SHORT COMMUNICATION

Fasting during promotion, but not during initiation, enhances the growth of methylnitrosourea-induced mammary tumours

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The purpose of this work was to investigate the effect of fasting on the induction and growth of chemically-induced mammary carcinogenesis. Female Sprague–Dawley rats were given methylnitrosourea (MNU) i.p. (50 mg/kg) at 50 days of age; a group of rats were exposed to 4 day fasting followed by 1 day of refeeding before the administration of the carcinogen, while another group was exposed to three cycles of 3 days fasting in 10 days, beginning 1 week after MNU injection. Fasting enhanced the development of mammary tumours only in rats fasted after carcinogen damage, while it did not affect the induction of tumours in rats fasted before MNU, if compared with full-fed controls. The enhanced growth of mammary tumours sustained by fasting during promotion was observed in the cervical-thoracic region. In addition, exposure to fasting made rats susceptible to the development of MNU-induced extra-mammary cancers. Different from the preventive effect of caloric restriction on tumor development, these data demonstrate that fasting affects the promotion phase of carcinogenesis by enhancing the growth of MNU-induced mammary tumours.

Introduction

Epidemiologic evidence has established a link between several human cancers and certain dietary components and conditions, such as fat and caloric intake (1). Both ‘overnutrition’ and body mass seem to enhance the risk of breast cancer (2,3), and obesity appears related to a poor prognosis in neoplastic patients (4).

The effect of diet on mammary tumours has been investigated experimentally in spontaneous tumours (5,6) and in chemical carcinogen-induced cancers (7). Both high caloric intake and dietary fat are known to increase carcinogenesis (8,9) as well as the growth of mammary tumours (10). On the contrary, restricted caloric intake permanently suppresses or, at least, markedly reduces mammary tumorigenesis, independently from the phase of the carcinogenic process (8,11). However, it is largely accepted that the effect of caloric intake, as well as other dietary perturbations, is stronger during promotion than during initiation. Amongst the possible mechanisms that lead to this effect, it is known that underfeeding depresses secretion of anterior pituitary hormones (11), IGF-I and insulin circulating levels (12), and reduces the proliferative rate (13).

Recently, cell death by apoptosis has been involved in the protective effect of chronic food restriction (14).

Despite the large body of evidences about the effects of caloric restriction on the development of mammary tumours, the understanding of the role of fasting on mammary tumorigenesis remains insufficient and, furthermore, contradictory (15–17). Recently, in our laboratory we have demonstrated that rats given a sub-necrogenic dose of diethylnitrosamine during refeeding after 4 days of fasting developed hepatocytic foci, nodules and cancer, while no lesions were observed in the full-fed controls (18,19). In addition, animals exposed to cycles of fasting/refeeding after initiation showed liver foci/nodules larger than the full-fed controls (20).

In the present study the effect of fasting on the various phases of carcinogenesis induced by methylnitrosourea (MNU*) was investigated in female Sprague–Dawley rats. Our results demonstrate that exposure to fasting during promotion, but not during initiation, markedly enhances the development of mammary tumours.

Methods

Thirty female Sprague–Dawley rats (Charles River, Como, Italy) of 40 days of age were fed a balanced diet (Piccioni, Brescia, Italy). Animals were divided into three groups (10 rats/group; Figure 1). At 50 days of age all the animals were given a single i.p. injection of 50 mg MNU/kg body wt (Sigma Chemical Co., Milan, Italy). Group 1 was fed ad libitum for the entire experimental period and used as full-fed controls. Rats of group 2 were fasted for 4 days and refed 24 h prior to carcinogen administration; group 3 was fasted for 3 successive days in 10, beginning 1 week after MNU injection for a total

*Abbreviation: MNU, methylnitrosourea.
of three cycles of intermittent fasting. Animals were palpated twice a week to monitor mammary tumour appearance and measurement by a venier caliper. Tumour area was calculated by the product of the largest perpendicular diameters per π/4 (21). The average tumour area was then determined as the mean of the areas of all tumours/rat. Rats were killed when the main tumour reached 2 cm in diameter (31 ± 9 weeks from MNU). At necropsy, all mammary tumours, suspected areas and any tissue with macroscopic lesion were collected, processed for histology and classified according to Young and Hallowes (22). Statistical analyses were performed using Student’s t-test.

**Results**

As expected, body wt of animals exposed to fasting both before and after MNU markedly dropped and completely recovered during the following refeeding (Figure 2). No significant difference in the incidence, in the average latency and in mean tumour size of mammary adenocarcinomas was observed among the experimental groups, despite a tendency to decrease in the latency of tumour appearance in rats fasted during initiation (Table I). The mammary tumour multiplicity (tumours/tumour-bearing animal) was significantly higher in animals exposed to three cycles of fasting after administration of the carcinogen in comparison with the full-fed controls. Four days of fasting prior to administration of the initiating agent had no effect on mammary tumour yield. The increased number of tumours per rat in the group exposed to cycles of fasting during promotion was due to an increment in only adenocarcinomas. No difference was observed in the benign tumours. The rate of occurrence of mammary carcinomas in the cervical-thoracic region in rats exposed to cycles of fasting/

