EFFECTS OF MELPHALAN ON THE DEVELOPMENT OF EXPERIMENTAL PANCREATIC CANCER

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SUMMARY

The effects of melphalan were studied in rats fed raw soya flour and injected with azaserine in order to determine the suitability of this experimental model for testing drugs potentially useful for the treatment of pancreatic cancer. While melphalan did not prevent or delay the development of pancreatic cancer in these rats, the drug significantly lessened the number and size of the premalignant proliferative lesions in the pancreas. It seems that the model is useful both for testing potentially useful therapeutic agents and for analysing some of the processes involved in the development of pancreatic cancer.

INTRODUCTION

We have previously reported that diets containing raw soya flour stimulate pancreatic growth in rats and that a small proportion (10–15%) of animals ultimately develop pancreatic cancer [5]. A diet of raw soya flour has also been shown to sensitise the raw pancreas to azaserine, a weak pancreatic genotoxic carcinogen [4,6].

In the present study, we have monitored the effects of melphalan (4-bis-(2-chloroethyl)-amino-L-phenylalanine), a drug used in the chemotherapy of human pancreatic cancer [2], on the development of proliferative and neoplastic lesions in the rat pancreas, in order to determine whether raw soya flour-fed, azaserine-treated rats provide a model for screening drugs potentially useful in the management of human pancreatic cancer.

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MATERIALS AND METHODS

Seventy-two male Wistar rats, 10 weeks old and weighing approximately 350 g were used in the study. The rats were divided into 4 groups and subjected to the following treatment regimens (Table 1).

1. Twenty-two rats were fed a diet of raw soya flour for as long as 90 weeks. Details of dietary composition and of vitamin and mineral supplements have been published previously [1].

2. Five rats were fed a diet of raw soya flour and were also given 1 mg/kg body wt of melphalan (Wellcome Foundation, London, U.K.) orally in syrup 5 times each week for 12 weeks and then 3 times each week for 40 weeks.

3. Twenty-five rats were fed a diet of raw soya flour as in group 1 and, in addition, received twice weekly intraperitoneal injections of 5 mg/kg body wt of azaserine (Calbiochem-Behring Corp., Bishops Stortford, U.K.) for as long as 60 weeks.

4. Twenty rats were fed raw soya flour and received twice weekly injections of 5 mg/kg azaserine for up to 60 weeks. These rats also received melphalan in a manner identical with rats of group 2.

After 2 years of study, all the animals had died or were killed. The pancreas and other organs were removed, weighed, examined macroscopically and prepared for histological study. Eight blocks were taken from each pancreas and sections were stained with haematoxylin and eosin. The volume densities of the pancreatic nodules were assessed according to the method of Weibcl [11], as described previously [6].

Pancreatic tissue from the animals killed after 24 weeks of study was also analysed for content of DNA and RNA by the method of Munro and Fleck [8]. Total protein was measured by the method of Papadopoulos et al., [9].

RESULTS

Body weights (Fig. 1)

In all groups, maximal weight was attained after 30 weeks of the study. Throughout the experimental period, the lightest rats were those which had only received a diet of raw soya flour (group 1). The weights of these rats were significantly less than rats which had also received melphalan (group 2) ($P < 0.02$).

Pancreatic weights (Fig. 2)

After 24 weeks of treatment, the azaserine-injected rats had significantly heavier pancreases ($P < 0.05$) than similarly treated rats of the same age which had also received melphalan. After 60 weeks or more of treatment, the lightest pancreases were found in the raw soya-fed rats given melphalan. A significant difference ($P < 0.05$) was observed between the weights of
## TABLE 1

**EFFECTS OF TREATMENT REGIMENS ON PROLIFERATIVE LESIONS IN RAT PANCREAS**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Duration of study (weeks)</th>
<th>No. of rats</th>
<th>Percent of pancreases with</th>
<th>Volume density of microscopic nodules</th>
<th>Percent of pancreases with carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Percent of pancreases with</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>microscopic nodules</td>
<td>Macroscopic nodules</td>
<td>Adenomatous nodules</td>
</tr>
<tr>
<td>1. RSF</td>
<td>24</td>
<td>7</td>
<td>43</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>61–90</td>
<td>15</td>
<td>100</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>2. RSF + M</td>
<td>61–90</td>
<td>5</td>
<td>100</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>3. RSF + AZA</td>
<td>61–90</td>
<td>19</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4. RSF + AZA + M</td>
<td>24</td>
<td>6</td>
<td>50</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>31–90</td>
<td>14</td>
<td>100</td>
<td>100</td>
<td>93</td>
</tr>
</tbody>
</table>

RSF = raw soya flour diet; M = melphalan; AZA = azaserine injections.

