Pharmacologic Studies of the Antitumor Agent
5-(Dimethyltriazeno)imidazole-4-carboxamide\(^1,2\)

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SUMMARY

In the dog, after an intravenous injection of 5-(dimethyltriazeno)imidazole-4-carboxamide (DIC) at 20 mg/kg (400 mg/sq m), the plasma clearance of the drug was fairly fast, with a half-life of about 36 min. The volume of distribution exceeded total-body water content. Excretion was complete in 6 hr, at which time the cumulative excretion was 17% of the injected dose. DIC appeared in the cerebrospinal fluid (CSF) of the dog 10 min after injection. When the drug was administered to the dog by constant intravenous infusion, an average CSF to plasma concentration ratio of 1:7 was obtained at steady state.

When administered intravenously to man at a dose ranging from 133 mg/sq m to 270 mg/sq m, the plasma clearance of DIC was similar to that seen in the dog: the plasma half-life was 35 min. The volumes of distribution likewise exceeded total-body water content. However, in 6 hr 43% of the dose was excreted as unchanged DIC. At least 1 unidentified biotransformation product was found in trace amounts in the urine of man and dogs treated with DIC.

The plasma half-time of DIC was 111 min when the drug was administered by mouth to patients at a dosage ranging from 30 mg/sq m to 260 mg/sq m. The drug persisted in the plasma for up to 6 hr following ingestion. The cumulative excretion of DIC when given orally was variable; on the average, 19% of the dose was found in the urine in 6 hr. These results suggest that gastrointestinal absorption of DIC was slow, incomplete, and variable.

INTRODUCTION

A series of triazenoimidazoles, analogs of AIC\(^3\) in which the 5-amino group has been replaced by various monoalkyl- and dialkyltriazeno groups, have been synthesized and evaluated for antitumor activity (12, 13, 15). One of these, DIC (NSC 45388), the dimethyl derivative (Chart I), has exhibited notable activity against mouse sarcoma 180, adenocarcinoma 755, and leukemia L1210 (14). In fact, DIC compares quite favorably with existing clinically useful agents such as cyclophosphamide and 6-mercaptopurine against the solid form of mouse Ehrlich carcinoma (5). For these reasons DIC is currently under clinical trial at several research centers. Concurrent pharmacologic investigations of DIC have been performed. We report here the results of such studies.

MATERIALS AND METHODS

DIC. Both crystalline DIC and the parenteral preparation for clinical application were supplied by the Drug Development Branch of the Cancer Chemotherapy National Service Center, National Cancer Institute. The material was chromatographically homogeneous (see below). For intravenous administration to the dog, DIC was dissolved in 0.1 N hydrochloric acid containing 0.88% of sodium chloride in which the maximal solubility was 20 mg/ml. Solutions of 7.5 mg/ml and 20 mg/ml had a pH of 1.3 and 2.5 respectively. Rapid intravenous injection of 3 ml/kg of the more acidic solution to dogs of 12 to 21 kg, or constant infusion of the same solution at 1.5 ml/kg/hr, caused only an insignificant change (less than 0.1) in the pH of the blood or CSF. Based on spectrophotometric and chromatographic evidence, the solutions were stable for at least 24 hours when stored in the dark at 25°C.

Colorimetric Determination. DIC was determined by a recently developed colorimetric method (6). The principle involves the photodecomposition by ultraviolet light irradiation of a solution of DIC in dilute acid in the presence of N-(1-naphthyl)-ethylenediamine (Bratton-Marshall reagent), followed by measurement of the intensity of the color of the azo compound thus formed. The method is specific for dialkyltriazeno compounds. However, photosensitive drugs such as prochlorperazine\(^4\) and chlor Diazepoxide\(^5\), which turn dark upon exposure to ultraviolet light, give very high blanks and interfere with the determination. Plasma and urine standards were prepared from plasma and urine samples collected just before each experiment. Plasma blanks vary from person to person but, for the same individual, variation within a 24-hr period was insignificant.

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\(^2\)Supported by Contract PH 43-66-1156 of the National Cancer Institute, NIH, USPHS.

\(^3\)Abbreviations used: AIC, 5-aminoimidazole-4-carboxamide; DIC, NSC45388, 5-(dimethyltriazeno)imidazole-4-carboxamide; CSF, cerebrospinal fluid.

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\(^4\)2-chloro-10-{3-(1-methyl-4-piperazinyl)propyl}-phenothiazine.

\(^5\)57-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide.
Chart 1. Structural formula of \(5\text{-}(\text{dimethyltriazeno})\text{imidazole-4-carboxamide}\).

RESULTS

Plasma Clearance and Cumulative Excretion of DIC in the Dog. The plasma clearance and cumulative excretion of DIC in the dog after a single i.v. dose is summarized in Table 1. Over a dose range of 5 to 20 mg/kg, the plasma half-time of DIC was 36 min (Chart 2A). Excretion was complete in 6 hr, at which time the cumulative excretion averaged 17% of the injected dose (Chart 3A, Table 1). In all cases, the volumes of distribution were estimated to exceed total-body water content by compartmental analyses of the plasma disappearance curves (11).

