Influence of Inulin and Oligofructose on Breast Cancer and Tumor Growth[1]

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ABSTRACT Because anticarcinogenic and tumor-growth–inhibiting effects of nonsoluble fibers have been described, similar actions of soluble fibers appear to merit investigation. In a preliminary study on methylnitrosourea-induced mammary carcinogenesis in Sprague-Dawley female rats, 15% oligofructose added to the basal diet modulated this carcinogenesis in a negative manner. There was a lower number of tumor-bearing rats and a lower total number of mammary tumors in oligofructose-fed rats than in the group fed the basal diet alone. The effect of dietary nondigestible carbohydrates (15% oligofructose, inulin or pectin incorporated into the basal diet) on the growth of intramuscularly transplanted mouse tumors, belonging to two tumor lines (TLT and EMT6), was also investigated. The results were evaluated by regular tumor measurements with a vernier caliper. The mean tumor surface in the experimental groups was compared with that in animals of the control group fed the basal diet containing starch as the only carbohydrate. The growth of both tumor lines was significantly inhibited by supplementing the diet with nondigestible carbohydrates. Such nontoxic dietary treatment appears to be easy and risk free for patients, applicable as an adjuvant factor in the classical protocols of human cancer therapy. J. Nutr. 129: 1488S–1491S, 1999.

KEY WORDS: • *inulin* • *oligofructose* • *breast carcinogenesis* • *tumor growth*

The protective and inhibitory influence of dietary components on cancer development and tumor growth is a topic of major interest (Milner 1994, Roberfroid 1991, Williams and Dickerson 1990). The identification of such dietary components, the understanding of their mechanisms of action as well as their development, and their use in the human diet are among the objectives of functional food science. In particular, certain dietary fibers were found to be factors preventing the initiation and possibly the promotion of carcinogenesis. These products were classified as anticarcinogens (Wattenberg 1992).

Carbohydrates such as inulin and oligofructose, which are nondigestible in the upper digestive tract, selectively promote the growth of certain types of bacteria, e.g., *Bifidobacteria* (Gibson et al. 1995); thus they are classified as prebiotics (Gibson and Roberfroid 1995) and as soluble dietary fiber (Roberfroid 1993).

Because it is generally recognized that dietary fibers may act as anticarcinogens (Wattenberg 1992), it appeared worthwhile to test the hypothesis that some of the recently identified soluble dietary fibers might behave in the same way in tumor pathology.

In line with this hypothesis, the work reported here had the following two objectives: *1*) to investigate the possible anticarcinogenic action of oligofructose in rat mammary carcinogenesis induced by methylnitrosourea; and *2*) to test the hypothesis that oligofructose, inulin or pectin might help to control the growth of two lines of transplantable mouse tumors.

MATERIALS AND METHODS

Young female Sprague Dawley rats were obtained from Iffa Credo, Brussels, Belgium. On d 45 after their birth, each rat in two groups of rats was injected subcutaneously with 50 mg/kg body weight of methylnitrosourea (*N*-methylnitrosourea, Sigma Chemical, St. Louis, MO) dissolved in 9g/L physiologic NaCl solution. One week after the carcinogen injection, one experimental group of nine rats received the basal diet for experimental animals AO4 (UAR, Villemoisson-sur-Orge, France) supplemented with 5 g/100 g oligofructose (Raftilose P_{g5}, Orafti, Tienen, Belgium). The next week, the oligofructose concentration was increased to 10 g/100 g, and from the third week until the end of the experiment, this carbohydrate was given at the level of 15 g/100 g. The control rats (also injected with methylnitrosourea) were fed a basal diet containing starch (from potatoes) as the only carbohydrate. Rats had free access to food and water.

From wk 4 after the carcinogen injection until the end of the experiment, the size, number and position of mammary tumors were manually assessed and their volume evaluated weekly by measuring three perpendicular dimensions with a vernier caliper. At wk 27, rats were anesthetized with diethylether and killed by exsanguination. A detailed autopsy was performed with tumor counting, measuring and description. The tumors and organs (liver, lung, kidneys, mammary glands and lymphatic nodes) were macroscopically examined, and specimens were taken for histopathologic examination (after fixation in 5% formalin solution, paraffin embedding and staining with hematoxylin-eosin).

