Endogenous and Exogenous Polyamines in Support of Tumor Growth

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ABSTRACT

The combination of inhibitors of ornithine decarboxylase and polyamine oxidase and of antibiotics suitable for the (partial) decontamination of the gastrointestinal tract with a polyamine-deficient diet reduced the growth rate of Lewis lung carcinoma by more than 80%. The formation of lung metastases was prevented by 70 to 100%, depending on the treatment. The reduction of tumor growth was accompanied by a decrease of tissue polyamine concentrations, a reduced rate of tumor cell proliferation, and protein synthesis. The comparison of the ornithine decarboxylase inhibitors Eflornithine [D,L-2-(difluoromethyl)ornithine] and (E)-2-(fluoromethyl)dehydroornithine ethylester confirmed the greater in vivo potency of the latter compound. Our method of growth inhibition by systematic polyamine deprivation is not tumor specific, but presumably generally applicable to rapid growth.

INTRODUCTION

Enzymes involved in the biosynthesis of the polyamines (i.e., of putrescine, spermidine, and spermine) are considered as targets for chemotherapy (1). An inactivator of ODC2 (EC 4.1.1.17), DFMO (Efornithine; MDL 71782) (2) gained prominence among all inhibitors of polyamine biosynthetic enzymes. Exposure of a great variety of tumor cell lines in culture to DFMO under conditions that cause the depletion of putrescine and spermidine concentrations causes an arrest of cell growth. Likewise, inhibition of tumor growth in some animal models by treatment with DFMO and with combinations of DFMO and other drugs has repeatedly been described (3, 4). In spite of these favorable premises, clinical chemotherapeutic attempts with DFMO in cancer were not satisfactory (5).

In addition to incomplete inactivation of ornithine decarboxylase the availability of putrescine from other endogenous sources, and also from exogenous sources, is a potential reason for the unsatisfactory therapeutic effects of DFMO. In an intact vertebrate organism putrescine can be mobilized by the interconversion reaction sequence in one tissue, and utilized in another, with the circulation functioning as transport system (6). The gastrointestinal tract is to be considered as the most important exogenous polyamine source. In agreement with these ideas, prevention of putrescine formation from N1-acetyl spermidine by specific inactivation of polyamine oxidase with MDL 72527 (7), administration of polyamine-free diet (8), and decontamination of the gastrointestinal tract (9) improved somewhat the cytostatic effects of DFMO in animal models of leukemia. However, only the systematic blockade of all three major sources of putrescine had a considerable effect on the survival time of L1210 leukemia-bearing mice and a profound effect on the growth of LLC (10).

We have continued our efforts to improve polyamine deprivation. In this paper we describe the effects of polyamine starvation on polyamine metabolism, tumor growth, and for-
0.9% NaCl solution. After counting the viable cells, tumors were
induced by intramuscular injection of 1.5 × 10^6 cells (in 0.1 ml) into
the hind legs of the mice. Palpable tumors appeared after 3 days. Tumor
growth was followed by determining the size using calipers (20). The
number of metastases was determined by counting under a magnifier
after fixation of the lungs in a 4% solution of formalin in physiological
saline.

**Drug Regimen.** Starting on Day 3 or Day 4 after tumor cell inocula-
cation, when all mice had developed well-measurable tumors, the ODC
inhibitor containing PDC (and tap water) was given ad libitum. Control
animals with and without LLC received standard rodent diet.

**Histology.** Tumors were frozen in liquid nitrogen and stored at
-80°C. Freeze-sections (10 µm) were prepared at -20°C, dried, and
fixed for 30 min in glutaraldehyde (4%) in phosphate-buffered saline,
pH 7.4, and stained by cresylviolet acetate (21).

Small intestines were fixed in formaldehyde (4% in phosphate-
buffered saline) for several days. Then they were embedded in Paraplast
(Labo Moderne, Paris, France), and 10 µm sections were prepared.
After removal of the Paraplast by toluene, dehydroxylation with alcohol-
formaldehyde, and dehydration with water, the sections were stained with
Harris’ hematoxylin and erythrosin.