*\(P < 0.01.\)

\(bP < 0.001.\)

\(cP,\) not significant (>0.05).
Fig. 1. Mean body weights of rats in the 4 treatment groups. Each point denotes mean ± S.E.M. RSF = raw soya flour (group 1); RSF + M = raw soya flour plus melphalan (group 2); RSF + AZA = raw soya flour plus azaserine (group 3); RSF + AZA + M = raw soya flour plus azaserine plus melphalan (group 4).

Fig. 2. Pancreatic weights of rats in the 4 treatment groups. Each point represents weight of 1 pancreas. Abbreviations as in Fig. 1. Abscissa shows the respective groups and the duration of treatment in weeks in each group.
these pancreases and those of raw soya-fed rats which had not received melphalan.

Pancreatic content of DNA, RNA and protein (Fig. 3)

After treatment for 24 weeks, the content of DNA ($P < 0.05$), RNA ($P < 0.01$) and total protein ($P < 0.01$) was significantly less in rats given additional melphalan (group 4) than rats given only a raw soya flour diet and azaserine injections (group 3).

Pancreatic nodules and cancer (Table 1)

Group 1. Raw soya flour diet: When fed raw soya flour for less than 30 weeks, the majority of rats did not show microscopically detectable nodules. After more than 60 weeks of study, all pancreases contained both macroscopic and microscopic nodules and three quarters of the animals had pancreases containing adenomatous nodules. None of these rats developed pancreatic carcinomas.

Group 2. Raw soya flour diet plus melphalan: After longer than 60 weeks of study, all pancreases contained both macroscopic and microscopic nodules. Three of the 5 rats also had adenomatous nodules. The volume density of the nodules was significantly less ($P < 0.01$) than the nodules of rats fed raw soya flour only (group 1).

![Fig. 3. Total pancreatic content of DNA, RNA and protein. Each point represents results from 1 animal. Abbreviations as in Fig. 1.](image-url)
Group 3. Raw soya flour diet plus azaserine injections: After 24 weeks of study, all pancreases contained both macroscopic and microscopic nodules. Over half of the animals (11/19) developed pancreatic carcinomas.

Group 4. Raw soya flour diet plus azaserine injections plus melphalan: After 24 weeks of study, 1 of 6 pancreases contained macroscopic nodules and a further 2 pancreases contained microscopic nodules. The number and volume density of the pancreatic nodules in this group of rats was highly significantly less ($P < 0.001$) than in rats fed raw soya flour and injected with azaserine only (group 3). After 30 weeks or more of treatment, 5 of the 14 rats developed pancreatic carcinomas.

DISCUSSION

Melphalan was selected as chemotherapeutic test drug in the present study because it had been used in the treatment of human pancreatic cancer [2] and also because it was considered particularly cytotoxic when used against rapidly proliferating cells [10].

We have previously reviewed the evidence for considering that azaserine initiates pancreatic cancer and that feeding raw soya flour promotes the development of the cancer in rats [7]. The present study has shown that while melphalan does not prevent or delay the ultimate development of pancreatic carcinoma in rats fed raw soya flour and injected with azaserine, the drug exerts an apparently significant inhibitory effect on the premalignant proliferative lesions of the pancreas. Melphalan decreased the number and size of the nodules in the rats fed raw soya flour throughout their life and even more significantly decreased the heightened expression of focal hyperplasia which characterises the pancreas of rats fed raw soya flour and receiving additional azaserine. The apparent inhibition of the histological manifestations of the focal proliferative tendency is confirmed by the finding that the DNA, RNA and total protein content of the pancreas of raw soya flour-fed, azaserine-injected rats is significantly less in the animals which have also received melphalan. We conclude that melphalan either prevents the development or promotes the regression of a considerable proportion of the hyperplastic nodules while not affecting the progression to malignancy.

It seems that our model of the azaserine-injected, raw soya flour-fed rat is a useful model not only for testing drugs which are potentially useful in the treatment of pancreatic cancer, but also for analysing some of the processes involved in the development of this type of cancer. This study has shown that melphalan does not affect the ultimate occurrence of frankly malignant pancreatic neoplasms but does significantly decrease the prevalence of the precursor proliferative foci produced by the combination of raw soya flour and azaserine. It is not yet clear whether this finding implies that there are two populations of proliferative foci — one sensitive to inhibition by melphalan and the other resistant to this type of inhibition.
Melphalan presumably does not affect the degree of initiation produced by azaserine and therefore probably interferes with the growth-stimulant effect of raw soya flour and the consequent promotion of the initiated cells. If some hypothesis such as this is correct, then comparison between foci in melphalan-treated and untreated animals may provide information about phenotypic changes predisposing to full neoplastic progression at an early stage in the development of the hyperplastic foci.

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REFERENCES