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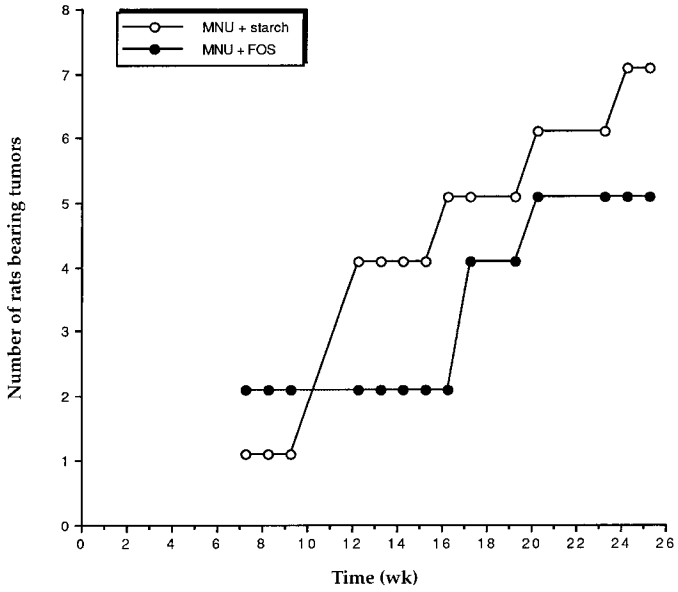

FIGURE 1 Effects of feeding rats a diet supplemented with15% oligofructose on the number of rats bearing mammary tumors. At d 45, both groups received methylnitrosourea (MNU; 50 mg/kg) as the mammary carcinogen.

In the second investigation, mice of the control groups were fed the basal diet for experimental mice A04 (UAR) and given free access to water. This basal diet contained 69% (wt/wt) carbohydrate (including 35% starch), 18% protein, 4% fiber, 3% lipids and 5% minerals and vitamins. The caloric value of this diet was 3.73 kcal/g. To 85 g of this basal diet, 15 g of oligofructose, inulin or pectin was added. The mice of both experimental groups were given 7 d before tumor transplantation and consumed the basal or experimental diets up to the end of experiment. Oligofructose (Raftilose) and inulin (Raftiline HP) were supplied by Orafti.

As a model for cancer growth, 10^6 viable neoplastic cells of two lines of transplantable mouse tumors were intramuscularly transplanted into the right thigh as follows: *1*) EMT6, a mammary carcinoma (Rockwell et al. 1972), was transplanted into young female BALB/c mice of ~20 g body weight (Iffa Credo); and *2*) TLT, a transplantable liver tumor (Taper et al. 1966), was transplanted in young male NMRI mice (Animalerie Facultaire, UCL, Brussels, Belgium). To quantify tumor growth, two perpendicular tumor dimensions were measured with a vernier caliper, and the mean tumor surface in mm^2 was calculated for each time period for 10–12 mice (TLT tumor) or 9–11 mice (EMT6 tumor) per group. These mean tumor surfaces in mm^2 for each group at individual time points are presented in the figures and were utilized for statistical analysis. For TLT tumor, the measurements started at d 6 after tumor transplantation and were performed twice weekly until the first animal died. Because the EMT6 tumor was growing more slowly, those measurements started 18 d after the tumor transplantation and were performed once weekly until the first mouse died. The results of each experiment were confirmed by a second experiment completed for each tumor line at another time. The results were cumulatively calculated for each tumor line.

Multiple ANOVA and the Scheffé test were used for statistical comparison of the results among the different experimental and control groups.

RESULTS

In the preliminary experiment on mammary carcinogenesis, tumor incidence (in terms of the number of rats bearing tumors) was always lower in the group of rats fed 15% oligofructose (**Fig. 1**). Similarly, the total number of tumors counted during the period of carcinogenesis was significantly lower in the oligofructose-fed group compared with the control group fed the basal diet with starch as the only carbohydrate (**Fig. 2**).

The results found after autopsy confirmed the above-mentioned findings, bringing at the same time some interesting details (**Table 1**), i.e., all mammary tumors were adenocarcinomas of different but equally distributed degree of malignancy in both groups of rats. However, only in the control group of rats fed the basal diet alone were tumors observed in other organs; there were two renal fibrosarcomas and two metastases of mammary carcinomas (one in lung, another one in lymphatic node). The mean volume of mammary tumors evaluated by a three-dimensional measurement of the lesions at autopsy was more or less the same in both groups. The lower number of rats bearing tumors and the lower total number of tumors per experimental group indicate that oligofructose addition in the diet modulated rat mammary carcinogenesis induced by methylnitrosourea in a negative manner by slowing down the kinetics of the appearance of malignant tumors, as well as by reducing the incidence of metastasis.