For histological examination of bone marrow, the femurs were
decalcified for 4 h with a solution of 1 volume of concentrated nitric
acid in 9 volumes of 10% formaldehyde. After rapid dehydration with
ethanol and n-butanol, the tissue samples were embedded in Histemed
Special 1 (Labo Moderne, Paris, France). After preparation of 10-µm
sections and deparaffinization, the sections were treated with colloidin
(0.1% in ethanol-diethyl ether), dehydrated in the usual manner, and
stained according to May-Grunwald.

All histological preparations were evaluated independently by two
investigators.

**Blood Analyses.** Blood samples (0.4 to 0.5 ml) of the decapitated
mice were collected in Complexon K3-containing plastic tubes. The
 cellular composition of the samples was determined by H. Dorne
(Laboratoire d’analyses médicales, Strasbourg, France).

**Autoradiographic Determination of the Labeling Index.** Tumor-bear-
ing mice received s.c. 20 µCi of [methyl-3H]thymidine (specific radio-
activity, 6.7 Ci mmol^{-1}) dissolved in 0.2 ml of physiological saline.
One h after thymidine administration, the tumors were isolated and
frozen in liquid nitrogen. Freeze sections (10 µm) were prepared and
fixed for 30 min in glutaraldehyde [4% in 50 mM phosphate buffer (pH
7.4)]. After washing in phosphate buffer and water, the sections were
dehydrated in the usual series of ethanol and xylene and subsequently
rehydrated in the reversed alcohol series and water. Before the slides
were covered with the emulsion, they were immersed in 0.3 M sodium
acetate buffer (pH 7.4). The photoemulsion (NTB3; Kodak) was heated
at 45°C and then diluted 1:1 with 0.6 M sodium acetate buffer (pH 7.4).
After dipping, the slides were air-dried overnight and then stored at
+4°C (in the presence of silica gel). After 4 wk the slides were developed
(D19; Kodak), fixed, and stained with toluidine blue (at 40–50°C). After
washing in water, ethanol, and xylene the slides were mounted as usual.
(For details of the method, see Refs. 22 and 23). The labeling index
was obtained by counting of 1000 tumor cells.

**Determination of Protein Synthesis Rate.** In essence the method of
Dunlop et al. (24) was used. The animals received s.c. 57.6 mg of L-
sine, containing 2 µCi of [3H]lysine (specific radioactivity, 6.7 Ci
mmol^{-1}) dissolved in 0.2 ml of water. After 1 h the tumors were isolated and homogenized in 5 volumes of
3% sulfoxalic acid. After a multistep washing procedure (see Ref. 24),
the protein pellet was dried. Five-mg portions of the protein were
dissolved at 37°C in 1 ml of 1 M hydroxide of hyamine in methanol
(Packard Instruments, Zurich, Switzerland) and, after addition of 10
ml of a scintillator cocktail (Aquasol; NEN Research, Boston, MA),
radioactivity was counted. The specific radioactivity of [14C]lysine was
determined in the sulfoxalic acid extract by counting the radioactivity
in aliquots and by determining the concentration of L-lysine, using
a published high-performance liquid chromatography method (25).

**Calculations.** Statistically significant differences between results fol-
lowing normal distribution were established by the Student t test. For
the establishment of significant differences of medians, the Kruskal-
Wallis test (26) was used.
RESULTS

Drug Intake. In contrast to our previous work, the PDC contained all drugs and was, therefore, the only source for drugs during the entire treatment period. Polyamine content and antibiotics were the same as previously reported (10). Food and water intake was monitored when tumors were established, during the first 3 days of treatment with drug-containing PDC, and at a late phase of tumor growth. The results are summarized in Table 3. It appears from this table that food (and drug) intake decreased with increasing tumor size by 43% (DFMO-PDC), 33% (MDL 72430-PDC), and 31% (controls). The decrease of food intake was roughly paralleled by a concomitant decrease of water intake.

Growth Rate of the Primary Tumor and Formation of Lung Metastases. Drug-containing PDC was usually given starting Day 4 after tumor cell inoculation. Both healthy mice and tumor-bearing mice showed a loss of body weight upon treatment with polyamine-deficient chow containing an ODC inhibitor, the polyamine oxidase inhibitor, and neomycin. However, the greatest loss was observed in untreated tumor-bearing mice. Treatment with DFMO-PDC and MDL 72430-PDC seemed to ameliorate the body weight loss of the tumor-bearing mice (Table 4). In contrast with the untreated controls, in both treatment groups tumor growth was very considerably retarded (Fig. 1). At Day 18 the weight of the tumors in the treated animals was less than 20% of that of controls (Table 4). There was no significant difference between the tumor size of animals treated with DFMO-PDC and MDL 72430-PDC.

