Transplantable Human Tumors in Experimental Chemotherapy
The Effects of Cytoxan on H.S. #1 and H.Ep. #3 in the Rat*

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SUMMARY
Cytoxan, a cyclic phosphamide ester of nitrogen mustard, was tested for tumor-inhibiting activity in the transplantable human tumor-rat host experimental chemotherapy systems with the use of the sarcoma H.S. #1 and the epidermoid carcinoma H.Ep. #3. The compound inhibited the growth of both tumors when the initial dose was given 24 hours after tumor transplantation, by the intraperitoneal or oral route of administration. Cytoxan was found to have limited ability to suppress growth of established human tumors.

Cytoxan, a cyclic phosphamide ester of nitrogen mustard, was synthesized by Arnold and Bourseaux (1) as a potentially enzyme-activated antitumor agent. Presumably, such compounds could provide a rational approach to cancer chemotherapy (1, 2, 8).

Arnold et al. (3) reported that cytoxan, at levels up to $10^{-4}$ mg/ml, was relatively inactive in vitro when incubated with tumor tissue at 37°C. There was no effect on tumor "takes" when the tissue was transplanted to animals. Similar results were obtained by Burchenal et al. (5) for leukemic cell lines. Wight et al. (12) found little or no inhibition by cytoxan of anaerobic glycolysis by Ehrlich ascites cells in vitro, but addition of tumor homogenates preincubated with cytoxan did result in inhibition of tumor glycolysis. Evidence of in vitro activation of cytoxan by mouse liver homogenates was reported by Foley et al. (7), but other normal tissues, tumor tissue, or blood from nontreated animals had little or no effect on cytoxan.

Cytoxan has been reported to be an effective tumor-growth inhibitor in experimental animals. Arnold et al. (3) reported cures of fully developed solid Yoshida, Walker 256, and Jensen sarcoma in rats treated with cytoxan. Druckrey et al. (6) found that cytoxan, but not Bayer E39 or mitomycin, would inhibit the growth of the DS carcino-sarcoma of the rat. Surgery plus combination chemotherapy with cytoxan and E39 gave 90 per cent cures, whereas surgical removal of subcutaneous tumor growths alone would result in recurrences or metastases in more than 70 per cent of the rats. Cytoxan was found by Venditti et al. (11) to be effective against sublines of leukemia L1210 resistant to antifolies or antipurines and was at least as effective as amethopterin in increasing the survival time of mice with advanced leukemia L1210.

The lack of activity of methyl bis(β-chloroethyl)amine in the human tumor-rat host experimental chemotherapy systems led to the testing of cytoxan in these systems. Results indicate important differences between cytoxan and nitrogen mustard.

MATERIALS AND METHODS
The two transplantable human tumors used were the sarcoma H.S. #1 and the carcinoma...
H.Ep. #3, growing subcutaneously in the conditioned rat. General conditioning, transplantation, and chemotherapy procedures have been described previously (9). For these experiments, rats x-radiated with a total-body dose of 150 r and given implants of the same tissue were divided randomly into groups of six each, one group being used as controls. Immediately after transplantation of the tissue, the rats were given a subcutaneous injection of cortisone acetate, near the nape of the neck, at a dose of 60 mg/kg of body weight. Rats bearing H.S. #1 tumors were given injections again on alternate days for a total of four doses; those bearing H.Ep. #3 tumors were given cortisone injections at 3-to 4-day intervals for a total of three doses. Therapy with cytoxan was started 24 hours after tumor transplantation and continued as single, daily, intraperitoneal (I.P.) injections (Sundays excepted). Oral treatment (p.o.) was by intubation once daily. H.S. #1-bearing rats received nine injections of the compound under this schedule and a total of seven injections when the initial dose was begun 4 days post-transplantation (post-4). Rats bearing H.S. #1 were sacrificed on the 9th or 11th day, 34-48 hours after the last dose had been given. H.Ep. #3-bearing rats received seven to eight injections of the compound when the initial dose was given 24 hours post-transplantation, and five to six injections for post-4 day tests on established tumors. Rats bearing H.Ep. #3 were sacrificed on the 9th or 11th day post-transplantation, also 24-48 hours after the last injection had been given. Tumors were excised, blotted, and weighed after removal of debris. Two or more tests at the same dose were pooled to give an average result. For H.S. #1, tumor inhibitions of 0-39 per cent were graded — (negative); 40-74 per cent, ±; and 75 per cent or greater, +. For H.Ep. #3, inhibitions of 0-24 per cent were —; 24-59 per cent, ±; and 60 per cent or greater, +. These graded effects are based upon the degrees of variability and reproducibility of results of these human tumor-rat host systems (10).

