Influence of Inulin and Oligofructose on Breast Cancer and Tumor Growth

Henryk S. Taper and Marcel Roberfroid

ABSTRACT Because anticarcinogenic and tumor-growth-inhibiting effects of non-soluble fibers have been described, similar actions of soluble fibers appear to merit investigation. In a preliminary study on methylnitrosoare-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 Ifa 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 (Rafitose Pp5, 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.

1 Presented at the conference Nutritional and Health Benefits of Inulin and Oligofructose held May 18–19, 1998 in Bethesda, MD. This symposium was supported in part by educational grants from the National Institutes of Health Office of Dietary Supplements, the U.S. Department of Agriculture and Orafti Technical Service. Published as a supplement to The Journal of Nutrition, Guest editors for the symposium publication were John A. Milner, The Pennsylvania State University, and Marcel Roberfroid, Louvain University, Brussels, Belgium.

2 To whom correspondence should be addressed.
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 $800 \text{ mm}^2$, which caused early mortality.

### 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 $800 \text{ mm}^2$, which caused early mortality.
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).

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 $\beta(2 \rightarrow 1)$ 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-

---

**TABLE 1**

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

<table>
<thead>
<tr>
<th>Diet group</th>
<th>Benign</th>
<th>Malignant</th>
<th>Other</th>
<th>Total</th>
<th>Mean(^1)</th>
<th>Metastasis</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>19</td>
<td>2</td>
<td>21</td>
<td>3.0 (2.7)</td>
<td>2</td>
<td>132</td>
<td>6.9</td>
</tr>
<tr>
<td>OFS</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>1.7 (1.3)</td>
<td>0</td>
<td>73</td>
<td>6.1</td>
</tr>
</tbody>
</table>

\(^1\) 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.
TUMOR INHIBITION BY INULIN OR OLIGOFRUCTOSE

1491S

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 carbohy-
drate. Tumors in other organs and metastases were observed
only in rats from the control group. Because the protective diet
gave during 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 trans-
plantable 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 in-
hibitory effect among all three dietary nondigestible carbohy-
drates 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 in-
volved in the inhibitory and/or anticarcinogenic 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 micro-
flora (Gibson et al. 1995, Wang and Gibson 1993). Such alter-
atations (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 azoxymeth-
ane-treated rats.

Moreover, it has been reported that a cell wall preparation
from Bifidobacterium infantis has a tumor-suppressive effect
(Sekine et al. 1994, Tsuuki et al. 1991); another report con-
cerned the antimelanoma activity of inulin (Cooper and Carter
the potential anticarcinogenic effect of nondigestible oligosac-
charides 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 oligosaccha-
rides 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 chicyc 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, Giovannucci et al. 1995) that hyperinsulinaemia 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
malignant 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 prom-
ising target for cancer therapy. Complementary to this idea are
recent observations that chicyc fructans, which inhibit tumor
growth, also decrease triglycerides, phospholipids and VLDL in
serum by lowering de novo lipogenesis in the liver (Fiordaliso

Further studies are required to elucidate which of the above-
mentioned mechanisms are essential in the tumor inhibitory
and/or anticarcinogenic 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 mecha-

isms 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.

LITERATURE CITED


52: 5794–5796.


Fiordaliso, M. F., Kok, N., Goethals, F., Desager, J. P., Debosyer, D., Roberfroid,
M. B. & Debienne, O. N. (1995) Dietary oligofructose lowers triglycerides, phos-
pholipids and cholesterol in serum and very low density lipoproteins of rats.

stimulation of Bifidobacteria in the human colon by oligofructose and inulin.


164–179.

Kok, N., Roberfroid, M. B. & Debienne, O. N. (1996) Involvement of lipogenesis in

G. R. (1994) Fatty acid synthesis: a potential selective target for antineo-

Milner, J. A. (1994) Reducing the risk of cancer. In: Functional Foods (Gold-

Reddy, G. V., Shabani, K. M. & Benerjee, M. R. (1973) Inhibitory effect of
yogurt on Ehrlich ascites tumor cell proliferation. J. Natl. Cancer Inst. 50:
815–817.

colon, mammary and liver carcinogenesis induced by 2-amino-3-methyl-
imidazo-[4,5-f]-quinoline, a food mutagen. Cancer Res. 53: 3914–3918.

Roberfroid, M. B. (1991) Dietary modulation of experimental neoplastic develop-
ment: role of fat and fibre content and caloric intake. Mutat. Res. 258:
351–362.

Roberfroid, M. (1993) Dietary fiber, inulin and oligofructose: a review compar-

a serially transplanted mouse mammary tumor and its tissue culture-adapted


Sekine, K., Watanabe-Seke, E., Ohta, J., Toida, T., Tatsuki, T., Kawashima, T. & Hashimoto,
Y. (1994) Induction and activation of tumoricidal cells in vivo
and in vitro by bacterial cell wall of Bifidobacterium infantis. Bifid. Microflora

transplantable mouse liver tumor of spontaneous origin. Cancer Res. 26:
143–148.

Tsuyuki, S., Yamazaki, S., Akashiba, H., Kamimura, H., Sekine, K., Toida, T.,
cell wall preparation, WPG, from Bifidobacterium infantis in germ free and

Characteristics of a serum- 
375–376.

Williams, G. M. & Dickerson, J. W. (1990) Nutrition and cancer. Some bio-