Parenteral gamma-linolenic acid administration in nude mice bearing a range of human tumour xenografts

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Summary
The nude mouse human tumour xenograft system was used as an in vivo model to investigate the possible effect of gamma-linolenic acid (GLA) both on established tumour xenografts and as a prophylactic agent prior to tumour induction. Eighty-nine nude mice bearing a range of different human tumours were studied and two solvents (each of which presented certain practical problems) were used to deliver GLA parenterally to the animals. GLA treatment was found to have no significant effect on the growth of any of the tumour xenografts investigated.

Recent reports have indicated that gamma-linolenic acid (GLA) has a marked effect on certain cell lines in vitro. The aim of this investigation was to determine whether or not these findings could be reproduced in vivo. Ghayur and Horrobin have reported inhibition of mammary tumour growth in rats after GLA administration. In the present study parenteral GLA (dissolved in sodium carbonate (Na₂CO₃)) was used to investigate whether this fatty acid had any effect on the growth of established human tumour xenografts in the nude mouse. Since this solution produced irritation at the injection site olive oil was used as solvent in a subsequent experiment in which treatment commenced prior to inoculation with tumour cells.

Materials and methods
The 89 BALB/C nude mice used in this study were maintained and bred under SPF-4 conditions. Tumours were induced in 4-6-week-old mice of both sexes by the subcutaneous inoculation of approximately 10⁶ cells of the continuous cell lines HCU 10, HCU 13 and HCU 39 or the tumour strain RB. The continuously growing cell lines HCU 10, HCU 13 and HCU 39 were derived respectively from poorly, well- and moderately differentiated human squamous cell carcinomas of the oesophagus. All lines were established using a dry explant technique and grown in Eagle's minimum essential medium supplemented with fetal calf serum. 'Take rates' and latent periods for tumours induced by cells of these lines have been reported previously.

Tumours induced in nude mice by the inoculation of HCU 39 cells were killed and subsequently re-inoculated into additional mice. Tumour passaging was continued and the HCU 39 tumours in the 9 mice in this study were at third passage level. The RB tumour strain, derived from a moderately differentiated invasive human tumour of the soft palate, was diced and inoculated subcutaneously into nude mice. The resulting tumours were re-inoculated and repeatedly passed through nude mice. This tumour strain was characterized by a 100% 'take rate' within 10 days of inoculation. The tumours in the 39 mice observed in this study were at the third and fourth passage level. Sterile solutions of GLA (1 mg/ml) were prepared using either 0,1M Na₂CO₃ or olive oil as solvents. Aliquots were stored frozen. The presence of GLA in both solutions was verified using thin-layer chromatography. As GLA is very unstable thin-layer chromatography was also used to confirm that the fatty acid was still present in the subcutaneous space 30 minutes after injection into the mouse.

The effects of GLA in Na₂CO₃ on a range of existing tumour xenografts with diameters exceeding 2 mm were examined in 68 nude mice. The mice received daily subcutaneous injections (0,1 ml) of Na₂CO₃ or the GLA in Na₂CO₃ solution or no treatment at all. Tumour sizes were measured during the experiment and at its termination, when all tumours were fixed in 10% formal saline and prepared for histopathological investigation.

To investigate the possible prophylactic effect of GLA, 21 nude mice were randomized into three groups of 7 each. All mice received daily subcutaneous injections (0,1 ml) from day 1 and all were inoculated with tumour strain RB on day 7. Group A received olive oil and group B GLA in olive oil for the duration of the experiment. Group C received olive oil until the day of tumour inoculation, whereupon GLA in olive oil was substituted. The experiment was terminated on day 23 since gross oil accumulation in the subcutaneous space hindered tumour palpation. Small nodular masses were seen on dissection and were examined microscopically.

Results
Inflammation and scabs developed at the injection sites in all mice which received Na₂CO₃ or GLA in Na₂CO₃. In the study using this solvent, tumour volumes calculated before and during treatment did not differ significantly between the three groups, although minor fluctuations occurred during the treatment period. Results of the histopathological investigations are shown in Table II.

