Correlations between Cytotoxicity, Biochemical Effects, Drug Levels, and Therapeutic Effectiveness of Daunomycin and Adriamycin on Sarcoma 180 Ascites in Mice

Rosella Silvestrini, Luigi Lenaz, Giovanni Di Fronzo, and Ornella Sanfilippo

Division of Experimental Oncology B, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

SUMMARY

The effects of adriamycin and daunomycin on mitotic activity, nucleic acid synthesis, and proliferative activity have been compared in Sarcoma 180 ascites in mice and related to the uptake and retention of the drugs by the ascites cells. The half-life of the drug in the cells after a single injection was 4 hr for daunomycin and 12 hr for adriamycin, while after repeated doses the uptake of the antibiotics was similar. The inhibition of mitotic activity and of thymidine incorporation into DNA was similar with daunomycin and adriamycin, while adriamycin inhibited uridine incorporation into RNA to a greater extent. When given according to different schedules to Sarcoma 180-bearing mice, adriamycin appeared able to inhibit tumor growth to a greater extent than did daunomycin and to induce a greater number of tumor-free long-term survivors. This effect has been observed also after a treatment schedule which results in similar intracellular levels of both antibiotics. The importance of mechanisms other than different cellular uptake of the drugs, operative in determining differences in the overall therapeutic efficacy in vivo, remains to be elucidated.

INTRODUCTION

The effectiveness of AM has been shown to be higher than that of DM, both in experimental tumors (4, 5) and in human neoplasms (2, 9). This has been partly related to a different pharmacokinetic behavior and metabolic fate, AM having been shown to be disposed more slowly (3) and to be metabolized to a smaller extent than DM (1, 3).

In studies on mammalian cells in culture, the effects of AM on mitotic and proliferative activities and nucleic acid synthesis have been described as equivalent (12) or inferior (8, 14) to those of DM. Furthermore, it was shown that high pharmacokinetic behavior and metabolic fate, AM than that of DM, both in experimental tumors (4, 5) and in human neoplasms (2, 9). This has been partly related to a different cellular uptake of the drugs, operative in determining differences in the overall therapeutic efficacy in vivo, remains to be elucidated.

Materials and Methods

Female Ha ICR mice (CD/1 line, Charles River Breeding Laboratory, Paris, France), 4 to 6 weeks old and weighing 20 to 25 g were used 5 days after the i.p. inoculation of 1 x 10^6 cells/mouse. All drug doses were given i.p. in a constant volume of 10 ml/kg body weight. For studies on drug and precursor incorporation and on proliferative activity, mice with hemorrhagic ascites were discarded. The content of cells other than Sarcoma 180 cells was negligible at the day studied.

Uptake Studies. Chromatographically pure, uniformly tritiated DM (specific activity, 347 μCi/mg) and AM (specific activity 19 μCi/mg), kindly supplied by Farmitalia Research Laboratories, Milan, Italy, were dissolved in pyrogen-free 0.9% NaCl solution and injected i.p. Mice were killed at different times by cervical dislocation; ascitic fluid was withdrawn and centrifuged at 1000 x g for 5 min, and the cells were washed in 0.9% NaCl solution at 4°. Radioactivity was determined in the ascites cells, after combustion in a Packard tritium oxidizer, by means of a Packard Model 3003 liquid scintillation spectrometer. Smears of ascites cells, after fixation in formalin solution at pH 7 (11), were processed for autoradiography.

Precursor Uptake. The nucleic acid precursors used were thymidine-3H (specific activity, 5 Ci/mmmole) and uridine-3H (specific activity, 25 Ci/mmmole) obtained from Radiochemical Centre, Amersham, England. Animals treated i.p. with various doses of the antibiotics received an i.p. injection of 20 μCi of thymidine-3H or 30 μCi uridine-3H 1 hr before they were killed. Smears of ascites cells were fixed in Bouin's solution and processed for autoradiography. Grains were counted in at least 300 to 500 labeled cells per mouse; the thymidine-labeling index was about 60%.

1 Presented in part at the Symposium on Mechanism of Action of Cytostatic Agents, Budapest, Hungary, October 26 to 28, 1972. Research supported in part by Grant 71.00789.04 from the Consiglio Nazionale delle Ricerche, Italy.

2 To whom reprint requests should be addressed: Istituto Nazionale degli Studio e Curati dei Tumori, Via Venezian 1, 20133 Milan, Italy.

