THREE-DIMENSIONAL CELL CULTURES AS A MODEL SYSTEM TO EVALUATE THE BIOLOGICAL ACTIVITY OF GEMCITABINE (2',2'-DIFLUORO-2'DEOXYCYTIDINE)


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Abstract. The cytotoxicity of Gemcitabine (dFdC) was 10 (ovarian cancer) to >10,000 (colon cancer) fold higher in monolayer compared to three-dimensional multilayer cell cultures. This selectivity was related to marked differences in dFdC activation and effects on ribonucleotides.

Gemcitabine (dFdC) is a cytidine analog currently under clinical evaluation, which has shown remarkable antitumor activity against ovarian and non-small cell lung cancer (partial response (PR) rates of 20-30%) but not against colon cancer (PR 4%)1. The compound needs activation to the triphosphate dFdCTP which can be incorporated into DNA and RNA2,3,4. In addition to inhibition of ribonucleotide reductase and dCMP deaminase5,6, considerable changes in ribo- and deoxyribonucleotide pools have been observed7,8. Initial preclinical in vitro evaluation was performed predominantly with leukemic cell lines2,8. In vivo evaluation showed considerable activity against ovarian and head and neck squamous cell carcinoma and variable activity against colon carcinoma9,10,11, only partly correlating with in vitro sensitivity of cell lines. Since solid tumors grow in a three-dimensional structure we further evaluated dFdC activity and metabolism in a culture system12, in which cells grow as three-dimensional multilayers and compared this with the two-dimensional monolayer system.

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MATERIALS AND METHODS.

Cells were cultured as monolayers and multilayers in 96-wells microtiter plates with flat-bottom and "V"-shaped wells, respectively, and were exposed to dFdC for 24 hr; monolayers at one day, multilayers at 5 days after plating, followed by culture in drug-free medium for 4 days. Using the sulphorhodamine B (SRB) test, growth inhibition was determined for the human ovarian cancer cell line A2780 and the human colon cancer cell lines SW620 and HT29. For monolayers the IC50 value (50% growth inhibition when compared to untreated cultures and corrected for initial cell count) and for multilayers the EC50 (concentration at which 50% of the cells in treated wells was present compared to untreated wells) was calculated. The metabolic effects of dFdC were determined by measurement of dFdCTP and that of normal nucleotides as described.

RESULTS AND DISCUSSION

Considerable differences in sensitivity were observed for the three cell lines (Table 1), with A2780 being the most sensitive cell line also when grown as multilayers. For the colon cancer cell lines almost no growth inhibition could be detected in multilayers, but in the A2780 cells dFdC was still cytotoxic. Both in mono- and multilayers of the A2780 cells the highest accumulation of the active metabolite dFdCTP was observed.

The concentrations of normal nucleotide pools were considerably lower in multilayers when compared to monolayers; dFdC caused major changes in these pools in both cell culture systems, which were cell line and concentration dependent. Exposure to 10 μM dFdC caused a 3-4 fold decrease in CTP pools in the colon tumor cells which was accompanied by a 2-3-fold increase in UTP pools (Table 1). This is consistent with an inhibition of CTP synthetase. However, at 1 μM dFdC only a 2-3-fold increase in UTP pools, but no decrease in CTP was observed in all cell lines. Interestingly, dFdC also induced a 2-3-fold increase in ATP and GTP pools in all monolayers, but only in A2780 cells a similar increase was observed in multilayers. Also in xenografts from ovarian cancer the increase of ATP was
TABLE 1: Summary of dFdC cytotoxicity, dFdCTP accumulation and effect on normal nucleotides in mono- and multilayered cultures.

<table>
<thead>
<tr>
<th></th>
<th>A2780</th>
<th>HT29</th>
<th>SW620</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 monolayers (nM)</td>
<td>6</td>
<td>26</td>
<td>36</td>
</tr>
<tr>
<td>EC50/IC50 ratio</td>
<td>16.3</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>dFdCTP accumulation*</td>
<td>2306</td>
<td>1364</td>
<td>1269</td>
</tr>
<tr>
<td>(pmol/10⁶ cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dFdCTP ratio mono/multi*</td>
<td>3.5</td>
<td>5.3</td>
<td>4.6</td>
</tr>
<tr>
<td>UTP ratio mono-multi⁺</td>
<td>3.2</td>
<td>1.7</td>
<td>2.9</td>
</tr>
<tr>
<td>CTP ratio mono-multi⁺</td>
<td>3.6</td>
<td>2.6</td>
<td>5.1</td>
</tr>
<tr>
<td>ATP ratio mono-multi⁺</td>
<td>3.9</td>
<td>1.4</td>
<td>2.2</td>
</tr>
<tr>
<td>UTP concentration (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mono</td>
<td>204</td>
<td>460</td>
<td>230</td>
</tr>
<tr>
<td>multi</td>
<td>252</td>
<td>167</td>
<td>177</td>
</tr>
<tr>
<td>CTP concentration (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mono</td>
<td>130</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>multi</td>
<td>254</td>
<td>36</td>
<td>25</td>
</tr>
<tr>
<td>ATP concentration (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mono</td>
<td>181</td>
<td>350</td>
<td>199</td>
</tr>
<tr>
<td>multi</td>
<td>200</td>
<td>132</td>
<td>142</td>
</tr>
</tbody>
</table>

Means of at least 3 separate experiments, with SE less than 20%.

⁺, 24 hr exposure to an equimolar concentration of 1 μM dFdC.

⁺, ratio of concentrations in untreated monolayers compared to multilayers.

Higher than in those from colon cancer⁷. It is noticeable that changes in ribonucleotide pools have not been reported in other studies²⁻⁸, but may be responsible for the observed differences between mono- and multilayer cultures. Also for other antimetabolites and other investigational and standard drugs we have observed differences in metabolism and sensitivity between the monolayers and multilayers¹²⁻¹³, which are in favor for the multilayer system to predict selective chemosensitivity in solid tumors.

In conclusion, dFdC shows a considerably different cytotoxicity pattern in cells grown in a three-dimensional structure compared to the two-dimensional monolayer cultures. This is accompanied by remarkable changes in its metabolism and the effect on normal nucleotide metabolism. It is
anticipated that the multilayer system may have a better predictive value for
in vivo systems than the monolayer system.

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REFERENCES

   19, 45-55.
   1988, 48, 4024-4031.
3. Huang, P.; Chubb, S.; Hertel, L.W.; Grindey, G.B.; Plunkett, W. Cancer
4. Ruiz Van Haperen, V.W.T.; Veerman, G.; Vermorken, J.B.; Peters,
5. Heinemann, V.; Xu, Y.Z.; Chubb, S.; Sen, A.; Hertel, L.W.; Grindey,
8. Shewach, D.S.; Hahn, T.M.; Chang, E.; Hertel, L.W.; Lawrence, T.S.
   Cancer Res. 1994, 54, 3218-3223.
11. Braakhuis, B.J.M.; Van Dongen, G.M.A.S.; Vermorken, J.B.; Snow,
12. Pizao, P.E.; Peters, G.J.; Van Ark-Otte, J.; Smets, L.A.; Smitskamp-
    1993, 29A, 1566-1573.