In the second investigation, the direct introduction of 15% oligofructose, inulin or pectin into the diet did not produce any gastrointestinal problems in mice, whereas in rats, it induced slight, but transitory diarrhea. As can be seen in **Figure 3**, the solid TLT tumor grew significantly more slowly in mice fed a diet containing 15% oligofructose, inulin or pectin compared with those fed the basal diet alone. This difference was observed from the beginning of the tumor measurements (d 6 after tumor transplantation) and was maintained until the end of the observation. Statistical analysis indicated a highly significant effect ($P < 0.01$) for all three experimental groups compared with the control group. Those highly significant effects for all three experimental groups compared with the control were found in both (separately performed) experiments with TLT tumor. Among the three groups of mice fed the experimental diets, tumor growth was not significantly different, at nearly 50% lower than in the control group. The TLT tumors grew rapidly up to a mean tumor surface of 800 mm^2, which caused early mortality,

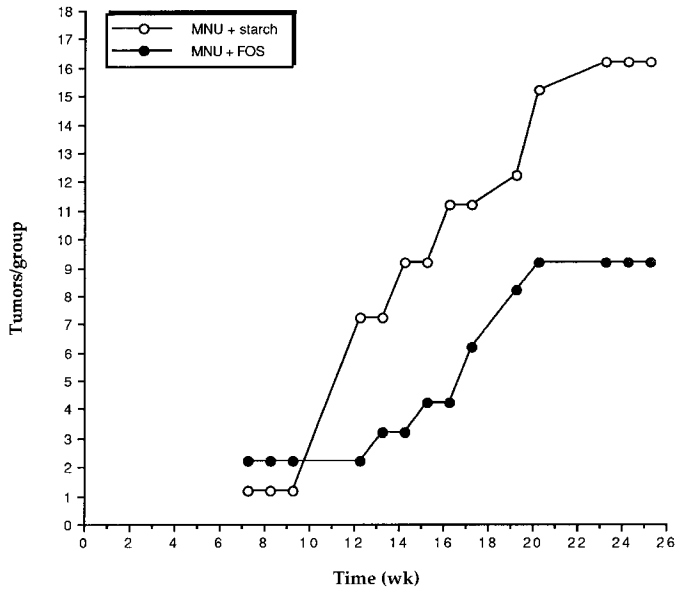

FIGURE 2 Effects of feeding rats a diet supplemented with15% oligofructose on the number of mammary tumors per group of rats injected at d 45 with methylnitrosourea (MNU; 50 mg/kg) as the mammary carcinogen.

TABLE 1

Effect of oligofructose (OFS) feeding on methylnitrosourea (MNU)-induced carcinogenesis in female rats

	Tumor						Volume (cm^3) of mammary tumors	
		Malignant						
Diet group	Benign	Mammary adenocarcinoma	Other	Total	Mean[1]	Metastasis	Total	Mean
Control	1	19	2	21	3.0 (2.7)	2	132	6.9
OFS	0	12	0	12	1.7 (1.3)	0	73	6.1

[1] Mean represents the total number of malignant tumors divided by the number of rats having tumors. Figure in parentheses is the total number of all types of tumors (including metastasis) divided by the number of rats in the assay.

justifying the interruption of the observation at d 24 after tumor transplantation. No mice survived in any of the investigated groups.

In both experiments performed with the EMT6 tumor line, each with 9–11 mice per group and presented cumulatively in **Figure 4**, the mean tumor growth was significantly inhibited in all three experimental groups fed oligofructose-, inulin- or pectin-supplemented diets, compared with the control group diet that contained starch as the sole carbohydrate. This tumor growth inhibition was more significant ($P < 0.01$) in the group of mice fed oligofructose than in mice fed inulin or pectin ($P < 0.05$). This inhibition by all three nondigestible carbohydrates of EMT6 tumor growth was observed from d 26 after tumor transplantation and was maintained until the end of the observation (i.e., until d 46).