3 On leave from Farmitalia Research Laboratory, Milan, Italy.

4 The abbreviations used are: AM, adriamycin; DM, daunomycin.

Received March 23, 1972; accepted August 6, 1973.
Determination of Mitotic and Proliferative Activities.
Mitotic activity was determined in smears of ascitic fluid
obtained at different times after various i.p. doses of the
antibiotics, on at least 2000 cells/mouse. For determination
of the effects on cell proliferation, ascitic fluid was with-
drawn and the abdominal cavity was washed with 0.9% NaCl
solution; cells were counted in a hemocytometer.

Chemotherapeutic Studies. Mice were treated i.p. accord-
ing to different schedules 5 days after the i.p. inoculum of 1
× 10^6 cells/mouse. The weight changes of the animals and
their survival times were recorded.

RESULTS

Uptake of Drug by Tumor Cells. At the earliest time
studied (0.1 hr), after the administration of DM or AM, 1
mg/kg, maximal drug levels were observed; subsequently, a
progressive label loss occurred, which was more rapid for
DM than for AM (Chart 1). The half-life time in the ascites
cells was, in fact, 4 hr for DM and 12 hr for AM. Radio-
autographic observations of the cells treated with
DM-3H showed that, at 0.1 hr after administration, all cells
were labeled and that radioactivity was localized exclu-
sively in the nuclei. The uptake and retention of DM and AM has
also been evaluated 6 hr after 1, 2, 3, and 4 doses of 0.25
mg/kg each at 6-hr intervals and 30 hr after the last
administration. Under these conditions (Chart 2) the levels
of radioactivity obtained for both antibiotics are completely
similar.

Effects on Mitotic Activity. The mitotic index was
markedly reduced by both drugs at 1.5 hr after treatment,
the inhibition being proportional to the dose given and quite
similar for both drugs (Chart 3). At 7 hr, a statistically
significant recovery of mitotic activity was observed only
after the lowest DM dose (0.125 mg/kg).

Effects on Precursor Incorporation into Nucleic Acids. No
impairment of the incorporation of thymidine-3H (Chart 4)
into DNA was observed 1.5 hr after treatment with both
drugs; moreover, a significantly increased incorporation
was observed after DM, 0.125 mg/kg, and AM, 0.5 mg/kg.
At 7 hr, on the contrary, thymidine-3H incorporation was at
control levels only at the lower DM and AM doses tested
while, at higher doses, a marked and statistically significant
reduction was observed that increased with increasing doses.
The extent of inhibition was similar for both drugs. At 1.5
hr after DM administration, the incorporation of uridine-3H
was significantly increased at lower doses (Chart 5), while a
highly significant reduction was observed after 1 mg/kg. At
7 hr, uridine-3H incorporation was in the range of the
controls at all doses tested. Conversely, after AM treat-
ment, the incorporation of uridine was decreased, at both
1.5 and 7 hr, to approximately 50% of control values at
doses of 0.25 mg/kg or higher.

Effects on Cell Proliferation. The effects of DM and
AM, 1 mg/kg, on cell proliferation have been studied; the
drug was given either in a single dose or was fractionated
to 0.5 mg/kg given twice with a 12-hr interval or to 4 doses of 0.25 mg/kg given 6 hr apart. Total cell counts of ascitic fluid show that each schedule of treatment induces a statistically significant effect versus 0.9% NaCl solution-treated controls (Table 1). At 24 hr after treatment, a 40% reduction from initial counts was observed for both drugs at each schedule tested. At 48 hr, ascites cell counts remained at these low levels in all cases except those in which DM was given in 4 doses of 0.25 mg/kg, 6 hr apart. After such treatment, in fact, a reduction from the initial cell counts of 70% was observed, this reduction being statistically highly significant, compared with results obtained with the other treatment schedules.