If treatment with DFMO-PDC started only at Day 8, i.e., at a later stage of tumor progression, when the average tumor weight was 0.9 ± 0.3 g, tumors continued growing, although at a significantly slower rate than the tumors in the control animals (Fig. 2).

In agreement with previous results (10, 11, 20), metastases were found in the lungs of all untreated tumor-bearing mice (Table 5). In the DFMO-PDC treatment group, only in two of seven animals were single metastases observed, whereas all animals of the MDL 72430-PDC group were free of lung metastases.

Histological Observations. Tissues for histological examinations were taken on Day 7 after LLC cell inoculations, 3 days after treatment with DFMO-PDC. Cresylviolet-stained freeze-sections of the tumors of control animals showed the usual appearance; large cells were generally closely associated in bundles or sheets. Cell boundaries were difficult to resolve by light microscopy. The cytoplasm was homogenous. Nuclei (one or two) had varying shapes. The chromatin formed a pattern uniformly dispersed in the nucleoplasm. Two or more nucleoli could be observed per cell. The density of inflammatory cells was low. In contrast, tumors from DFMO-PDC-treated mice contained a considerable number of inflammatory cells, especially neutrophils and lymphocytes. None of the tumors had necrotic fields. Harris hematoxylin-stained sections of the small intestine from controls and from mice treated with DFMO-PDC or with MDL 72430-PDC exhibited a normal histological aspect. In treated animals, intestinal mucosa showed normal plicae circulares supporting an unusual number of intestinal villi. Absorptive cells, goblet cells, and basal granular cells were present without noticeable alteration in their number and in their cytological appearance. No histological abnormalities were noticed in the crypts of Lieberkühn.

Purely based on morphological criteria for the identification of bone marrow cell types, the examination of bone marrow from controls and DFMO-PDC- and MDL 72430-PDC-containing mice was free of lung metastases.

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Table 3 Food, water, and drug intake by LLC-bearing C57BL mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after tumor cell inoculation</th>
<th>Av. tumor cross-section (mm²)</th>
<th>Av. food intake (g/animal)</th>
<th>Drug intake (g·kg⁻¹/24 h)</th>
<th>Water intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DFMO</td>
<td>MDL 72430</td>
</tr>
<tr>
<td>None (SC)*</td>
<td>4-6</td>
<td>28</td>
<td>2.9</td>
<td>3.5</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>18-21</td>
<td>456</td>
<td>2.0</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>DFMO-PDC</td>
<td>4-6</td>
<td>15</td>
<td>3.0</td>
<td>4.9</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>18-21</td>
<td>100</td>
<td>1.6</td>
<td>2.8</td>
<td>0.047</td>
</tr>
<tr>
<td>MDL 72430-PDC</td>
<td>4-6</td>
<td>15</td>
<td>2.7</td>
<td>3.0</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>18-21</td>
<td>100</td>
<td>1.9</td>
<td>2.3</td>
<td>0.057</td>
</tr>
</tbody>
</table>

* SC, standard rodent chow; DFMO-PDC, polyamine-deficient chow containing 3% DFMO, 0.05% MDL 72527, and 0.2% neomycin; MDL 72430-PDC, polyamine-deficient chow containing 2% MDL 72430, 0.05% MDL 72527, and 0.2% neomycin. For details of the composition of the artificial diet, see "Materials and Methods."

Table 4 Effect of treatment with D.L-2-(difluoromethyl)ornithine (MDL 71782; DFMO) or D,L-(E)-2-(fluoromethyl)dehydroornithine ethylester (MDL 72430) and N,N'-bis(2,3-butadienyl)putrescine-2HCl (MDL 72527) in combination with PDC, showing body and tumor weights of healthy and Lewis lung carcinoma-bearing C57Bl mice

The initial body weight was before LLC cell inoculation. The final body and tumor weights were taken on Day 18 after LLC cell inoculation. The data in this table and in Table 5 were obtained from the same animals. For details of the treatment schedule, see "Materials and Methods."

