with the same dose of X-rays, but in mice fed normal diet without inulin or oligofructose. This radiotherapy potentiation was similar for inulin and oligofructose (28).

Discussion

The results of our experiments on the effects of dietary treatment with inulin or oligofructose demonstrated: 1. reduction of the incidence of mammary tumors induced by methylnitrosourea in Sprague-Dawley female rats; 2. inhibition of the growth of transplantable tumors in mice; 3. decreased incidence of lung metastases of a malignant tumor intramuscularly implanted in mice; 4. significant potentiation of the results of a) chemotherapy and b) radiotherapy of transplantable tumors in mice. In all our experiments, there was no functional or morphological sign of toxicity after inulin or oligofructose administration. The non-toxic character of dietary treatment with these fructans was confirmed by the increase of survival time of inulin- or oligofructose- fed mice (22-24).

It should also be mentioned that lower doses of inulin or oligofructose (10%) produced similar effects on cancer therapy to 15% content in animal food (unpublished results).

Hypothetical mechanisms of anti-cancer effects of inulin or oligofructose. Several hypothetical mechanisms are probably involved in the cancer growth inhibition and cancer therapy potentiating effects of inulin or oligofructose. These carbohydrates are non-digestible by endogenous enzymes, but they are actively fermented by the colonic bacteria, selectively promoting the growth of some of them, especially the Bifidobacteria (14, 29). Such changes in the composition of the colonic microflora have been reported to reduce tumor incidence and/or growth (10). Moreover, it has been reported that cell wall preparations from *Bifidobacterium infantis* have a tumor suppressive effect (13, 30) and it has also been shown that inulin and oligofructose reduce the incidence of aberrant crypt foci in the colon of rats previously injected with a chemical carcinogen (31, 32). Since inulin or oligofructose selectively promoted the growth of certain types of bacteria, which produced inhibitory effects on tumor incidence and growth, they were classified as prebiotics (15).

The growth and proliferation of tumor cells depend on glucose availability, because these cells acquire the major part of their energy from the glycolytic pathway (33). The non-digestible carbohydrates have been reported to decrease the serum glucose level in rats and humans (34, 17), an effect that might deprive cancer cells of their essential substrate.

Kuhajda *et al.* (35) have demonstrated that *in vitro* human cancer cells do require endogenous fatty acid synthesis for their growth and that the inhibition of this metabolic pathway can be considered as a new and promising target for cancer therapy. Recent experiments have demonstrated that inulin and oligofructose, which inhibit tumor growth, also decrease triglycerides, phospholipids and low-density-lipoproteins in serum by lowering the *de novo* lipogenesis in

the liver (16, 17). Such a metabolic effect might also be related to the tumor inhibitory effect reported above.

Conclusion

More advanced investigations are necessary to elucidate further which of the above-mentioned or other mechanisms are involved in the reduction of the cancer risk and in the cancer chemo- and/or -radiotherapy-potentiating effects of dietary inulin or oligofructose. Such non-toxic dietary components might, however, prove to be very useful.

Further investigations on these topics may lead to improved efficacy of cancer therapy by allowing a potentiation of classic clinic treatments, with no site effects.

Acknowledgements

This program was financially supported by ORAFI, Tienen, Belgium. The authors thank Mrs. R. M. Dujardin for her very competent technical assistance and Mrs. E. Ferdinand for typing the manuscript.

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Received March 23, 2004

Revised November 19, 2004

Accepted December 20, 2004