Methotrexate increases the activation and cytotoxicity of 5-fluorouracil: detected by \(^{19}\text{F}\) n.m.r. in vivo

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In the rat Walker 256 carcinosarcoma the cytotoxicity of the anticancer drug 5-fluorouracil (FU) should be thought to be mediated by the 5-fluoronucleotide (FNuct) FUTP, which interferes with RNA metabolism [1]. Total FNuct can be detected in vivo, as a composite peak, by \(^{19}\text{F}\) n.m.r. and we have found in the Walker 256 carcinosarcoma that decreased concentrations of FNuct (mainly FUTP) formed in vivo accompanied decreased cytotoxicity [2]. Sequential treatment of Walker tumours with methotrexate (MTX) followed by FU (MTX–FU) is known to produce increased cytotoxicity compared with either drug alone or to the reverse schedule (FU–MTX) [3]. This effect may be due to MTX increasing intracellular pyrimidine ribosylpyrophosphate (PRPP) and thus increasing anabolism of FU to FNuct [4]. The aim of this study was to use \(^{19}\text{F}\) n.m.r. to determine whether MTX pretreatment increases FNuct formation in vivo and to correlate this with cytotoxicity.

Methods

Walker tumours grown subcutaneously in female Wistar rats were used for n.m.r. studies when > 4 g (determined from the formula \([\text{width}^2 \times \text{length}/2] \approx \text{g}\) [2]). Rats (200 g, three in each group) were anaesthetized with sodium pentobarbitone 30 min before being placed in the 1.9 T horizontal bore magnet of an ORS TMR-32 spectrometer and receiving 50 mg of FU/kg as an intravenous bolus. Tumour spectra \((75.5\text{ MHz, with a } 4\text{ kHz bandwidth})\) were obtained immediately with a 15 mm surface coil using 480 15 ps pulses and a 1 s repetition time. All spectra received 19 Hz line broadening and peak areas were determined using the ORS analysis programme. Pretreated rats received 20 mg of MTX/kg intraperitoneally 24 h before the FU injection (MTX–FU schedule).

To measure tumour growth inhibition, 40 rats (four groups of 10) were injected subcutaneously with Walker tumour cells on day 0. When tumours reached a group mean of at least 4 g they were treated with 0.9% (w/v) NaCl on days 6 and 7 (control), or MTX (20 mg/kg) and FU (50 mg/kg) on days 6 and 7, respectively (MTX–FU), or the reverse schedule (FU–MTX), or with a single dose of MTX alone on day 6.

Abbreviations used: FU, 5-fluorouracil; FNuct, 5-fluoronucleotide; MTX, methotrexate; PRPP, pyrimidine ribosylpyrophosphate.

Results and discussion

\(^{19}\text{F}\) spectra taken in vivo showed that tumours receiving FU alone metabolized the drug to FNuct until 67 min, when the FU and FNuct peak areas were similar. MTX–FU caused a large and significant increase in FNuct compared with FU (Table 1). Analysis of tumour extracts by \(^{19}\text{F}\) n.m.r. and h.p.l.c. (results not shown) confirmed that, in comparison to FU, MTX–FU led to a 3-fold increase in total FNuct, as well as a 2.5-fold increase in FUTP. These results are consistent with MTX raising intracellular levels of PRPP and thus enhancing RNA-directed cytotoxicity of FU.

Pretreatment of the Walker tumour by MTX 24 h before FU (MTX–FU) led to significant inhibition of tumour growth (Table 1). Gabriel’s analysis of variance showed that the MTX–FU schedule was also significantly different to the FU–MTX schedule or MTX alone \((P<0.001)\). These results suggest that n.m.r. could be used in the clinic to optimize the MTX–FU regime for tumours sensitive to RNA-directed cytotoxicity.

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Table 1. Effect of FU–MTX combination therapy on FU and FNuct signals in Walker tumours and on tumour growth

<table>
<thead>
<tr>
<th>Schedule</th>
<th>FU peak area (arbitrary units)</th>
<th>FU-MTX peak area (arbitrary units)</th>
<th>FNuct peak area (arbitrary units)</th>
<th>Mean tumour weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FU</td>
<td>1045 ± 133</td>
<td>N.A.</td>
<td>841 ± 203</td>
<td>13.5 ± 1.9</td>
</tr>
<tr>
<td>FU-MTX</td>
<td>734 ± 172</td>
<td>N.A.</td>
<td>2432 ± 343*</td>
<td>7.5 ± 2.4†</td>
</tr>
<tr>
<td>MTX-FU</td>
<td>133 N.A.</td>
<td>734 N.A.</td>
<td>133 N.A.</td>
<td>12.1 ± 4.0</td>
</tr>
</tbody>
</table>

*P<0.05 compared with FU (Gabriel’s analysis of variance).
†P<0.02 compared with control tumours (20.7 ± 2.1 g, n = 10; Gabriel’s analysis of variance).