In the study using the olive oil as solvent it was impossible to palpate tumours at any time because, from day 14 onwards, large subcutaneous oil depots developed. However, on dissection non-invasive nodules were discovered in all groups: 4 in group A, 6 in group B and 5 in group C. These were all found to be poorly differentiated carcinomas. Some cytoplasmic vacuolation was observed in tumours from groups B and C. Mice in all three
Tumour cells
inoculated | Treated with | Treated with | Duration of
          | Na₂CO₃ | GLA in Na₂CO₃ | treatment (d) |
----------|---------|-------------|--------------|
HCU 10    | 4       | 0           | 8            | 10 |
HCU 13    | 4       | 0           | 4            | 10 |
HCU 39    | 4       | 0           | 5            | 10 |
RB        | 4       | 17          | 18           | 10 |

Table II: Differentiation and Necrosis of Treated and Untreated Tumours in the Study Using Sodium Carbonate as Solvent (Number of Mice in Brackets)

<table>
<thead>
<tr>
<th>Tumour cells inoculated</th>
<th>Summary of treatment</th>
<th>Degree of differentiation</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLA in Na₂CO₃</td>
<td>Well (8)</td>
<td>Central (4)</td>
</tr>
<tr>
<td></td>
<td>Untreated (4)</td>
<td>Well (3)</td>
<td>Central (1)</td>
</tr>
<tr>
<td>HCU 10</td>
<td>GLA in Na₂CO₃</td>
<td>Well (4)</td>
<td>10-50% central (4)</td>
</tr>
<tr>
<td></td>
<td>Untreated (4)</td>
<td>Well (4)</td>
<td>None (4)</td>
</tr>
<tr>
<td>HCU 13</td>
<td>GLA in Na₂CO₃</td>
<td>Moderate (5)</td>
<td>Small areas (4)</td>
</tr>
<tr>
<td></td>
<td>Untreated (4)</td>
<td>Moderate (4)</td>
<td>Minimal (1)</td>
</tr>
<tr>
<td>HCU 39</td>
<td>GLA in Na₂CO₃</td>
<td>Poor (18)</td>
<td>Variable (18)</td>
</tr>
<tr>
<td></td>
<td>Untreated (4)</td>
<td>Poor (17)</td>
<td>Variable (17)</td>
</tr>
<tr>
<td></td>
<td>Na₂CO₃ (18)</td>
<td>Poor (4)</td>
<td>Few areas (1)</td>
</tr>
<tr>
<td></td>
<td>Na₂CO₃ (17)</td>
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</table>

Discussion

The results of several in vitro and a single in vivo study have supported the hypothesis of Horrobin that GLA supplementation may inhibit tumour growth. However, in the present study GLA administration had no significant effect on various human tumour xenografts in nude mice.

In an attempt to parallel the in vitro studies Na₂CO₃ was used as a solvent. In this experiment tumour volumes did not differ significantly between control mice and those receiving GLA. Thus GLA did not affect the growth of any of the four tumour types treated. GLA was also without effect on the degree of tumour differentiation. While GLA-treated tumours showed a greater incidence of necrosis than those of untreated controls, comparison of the test group with that receiving Na₂CO₃ alone revealed equivalent degrees of necrosis. This indicates that cell death was probably not due to the GLA but rather to the solvent. The necrotic changes may be a consequence of the inflammatory process induced by this intensely irritant substance. While the irritant effects of Na₂CO₃ clearly made it an unsuitable solvent for further in vitro studies, thin-layer chromatography indicated that GLA was present in the mouse long enough after administration for some of it to reach the tumour. In fact, the study closely paralleled the in vitro work since some of the tumours investigated were derived from the same cell lines as those studied in culture. Despite this, the in vitro findings were not reproduced.

While the use of olive oil as a solvent overcame the irritation problem, accumulation of oil in the subcutaneous space interfered with tumour growth. Small tumours did develop, however, and the incidence of these was unrelated to the presence of GLA. Such findings are contrary to those of Ghayur and Horrobin, who reported that subcutaneous administration of evening primrose oil inhibited mammary tumour growth in rats. The conflicting findings may be related to such factors as immunocompetence of the animal host, metabolic capacity of the treated tumours and the dose of oil.

In summary, the present study showed that GLA had no effect on the various tumours studied, regardless of solvent used. It appears, therefore, that the published in vitro findings do not hold in the in vivo model used here, and for the present should accordingly be interpreted with caution.

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REFERENCES