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial body wt (g)</th>
<th>Final body wt (Day 18, g)</th>
<th>Final body wt minus tumor wt (g)</th>
<th>Body wt difference (g)</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>17.6 ± 0.5*</td>
<td>18.4 ± 0.8</td>
<td>+0.8 ± 1.3</td>
<td>-1.0 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>DFMO-PDC</td>
<td>16.9 ± 0.9</td>
<td>15.9 ± 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLC tumor-bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>19.0 ± 0.8</td>
<td>20.7 ± 0.8</td>
<td>+1.7 ± 2.2</td>
<td>+1.8 ± 0.4</td>
<td>5.6 ± 0.8</td>
</tr>
<tr>
<td>DFMO-PDC</td>
<td>18.3 ± 0.8</td>
<td>17.0 ± 0.8</td>
<td>+1.3 ± 2.4</td>
<td>+1.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>MDL 72340-PDC</td>
<td>18.3 ± 1.0</td>
<td>16.6 ± 0.5</td>
<td>+1.7 ± 2.2</td>
<td>+1.8 ± 0.4</td>
<td>5.6 ± 0.8</td>
</tr>
</tbody>
</table>

* Mean ± SD (n = 7)

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treated mice did not reveal any major differences in the abundance of hemopoietic marrow cells and in the cytological aspects of cell lineages.

Cellular Composition of the Blood. Blood analyses were carried out on Day 18 after tumor cell inoculation. The results are summarized in Table 6. The untreated tumor-bearing mice had a dramatically reduced number of red blood cells, but an approximately normal hemoglobin content per cell. Leukocyte and platelet numbers were within the normal range. Polyamine deprivation caused no further loss of red blood cells, but a decrease of total leukocytes and a drop in blood platelets was observed.

The most conspicuous change in LLC-bearing mice was the dramatic increase of the proportion of neutrophils and a corresponding decrease in lymphocytes. Treatment with either DFMO-PDC or MDL 72430-PDC did not restore a normal leukocyte pattern, but there was a tendency toward normalization.

Ornithine Decarboxylase, S-Adenosylmethionine Decarboxylase, and Polyamines. Owing to the ill-defined time between drug intake and tissue isolation, the determination of the activity of enzymes with such high turnover rates as those of ODC and AdoMet-DC (1) can only give a crude impression of the average changes of enzyme activities. ODC activity was determined immediately after the tissues had been isolated in the morning of Day 13 after tumor cell inoculation. Treatment with MDL 72430-PDC had a clearly greater effect on ODC activity in tumor and kidney than treatment with DFMO-PDC (Table 7).

AdoMet-DC activity was determined in tumors, part of which was used for polyamine determinations. Treatment with both, DFMO-PDC and MDL 72430-PDC, caused a considerable enhancement of AdoMet-DC activity (Table 7).

Polyamine determinations were carried out with a faster version of the previously published method (16). Fig. 3 shows representative chromatograms of a polyamine standard mixture and of a perchloric acid extract of an LLC. By using a shorter column (3-μm spherical particles with C18-residues) and by increasing the flow rate, it was possible to obtain polyamine separations of the same quality as those reported previously (27, 28), however, within half the time. Due to the narrow peaks the present version is somewhat more sensitive and has a reproducibility comparable to that of the reported method.

In Table 8 the polyamine data of tumors and tissues are summarized. The tissues were isolated on Day 14 after LLC cell inoculation. Putrescine and spermidine concentrations were significantly decreased in all tissues, and spermine concentrations were enhanced in those tissues that exhibit the most important depletion of spermidine, in agreement with previous experience (10).

In comparison with results obtained from animals that had received DFMO (as a 2% solution) and the polyamine oxidase inhibitor MDL 72527 with the drinking water (10), the depletion of putrescine and spermidine was more complete in all tissues in the present experiment, in agreement with the higher dose of DFMO and the greater potency of MDL 72430 (11), respectively. There was no statistically significant difference between the polyamine data of DFMO-PDC- and MDL 72430-PDC-treated mice, but the latter showed in nearly all tissues slightly higher spermidine concentrations.

Labeling Index of Tumor Cells. Three days after LLC cell inoculation, treatment with DFMO-PDC started. On Day 6 after tumor cell inoculation (3 days of treatment), 20 μCi of [methyl-3H]thymidine were administered s.c.; tumors were iso-
Table 6 Effect of treatment with D,L-2-(difluoromethyl)ornithine (MDL 71782; DFMO) or D,L-(E)-2-(fluoromethyl)dehydroornithine ethylester (MDL 72430) and N,N'-bis(2,3-butadienyl)putrescine 2HCl (MDL 72527) in combination with PDC, showing cellular composition of the blood 18 days after LLC cell inoculation.