Cytoxan was prepared in physiological saline fresh daily in concentrations such that the dose was administered in 0.5-0.6 ml. Control rats received equal amounts of the diluent.

RESULTS

The effects of cytoxan on the subcutaneous growth of H.S. #1 and H.Ep. #3 are presented in Tables 1 and 2, respectively. At a dose of 62.5 mg/kg, cytoxan was lethal to rats bearing H.S. #1, with all treated rats dying between the 8th and 11th day. An average weight loss of 10 gm. and a mortality of 2/6 occurred at 32 mg/kg. At 16 and 8 mg/kg, H.S. #1 was inhibited 62 per cent and 53 per cent, respectively. These were ± effects in the grading system employed. The inhibition at 4 mg/kg averaged 20 per cent (—) for three tests at this dose, a ± inhibition in the first test not being repeated in two additional tests. In one test at this dose the three deaths in the treated group were considered accidental but were included for the record. An average inhibition of 70 per cent (±) occurred against established tumors (post-4 day injection) at 32 mg/kg, and a negative result was obtained at one-half this dose, 16 mg/kg. When cytoxan was given orally, H.S. #1 was inhibited 78 per cent (+) at 32 mg/kg, and inhibitions of 51 per cent and 40 per cent occurred at 16 and 8 mg/kg, respectively, these last being ± effects.

Rats given transplants of H.Ep. #3 and treated intraperitoneally with cytoxan at 32 mg/kg lost an average of 5 gm., and tumor growth was inhibited 68 per cent (+ effect). H.Ep. #3 was inhibited 55 per cent at 16 mg/kg and 50 per cent at 8 mg/kg (± effect). Established tumors were inhibited 42 per cent (±) at a dose of 32 mg/kg accompanied by host toxicity, and a negative effect occurred at 16 mg/kg. Orally administered cytoxan inhibited H.Ep. #3 an average of 77 per cent (+) at 32 mg/kg, and inhibitions of 64 (+), 54 (±), and 50 per cent (±) were obtained at 16, 8, and 4 mg/kg, respectively. No inhibition occurred at 2 mg/kg.

DISCUSSION

Cytoxan was found to inhibit the growth of two human tumors, transplanted into the rat, when given either I.P. or p.o. The therapeutic index of this compound was reported to be significantly higher than that of other cytostatic agents in a series tested by Brock and Wilmanns (4). Since toxicological studies were not carried out in rats bearing H.S. #1 and H.Ep. #3, therapeutic indices could not be calculated. On the other hand, the ratio of maximum tolerated dose to minimum effective dose (MTD/MED) for cytoxan against the epidermoid carcinoma H.Ep. #3 was unusually high for transplantable human tumors. This ratio was 4 for the I.P. route of administration and 8 when cytoxan was administered p.o. In comparison, the MTD/MED ratio for the sarcoma H.S. #1 was 2 for the intraperitoneal route and 4 for the oral route of administration. Tested post-4 days against established tumors, cytoxan inhibited H.S. #1 an average of 70 per cent, and H.Ep. #3 an average of 42 per cent with a large host weight loss, at 32 mg/kg. At one-half this dose, 16 mg/kg, tumor inhibition dropped into
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the negative range. This indicated limited activity against established growths, at least under these experimental conditions.

The tumor-inhibiting effects of this cyclic phosphamide of nitrogen mustard were not seen in tests with nitrogen mustard (methyl bis[b]-chloroethyl]amine) itself in the human tumor-rat host systems. HN2 was reported to be inactive against both H.S. #1 and H.Ep. #3 in the rat at a maximum tolerated dose of 0.13 mg/kg (9). Although the human tumors may be resistant to HN2 under these conditions, it is possible that an effective concentration of the compound cannot be made available to the tumor because of extreme host toxicity at higher dosages. On the other hand, the larger doses permissible with cytoxan may make available a larger amount of the active principle to inhibit tumor growth.