**Table I.** The effect of fasting on MNU mammary tumorigenesis

<table>
<thead>
<tr>
<th></th>
<th>Incidence</th>
<th>Cancer free time (weeks)</th>
<th>No. of T</th>
<th>Mean T area (cm²)</th>
<th>BMT a</th>
<th>p</th>
<th>c</th>
<th>i</th>
<th>MMT AC</th>
<th>PC</th>
<th>APC</th>
<th>No. of MMT C-T A-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>100</td>
<td>25.4 ± 8.4</td>
<td>1.5 ± 1.0</td>
<td>2.7 ± 0.7</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>Controls</td>
</tr>
<tr>
<td>F on Initiation</td>
<td>100</td>
<td>20.5 ± 7.1</td>
<td>1.4 ± 0.7</td>
<td>3.1 ± 1.0</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>F on Initiation</td>
</tr>
<tr>
<td>F on Promotion</td>
<td>100</td>
<td>24.1 ± 7.0</td>
<td>2.9 ± 1.4*</td>
<td>2.3 ± 1.3</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>F on Promotion</td>
</tr>
</tbody>
</table>

Controls: the full-fed rats; F = fasting; T = tumours, BMT = benign mammary tumours; MMT = malignant mammary tumours; AC = adenocarcinoma; PC = papillary; APC = adenosubpapillary; a = adenoma; p = papilloma; c = cysts; i = hyperplasia. C-T = cervical-thoracic; A-I = abdominal-inguinal; mean ± SD. The tumour size was expressed as mean tumour areas ± SD. *Significantly different from controls; P < 0.05.

**Fig. 2.** Growth curves of different animal groups. Arrows indicate the start of cycles of fasting. Day 0 corresponds to the time of administration of MNU as described in Methods. SD is within 10%.

( ) Controls; ( ) Fasting/refeeding on initiation; ( ) Fasting/refeeding on promotion.

**Table II.** Non-mammary tumours induced by MNU

<table>
<thead>
<tr>
<th></th>
<th>Non-mammary tumours</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F on initiation</td>
<td>2 mesenchymal tumours, kidney</td>
<td>0</td>
</tr>
<tr>
<td>F on promotion</td>
<td>2 mesenchymal tumours, kidney</td>
<td>0</td>
</tr>
</tbody>
</table>

refeeding during promotion was more than two-fold that of the control group. Moreover, fasted rats, both prior and after the administration of MNU, developed about three-fold more mammary tumours in cervical-thoracic area as in the abdominal-inguinal gland chains.

Interestingly, the exposure for 3–4 days of fasting, during the initiation and the promotion phase, enhanced the susceptibility of the genito-urinary tract to MNU-induced carcinogenesis (Table II).

**Conclusions**

The main finding of our work is that exposure to cycles of fasting during promotion enhances the development of rat mammary cancers induced by MNU, while no effect was observed in rats fasted before initiation. One day in two of fasting for the life span of the animals increased the survival time and also decreased the incidence of spontaneous mammary tumours (15), while no effect was observed when rats were exposed to 1-day fasting in 3 (16). When spontaneous or transplantable tumours were already macroscopically evident the exposure of mice to 2 or 3 day-fasting resulted in suppression of cell proliferation and in tumour regression; the refeeding re-established the pre-fasting growth rates (17). We have recently reported that one or three cycles of fasting after initiation by diethylnitrosamine, stimulated the growth of foci/nodules in the liver (20). Interestingly, liver cancer was induced by a non-initiating dose of diethylnitrosamine when exposure to fasting occurred before the administration of the carcinogen (18,19). The exposure of rats to fasting either before or after MNU administration made organs or tissues, other than mammary glands, more susceptible to carcinogen damage. The responsiveness of various tissues to MNU is generally dependent on the dose and the method of administration (23). In the experimental conditions used here, no primary tumours except the mammary ones were observed in rats treated only with MNU. Nevertheless, some rats exposed to fasting during
either initiation or promotion developed extra-mammary tumours.

The effect of fasting on mammary carcinogenesis could be related to general mechanisms or organ-specific factors. Both cell proliferation and cell death play a pivotal role in the development of cancer. Fasting depresses cell division while refeeding stimulates cell proliferation in several organs, including the mammary glands (17). However, exposure of rats to fasting induced liver cell death by apoptosis (14), which could be the stimulus for cell proliferation that occurs during refeeding. The hormonal state plays a critical role in mammary gland development and carcinogenesis. Ovariectomy causes tumour regression, while hypotalamic lesions or pituitary graftings enhance tumour growth. Oestrogens seem involved in the initiation phase (24), while prolactin affects promotion (25), with both stimulating tumorigenesis. In our experimental conditions fasting could depress the levels of oestrogens and prolactin, while refeeding might restore/increase their levels over the basal value.

The lack of effect of fasting during the initiation of the trial on mammary tumours could depend on the high dose of carcinogen. In fact, fasting has no effect at all on liver carcinogenesis when rats were initiated with the carcinogenic dose of diethylnitrosamine (20). Alternatively, fasting could increase the number of molecules of ‘terminal’ carcinogen. It is unlikely that fasting enhances the activation of MNU, because it is a direct-acting carcinogen that does not require metabolic activation, as has been previously reported (26).

Regardless of the mechanisms involved, the enhancing effect of fasting on carcinogenesis differs from the protective action of caloric restriction on several neoplasia (27), included mammary cancers (8,11,28). Chronic caloric restriction causes in the animal various alterations (metabolic, hormonal, energy balance etc.) that remain constant for a long period of time, while cycles of fasting induce acute responses, which are reversed during refeeding. Our data suggest that fasting/reefing appears to trigger different metabolic, hormonal and proliferative responses that need further investigation in order to understand how dietary habits can interfere with cancer.

Acknowledgements

This work was supported by grants from CNR (No. 95.02456.CT04) and MURST, Rome and Associazione Italiana per la Ricerca sul Cancro, Milan. We are grateful to Dr P.Pani for helpful comments.

References


Received on October 22, 1996; revised on April 21, 1997; accepted on April 22, 1997

Fasting effects of chemically-induced mammary cancers