The blood samples were taken from the animals for which results are shown in Tables 4 and 5.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Untreated normal controls</th>
<th>Untreated tumor bearing</th>
<th>DFMO-PDCa</th>
<th>MDL 72430-PDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g (100 ml)-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pg per cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.8 ± 0.5</td>
<td>18.0 ± 1.2</td>
<td>13.3 ± 1.2</td>
<td>18.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g (10³ mm-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 ± 2</td>
<td>41 ± 11f</td>
<td>31 ± 6f</td>
<td>26 ± 2f</td>
<td></td>
</tr>
<tr>
<td>Basophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>86 ± 3</td>
<td>52 ± 6f</td>
<td>64 ± 4f</td>
<td>70 ± 4f</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 ± 3</td>
<td>8 ± 5</td>
<td>5 ± 2</td>
<td>4 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

* DFMO-PDC, polyamine-deficient chow containing 3% DFMO, 0.05% MDL 72527, and 0.2% neomycin; MDL 72430-PDC, polyamine-deficient chow containing 2% MDL 72430, 0.05% MDL 72527, and 0.2% neomycin.

a Significant (P < 0.01) difference between normal tumor-free controls and LLC-bearing animals.

For details of the treatment schedule, see "Materials and Methods." Tumors were analyzed on Day 13 after LLC cell inoculation.

Table 7 Effect of treatment with D,L-2-(difluoromethyl)ornithine (MDL 71782; DFMO) or D,L-(E)-2-(fluoromethyl)dehydroornithine ethylester (MDL 72430) and N,N'-bis(2,3-butadienyl)putrescine 2HCl (MDL 72527) in combination with PDC, showing ODC and AdoMet-DC activities in the tumor of LLC-bearing C57BL mice.

For details of the treatment schedule, see "Materials and Methods." Tumors were analyzed on Day 13 after LLC cell inoculation.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>ODC (nmol-g⁻¹-h⁻¹)</th>
<th>AdoMet-DC (nmol-g⁻¹-h⁻¹)</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>None</td>
<td>31 ± 6f</td>
<td>45 ± 1</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>DFMO-PDCa</td>
<td>12 ± 4f</td>
<td>390 ± 105f</td>
<td>0.6 ± 0.2f</td>
</tr>
<tr>
<td></td>
<td>MDL 72430-PDC</td>
<td>2 ± 1f</td>
<td>254 ± 90f</td>
<td>0.48 ± 0.03f</td>
</tr>
</tbody>
</table>

* Mean ± SD (n = 3).

a DFMO-PDC, polyamine-deficient chow containing 3% DFMO, 0.05% MDL 72527, and 0.2% neomycin; MDL 72430-PDC, polyamine-deficient chow containing 2% MDL 72430, 0.05% MDL 72527, and 0.2% neomycin.

DISCUSSION

In order to demonstrate a role in tumor growth of putrescine that is not deriving from the intracellular decarboxylation of ornithine, it was essential to choose an animal tumor model which is not sensitive to treatment with ODC inhibitors. It has been shown that the growth of LLC can be affected by DFMO (7, 11, 20) if treatment starts immediately after tumor cell inoculation. However, once a palpable tumor has been established, administration of DFMO (10) but also of more potent ODC inhibitors, including MDL 72430, has little effect on LLC progression (11).

Our present results are in full support of previous findings...
The observed induction of AdoMet-DC and the changes of polyamine concentrations, especially also the increase of spermine in some tissues, can be understood as a consequence of the role of the polyamines in the regulation of their cellular content (29). It was confirmed in this work that it is necessary to prevent, in addition to their endogenous formation, the utilization of polyamines from the gastrointestinal tract. The lectin-induced growth of small intestines (30, 31) and the obstruction-induced colonic mucosal hyper trophy (32) are further recent examples for a role of gastrointestinal polyamines in growth processes.