<table>
<thead>
<tr>
<th>No.</th>
<th>Weight (kg)</th>
<th>Dose (mg/kg)</th>
<th>Plasma t1/2 (min)</th>
<th>Excretion in 6 hr (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>17.3</td>
<td>5</td>
<td>140</td>
<td>45</td>
</tr>
<tr>
<td>13</td>
<td>19.4</td>
<td>10</td>
<td>298</td>
<td>30</td>
</tr>
<tr>
<td>15</td>
<td>14.9</td>
<td>10</td>
<td>258</td>
<td>30</td>
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<tr>
<td>193</td>
<td>19.7</td>
<td>18</td>
<td>540</td>
<td>45</td>
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<tr>
<td>198</td>
<td>21.0</td>
<td>20</td>
<td>633</td>
<td>45</td>
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<tr>
<td>233</td>
<td>16.4</td>
<td>20</td>
<td>544</td>
<td>30</td>
</tr>
<tr>
<td>234</td>
<td>16.6</td>
<td>20</td>
<td>548</td>
<td>30</td>
</tr>
<tr>
<td>276</td>
<td>19.8</td>
<td>20</td>
<td>604</td>
<td>40</td>
</tr>
<tr>
<td>277</td>
<td>20.3</td>
<td>20</td>
<td>612</td>
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</tr>
<tr>
<td>278</td>
<td>21.6</td>
<td>20</td>
<td>636</td>
<td>30</td>
</tr>
</tbody>
</table>

Average: 36

Plasma clearance and cumulative excretion of \(5\text{-}(\text{dimethyltriazeno})\text{imidazole-4-carboxamide}\) in the dog after a single i.v. dose.

\*Dose in mg/sq m is obtained by multiplying the dose in mg/kg by a \(K_m\) factor. \(K_m\) for the dog is related to its body weight \(W\) in kg; thus, \(K_m = (W + 15)/1.16\) (3).

Entry of DIC into the CSF of the Dog. After a priming dose of DIC, a constant plasma level of the drug was maintained in the dog by constant infusion. Periodic samples of blood and CSF were collected and analyzed for DIC. The drug appeared in the CSF within 10 min of i.v. injection. At steady state, an average CSF to plasma DIC ratio of 1:7 (corrected for protein binding) was attained in 5 experiments (Table 2).

Plasma Clearance and Cumulative Excretion of DIC in patients after a Single i.v. Dose. DIC was administered to 6 patients by a single rapid i.v. injection. The plasma clearance and cumulative excretion in 6 hr in terms of percentage of administered dose are presented in Table 3. The average plasma half-time was 38 min. Excretion was complete in 6 hr and the average cumulative excretion in 6 hr was 43% of the injected dose. For comparison, some of the clinical graphic data (Charts 2B and 3B) are plotted side by side with those of the dog. Similar to the dog the volumes of distribution exceeded total-body water content.

Plasma Clearance and Cumulative Excretion of DIC in Patients after a Single Oral Dose. Similar determinations were made on 4 patients who received the drug orally while fasting (Table 3). The plasma levels were measured in 3 of the 4
patients; the average half-time was 111 min. The drug persisted in the blood stream for up to 6 hr after ingestion. The cumulative excretion averaged 19.2% of the ingested dose. Two of these patients (O. A. D. and McD.) were given DIC first by mouth, followed by i.v. injection several weeks later.

**Plasma Clearance and Cumulative Excretion of DIC in Patients on a 5-Day Course.** In current clinical practice, DIC is administered i.v. to patients at 150 mg/sq m daily for 5 days. To study the possible effect of DIC on its own pharmacology in the course of this treatment schedule, plasma levels and cumulative excretion were determined in the same patient on the first and fifth days of treatment. The results are shown in Charts 4 and 5. The average plasma half-time was 37 min on the first day and 43 min on the fifth day. The 6-hr cumulative excretion was 43% on the first day and 50% on the fifth day.

**Binding of DIC to Plasma Protein.** DIC was not appreciably bound to human plasma protein in solutions more concentrated than 5 μg/ml. In dilute human plasma solutions of 0.5 to 5 μg/ml, it was about 20% bound. In canine plasma solutions of 5 to 10 μg/ml, DIC was 27.5% bound. Assuming that the binding involved plasma albumin alone and assuming an average plasma albumin concentration of 5 X 10^{-4} M (7), the average drug-protein association constant at 25°C was 4.4 X 10^2 per mole for human plasma and 8.1 X 10^2 per mole for dog plasma.