**TABLE 1**

**EFFECT OF CYTOXAN ON H.S. #1 GROWING SUBCUTANEOUSLY IN THE CONDITIONED RAT**

<table>
<thead>
<tr>
<th>Route*</th>
<th>Dose (mg/kg)</th>
<th>Time of first dose</th>
<th>Toxicity</th>
<th>Av. Wt. Δ (gm.)</th>
<th>Mortality</th>
<th>Av. tumor wt. (gm.)</th>
<th>Per cent inhibition</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T†</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I.P.</td>
<td>62.5</td>
<td>24 hr.</td>
<td>-10</td>
<td>+13</td>
<td>6/6</td>
<td>0/6</td>
<td>68</td>
<td>Lethal</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td></td>
<td>+3</td>
<td>+16</td>
<td>2/6</td>
<td>0/6</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td></td>
<td>+9</td>
<td>+20</td>
<td>1/12</td>
<td>0/12</td>
<td>55</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>+4</td>
<td>+11</td>
<td>3/18</td>
<td>0/18</td>
<td>20</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Post-4 day</td>
<td>+3</td>
<td>+21</td>
<td>0/12</td>
<td>0/12</td>
<td>70</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td></td>
<td>+8</td>
<td>+23</td>
<td>0/6</td>
<td>0/6</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>p.o.</td>
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<td>24 hr.</td>
<td>+3</td>
<td>+34</td>
<td>0/6</td>
<td>0/6</td>
<td>78</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td></td>
<td>+8</td>
<td>+15</td>
<td>0/13</td>
<td>0/12</td>
<td>51</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>+5</td>
<td>+11</td>
<td>0/6</td>
<td>0/6</td>
<td>42</td>
<td>±</td>
</tr>
</tbody>
</table>

* I.P. = interperitoneal; p.o. = per os.
† T = treated; C = control.
‡ Toxic dose: average host weight change ± 6 gm. and/or mortality of 2/6, relative to controls, or rats in debilitated condition.
§ ± in one test, not repeated in two other tests.

**TABLE 2**

**EFFECT OF CYTOXAN ON H.EP. #3 GROWING SUBCUTANEOUSLY IN THE CONDITIONED RAT**

<table>
<thead>
<tr>
<th>Route*</th>
<th>Dose (mg/kg)</th>
<th>Time of first dose</th>
<th>Toxicity</th>
<th>Av. Wt. Δ (gm.)</th>
<th>Mortality</th>
<th>Av. tumor wt. (gm.)</th>
<th>Per cent inhibition</th>
<th>Effect</th>
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<td>C</td>
<td>T</td>
<td>C</td>
<td></td>
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</tr>
<tr>
<td>I.P.</td>
<td>32</td>
<td>24 hr.</td>
<td>-5</td>
<td>0</td>
<td>0/12</td>
<td>0/12</td>
<td>68</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td></td>
<td>+2</td>
<td>0</td>
<td>0/12</td>
<td>0/12</td>
<td>55</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>+10</td>
<td>+12</td>
<td>0/6</td>
<td>0/6</td>
<td>50</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>+6</td>
<td>+12</td>
<td>0/6</td>
<td>0/6</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Post-4 day</td>
<td>-8</td>
<td>+22</td>
<td>0/6</td>
<td>0/6</td>
<td>± T†</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td></td>
<td>+14</td>
<td>+23</td>
<td>0/6</td>
<td>0/6</td>
<td>18</td>
<td>±</td>
</tr>
<tr>
<td>p.o.</td>
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<td>24 hr.</td>
<td>+1</td>
<td>+16</td>
<td>0/12</td>
<td>0/12</td>
<td>77</td>
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<td>+22</td>
<td>0/6</td>
<td>0/6</td>
<td>64</td>
<td>±</td>
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<td></td>
<td>8</td>
<td></td>
<td>+10</td>
<td>+12</td>
<td>0/6</td>
<td>0/6</td>
<td>54</td>
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<td>50</td>
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<td>+13</td>
<td>+20</td>
<td>0/6</td>
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<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* For explanation of notations, refer to Table 1.
† See footnote †, Table 1.
‡ See footnote ‡, Table 1.
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REFERENCES

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