The combination of all drugs, antibiotics, ODC inhibitor, and polyamine oxidase inhibitor in the polyamine-free diet is not only convenient, but it allowed the administration of the ODC inhibitors in higher amounts than were consumed when administered with the drinking fluid. The increased drug intake is, among others, evident from the tissue putrescine and spermidine concentrations which were throughout lower than in our previous experiments (10). We believe, therefore, that the inclusion of the drugs into the diet has considerable advantages over their administration with the drinking water.

**Table 8. Effect of treatment with d,L-2-(difluoromethyl)ornithine (MDL 71782; DFMO), or d,L-(E)-2-(fluoromethyl)dehydroornithine ethylester (MDL 72430) and N,N'-bis(2,3-butadienyl)putrescine -2HCl (MDL 72527) in combination with PDC, showing polyamines in tumor and tissues of LLC-bearing C57BL mice**

Treatment with drug-containing PDC started 4 days after i.m. (hind leg) inoculation of 1.5 x 10⁶ LLC cells and was continued for 10 days. On Day 14 after tumor cell inoculation the organs were isolated, and the polyamines were determined in 0.2 M perchloric acid extracts.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Putrescine</th>
<th>N⁴-Acetyl spermidine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>N⁴-Acetyl spermine</th>
<th>Tumor or organ wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N⁴-Acetyl spermidine</td>
<td>341 ± 121</td>
<td>249 ± 55</td>
<td>315 ± 49</td>
<td>174 ± 59</td>
<td>204 ± 42</td>
<td>190 ± 15</td>
</tr>
<tr>
<td>Spermidine</td>
<td>610 ± 54</td>
<td>429 ± 38</td>
<td>334 ± 25</td>
<td>170 ± 12</td>
<td>203 ± 42</td>
<td>180 ± 15</td>
</tr>
<tr>
<td>Spermine</td>
<td>910 ± 78</td>
<td>653 ± 52</td>
<td>334 ± 25</td>
<td>170 ± 12</td>
<td>203 ± 42</td>
<td>180 ± 15</td>
</tr>
</tbody>
</table>

* a Mean ± SD (n = 3).
* b.d.l., < 2 nmol/g. DFMO-PDC, polyamine-deficient chow containing 3% DFMO, 0.05% MDL 72527, and 0.2% neomycin; MDL 72430-PDC, polyamine-deficient chow containing 2% MDL 72430, 0.05% MDL 72527, 0.2% neomycin.
* c Statistically significant (P < 0.01) difference between the total tissue polyamine content of untreated and treated mice.
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accessible and allowed the tumor cells to proceed into the S phase. This explanation is supported by histological observations, but needs to be confirmed by future experiments.

Since all tissues had decreased spermidine concentrations, and since decreased polyamine concentrations are a reason for enhanced polyamine uptake (35), competition between tumor and tissues for circulating polyamines will certainly occur. We believe that the successful competition of normal tissues with the tumor for the available polyamines is a major reason for the considerably reduced tumor growth rate which occurs in spite of a rather incomplete inhibition of the endogenous formation of putrescine, and in spite of the incomplete blockade of polyamine import from exogenous sources. The intestines with their ability to resorb polyamines from the lumen (30, 32) are probably the most efficient competitors for the available putrescine and spermidine, as appears from the apparent lack of pathological alterations of the small intestines, even after 3 wk of severe polyamine deprivation.

Severe losses especially of blood platelets, but also of leukocytes, were observed. This finding is in agreement with a previous observation in rats which were treated with DFMO (36). In humans, thrombocytopenia and also leukopenia are frequent adverse (though transitory) effects of treatment with DFMO (5).

In contrast with normal controls, LLC-bearing mice had considerably higher proportions of neutrophils and correspondingly lower proportions of lymphocytes. Treatment with DFMO-PDC or MDL 72430-PDC did not restore normal composition of leukocytes, but produced a shift toward normal proportions (Table 6). Both with regard to its (adverse) effects on the number of blood cell elements, and with regard to the shifting of neutrophil and lymphocyte numbers toward normality, feeding of MDL 72430-PDC was somewhat more effective than of DFMO-PDC.

Polyamines are a general requirement for growth (1, 29, 37, 38). We assume, therefore, that our method of growth inhibition by systematic polyamine deprivation is in principle generally applicable, provided the utilization of exogenous polyamines and their intracellular formation and distribution within the organism can be sufficiently restricted.

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Endogenous and Exogenous Polyamines in Support of Tumor Growth

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