Chemotherapy Studies. The same treatment schedules used for the studies of effects on cell proliferation were used for studying effects on tumor progression and survival of treated mice. The weights of the treated animals are given in Chart 6; it can be observed that a rapid weight loss occurs after treatment, this loss being particularly evident in the animals treated with 4 doses of DM, 0.25 mg/kg. However, the recurrence of tumor growth in this group is rapid. This effect is paralleled by a shorter life-span versus the other groups (Table 2). AM appears to be able to induce

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount given (mg/kg)</th>
<th>No. of doses given</th>
<th>Total cell counts x 10^-4 at following times after beginning of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td>137 ± 13.4*</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>0.25</td>
<td>2</td>
<td>81 ± 7.9</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>0.25</td>
<td>4</td>
<td>86 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>4</td>
<td>80 ± 11.0</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
DISCUSSION

In experiments on L1210 leukemia cells cultured in vitro (8, 12), the uptake of DM occurs more rapidly and to a greater extent than that of AM, and DM more effectively inhibits precursors incorporation into nucleic acids. On the same tumor system in mice given doses in the range of the 50% lethal dose, DM was as effective as AM in inhibiting precursor incorporation (14). The highest dose used in our study, 1 mg/kg, is well tolerated by mice also when given i.p. for each of 5 successive days.

As already described for other mouse tissues (3), after a single injection, AM was retained by the ascites cells for a longer time than DM.

This longer retention of AM did not result in increased inhibition of mitotic activity and of thymidine incorporation. On the contrary, AM treatment resulted in an earlier inhibition of uridine incorporation into RNA than DM, and also lower doses were effective. Thus, while with DM it is possible to observe doses at which only inhibition of DNA synthesis occurs, all AM doses tested are effective in inhibiting precursor incorporation into both DNA and RNA. The extent of inhibition of precursor incorporation into nucleic acids in our experiments is lower than that described by others (8, 14). This may be accounted for by the fact that we used relatively low doses.

It was of interest to determine whether different schedules of treatment could result in different effects on the proliferative activity of the ascites cells. We used a total dose of 1 mg/kg of both drugs given either as a single injection or fractionated to 2 and 4 treatments. The fractionations of the dose and the interval between the injections were based on the knowledge that DM is rapidly lost from the cells, that DM doses of 0.5 mg/kg or lower have a selective effect on DNA synthesis, and that the duration of the S phase is approximately 13 hr in the system tested (13). With the fractionated dose schedule we could expect to maintain constant levels in the cells for both DM and AM as determined by the uptake of tritiated drugs given according to this schedule. The results indicate that, under these conditions, DM appears to be schedule dependent, as treatment with four 0.25 mg/kg doses given 6 hr apart is the most effective, while AM effects were similar for all schedules. As this DM dose selectively inhibits DNA synthesis, it is conceivable that this marked effect is due to the induction of a state of unbalanced inhibition (7, 10). From the data obtained in mice, however, it would appear that the rate of proliferation of the cells that escape rapid death after DM treatment is not impaired, resulting in a short remission followed by rapid recurrence of tumor growth and little or no effect on the survival of treated mice.

When AM is used, more cells appear to be able to escape death shortly after treatment, but only few cells are able to start to proliferate again, resulting in a slow tumor growth in a limited number of animals, while approximately one-third of the animals are eventually cured. It has been suggested (3–6) that the longer retention of AM versus DM is the major reason for its increased antitumor activity. Our data indicate that this, at least in ascites Sarcoma 180, might not be the only reason, since similar levels of the 2 drugs observed after repeated doses result in different effects. Whether or not the greater effect of AM on RNA synthesis plays a major role in the higher activity of AM remains to be demonstrated. Other mechanisms such as different rates of repair of the damage induced by the binding of the drugs to DNA in relation to the loss of proliferative activity of the ascites cells in vivo might be involved; these factors need further comparative studies.

ACKNOWLEDGMENTS

The authors are indebted to M. Cattedri and T. Comisso for their excellent technical assistance.

REFERENCES


AM and DM in Sarcoma 180 Ascites

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount given (mg/kg)</th>
<th>No. of doses given</th>
<th>Av. survival time (days)</th>
<th>Long-term survivors (no. alive at 60 days/no. tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td>15.5 ± 0.4*</td>
<td>0/10</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>1</td>
<td>1</td>
<td>18.6 ± 0.5</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>4</td>
<td>19.5 ± 1.3</td>
<td>0/10</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>1</td>
<td>1</td>
<td>26.6 ± 4.3</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>4</td>
<td>31.1 ± 4.9</td>
<td>3/10</td>
</tr>
</tbody>
</table>

* Mean ± S.E.


Correlations between Cytotoxicity, Biochemical Effects, Drug Levels, and Therapeutic Effectiveness of Daunomycin and Adriamycin on Sarcoma 180 Ascites in Mice

Rosella Silvestrini, Luigi Lenaz, Giovanni Di Fronzo, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/33/11/2954

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.