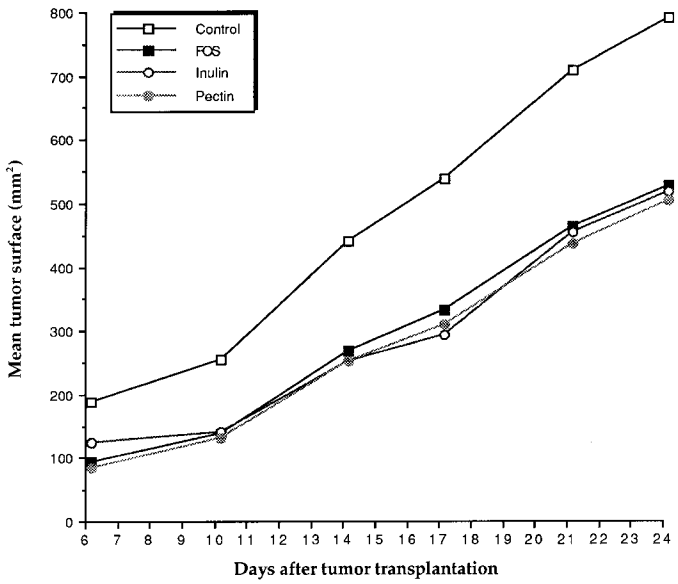

FIGURE 3 Effect of inulin, oligofructose and pectin added (15%) to a standard basal diet fed to mice transplanted intramuscularly with TLT cancer cells on the mean growth of the tumor (n = 20–22 mice). In the three treated groups, the kinetics of tumor growth were found to be significantly different (ANOVA $P < 0.01$) compared with the control group. SEM values for each time point and each experimental group are presented in the following table:

SEM (mm^2)	n	d 6	d 10	d 14	d 17	d 21	d 24
Control	22	18,1	20,6	23,5	22,6	27,8	24,5
Oligofructose	21	10,9	10,8	17,8	24,2	23,1	20,4
Inulin	21	11,7	9,9	13,3	13,7	20,1	20,3
Pectin	22	8,4	8,6	14,8	14,7	14,3	16,2

As can also be seen by comparing Figures 3 and 4, the EMT6 tumor grew considerably more slowly than the TLT tumor, thus allowing a longer period of observation. In EMT6 implanted mice, the mortality started 46 d after tumor transplantation. As in the TLT tumor investigation, in the experiments on EMT6 tumor, none of the tumor-bearing mice survived.

DISCUSSION

Inulin and oligofructose are natural food ingredients that are present in many edible plants such as onion, garlic, asparagus, wheat, leeks, chicory and artichokes (Edelman and Dickerson 1966, Van Loo et al. 1995). The average daily consumption is estimated to be of the order of a few grams. Like pectin, these $\beta(2\rightarrow1)$ fructans are classified as resistant carbohydrates to which the dietary fiber concept applies. The beneficial role of such food ingredients on carcinogenesis remains an important topic for scientific research.

Supplementation of a rat diet with 15% oligofructose neg-

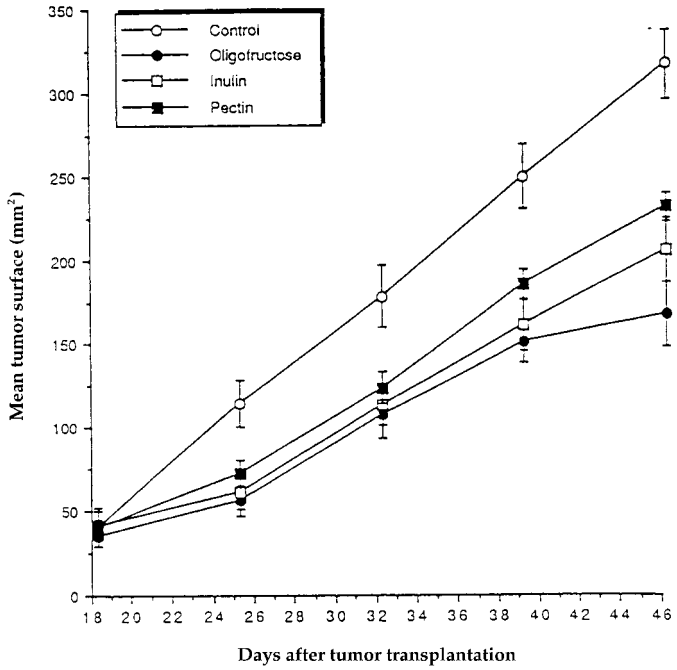

FIGURE 4 Tumor growth inhibitory effect of inulin, oligofructose or pectin added (15%) to a standard basal diet fed to mice transplanted intramuscularly with EMT6 cancer cells. In the group of mice fed oligofructose, the inhibition of tumor growth was significantly higher ($P < 0.01$) than in the inulin- or pectin-fed mice ($P < 0.05$). The graph presents the mean ± SEM for each group.

atively modulated rat mammary carcinogenesis induced by methylnitrosourea by decreasing the incidence of tumors (in term of the number of rats bearing the tumors) and the total number of tumors per group when compared with a control group fed a basal diet containing starch as the only carbohydrate. Tumors in other organs and metastases were observed only in rats from the control group. Because the protective diet was given after the phase of initiation, during the phase of promotion and progression, this anticarcinogenic effect can be considered as antipromoting and/or antiprogressing. However, the interesting results of this preliminary experiment require confirmation in a larger experiment.

The growth of solid tumors made of two different transplantable tumor cell lines is distinctly inhibited in mice fed a 15% oligofructose-, inulin- or pectin-supplemented diet. There was practically no difference in the tumor growth inhibitory effect among all three dietary nondigestible carbohydrates in the experiments on TLT tumor, but oligofructose appeared to be slightly more active than inulin or pectin on EMT6 tumor. In both tumors, this inhibitory effect reached almost 50% compared with mice fed the control diet.

There are several hypothetical mechanisms that may be involved in the inhibitory and/or anticancinogenic effect of these nondigestible carbohydrates on tumor growth and/or appearance. These carbohydrates are nondigestible by endogenous enzymes, but they are actively fermented by colonic bacteria. In addition, the chicory fructans selectively promoting *Bifidobacteria* are acting as prebiotics, thus modifying the composition of colonic microflora (Gibson et al. 1995, Wang and Gibson 1993). Such alterations (and others that are similar) of the colonic microflora have been reported to have an inhibitory action on tumor incidence and/or growth (Reddy et al. 1973, Reddy and Rivenson 1993). The same investigators are reporting that inulin and oligofructose reduce the incidence of colonic aberrant crypt foci in azoxymethane-treated rats.

Moreover, it has been reported that a cell wall preparation from *Bifidobacterium infantis* has a tumor-suppressive effect (Sekine et al. 1994, Tsuyuki et al. 1991); another report concerned the antimelanoma activity of inulin (Cooper and Carter 1986). More recently, Rumney and Rowland (1995) reviewed the potential anticarcinogenic effect of nondigestible oligosaccharides and concluded that the following two lines of evidence are suggestive of such an effect: *1)* "certain biomarkers thought to be affected by cancer risk are beneficially affected by oligosaccharides consumption in animals and man;" *2)* "they increase the numbers of lactic acid bacteria in the gut, bacteria which show antigenotoxic and anticarcinogenic effects. "

Although tumor cell proliferation is dependent on glucose availability because these cells acquire the major part of their energy from the glycolytic pathway (Cay et al. 1992), it has been reported that chicory fructans decrease serum glucose (Kok et al. 1996, Yamashita et al. 1984) and insulin levels (Kok et al. 1996), and it has been hypothesized (Basserga 1995, Giovanucci 1995) that hyperinsulinemia could be a key factor in carcinogenesis and tumor development. Change in insulin sensitivity could thus be part of the mechanism of the tumor growth inhibition by nondigestible carbohydrates.

Finally, Kuhajda et al. (1994) demonstrated that human cancer cells cultivated in vitro strongly 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. Complementary to this idea are recent observations that chicory fructans, which inhibit tumor growth, also decrease triglycerides, phospholipids and VLDL in serum by lowering de novo lipogenesis in the liver (Fiordaliso et al. 1995, Kok et al. 1996).

Further studies are required to elucidate which of the above-mentioned mechanisms are essential in the tumor inhibitory and/or anticancinogenic effect of nondigestible carbohydrates. It is possible that all or some of them are necessary to create a metabolic chain reaction conditioning these beneficial effects. More advanced investigations on other tumors and on the mechanisms involved may lead to a considerable improvement in the understanding of their action, thus enabling their introduction as food components that reduce the risk of cancers.

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