**Chromatography of DIC and Metabolite.** The R_F values of authentic DIC in solvent systems A, B, C, and D were 0.26, 0.60, 0.60, and 0.53 on Whatman 3 MM paper and 0.25, 0.40, 0.60, and 0.52 on cellulose MN 300 thin-layer plates. The CSF of a dog (No. 226) 2 hr after constant infusion with DIC contained a material chromatographically indistinguishable from authentic DIC in all of the solvent systems. The urine of DIC-treated dogs and patients behaved likewise, except for the presence of a very faint second spot discernible both under 366 μm illumination and after spraying with Bratton-Marshall reagent. On Whatman 3 MM paper, this faint spot had the same R_F value of 0.30 in solvent systems C and D. It persisted in the urine up to 5 hr after drug administration.

**DISCUSSION**

After i.v. administration the plasma half-time of DIC is the same in the dog (Table 1) as in humans (Table 3), but the cumulative excretion in 6 hr in the dog is only 17% of the
Table 3

<table>
<thead>
<tr>
<th>Patient</th>
<th>Body weight (kg)</th>
<th>Dose mg/kg</th>
<th>Dose mg/sq.m</th>
<th>Route</th>
<th>Plasma $t_{1/2}$ (min)</th>
<th>Excretion in 6 hr (% of dose)</th>
<th>BUN (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. V.</td>
<td>20.8</td>
<td>4.8</td>
<td>103</td>
<td>i.v.</td>
<td>35</td>
<td>46</td>
<td>3</td>
</tr>
<tr>
<td>O. A. D.</td>
<td>38.9</td>
<td>5.2</td>
<td>133</td>
<td>i.v.</td>
<td>20</td>
<td>51</td>
<td>11</td>
</tr>
<tr>
<td>J. M. L.</td>
<td>75.0</td>
<td>5.2</td>
<td>200</td>
<td>i.v.</td>
<td>30</td>
<td>38</td>
<td>17</td>
</tr>
<tr>
<td>R. L. A.</td>
<td>60.0</td>
<td>5.3</td>
<td>200</td>
<td>i.v.</td>
<td>40</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>R. J. K.</td>
<td>64.5</td>
<td>6.8</td>
<td>250</td>
<td>i.v.</td>
<td>45</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>McD.</td>
<td>44.5</td>
<td>9.0</td>
<td>270</td>
<td>i.v.</td>
<td>55</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. T. C.</td>
<td>60.0</td>
<td>0.8</td>
<td>30</td>
<td>p.o.</td>
<td>Not Done</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>O. A. D.</td>
<td>44.3</td>
<td>2.3</td>
<td>67</td>
<td>p.o.</td>
<td>109</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>C. A. G.</td>
<td>54.6</td>
<td>5.5</td>
<td>192</td>
<td>p.o.</td>
<td>79</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>McD.</td>
<td>47.3</td>
<td>8.5</td>
<td>260</td>
<td>p.o.</td>
<td>146</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>111</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

Plasma clearance and cumulative excretion of 5-(dimethyltriazeno)imidazole-4-carboxamide in patients after a single dose.

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being sprayed with Bratton-marshall reagent and after exposure to ultraviolet light, it must have the intact 5-diazoimidazole moiety.

The volume of distribution of DIC in both dogs and men exceeded total-body water content, suggesting localization of the drug in some body tissue. Preliminary work in our laboratories (G. E. Housholder, personal communication) showed that radioactivity was highest in the liver of the mouse after an i.p. injection of DIC-2-14C.

DIC enters the CSF of the dog fairly rapidly, within 10 min after an i.v. injection. However, the extent of entry is relatively low, only 14% of the plasma equilibrium level as determined by constant infusion experiments (Table 2). This accords with the observation that DIC is not effective against intracranial L1210 mouse leukemia (5). The relatively low equilibrium concentration of DIC in the CSF is consistent with the insolubility of DIC in lipid solvents such as ether, chloroform, and ethyl acetate. Furthermore, the basicity of the dimethyltriazeno group in DIC is probably comparable to that of the amino group in AIC. Therefore, it is likely that at body pH DIC is partially ionized.

The sustained plasma level of DIC and the diminished 6-hr cumulative excretion after p.o. administration in man (Table 3) indicates that gastrointestinal absorption of DIC is slow, incomplete, and variable. This is compatible with animal toxicity studies in which two to four times the parenteral dose is required for oral administration to achieve comparable toxicity (H. E. Skipper, personal communication). Initially, clinical trials of DIC were conducted with oral preparations because parenteral solutions were unavailable. Under these circumstances, the tolerated oral dose was highly variable. For agents with steep dose-response curves and the potential for serious and even fatal toxicity, as are most antitumor drugs, oral administration should be avoided unless absorption is complete.

Repeated administration of a drug frequently induces the enzymes which biotransform the same drug, thus grossly affecting its pharmacology (2). Clinical observations have suggested that the pharmacology of DIC may be altered by prior administration of DIC or other agents. Nausea and vomiting, prominent during the first day or two of a five-day course,
essentially disappear towards the end of the course. In patients prior administration of phenobarbital is the most effective means to reduce these side-effects (E. Frei, III, personal communication). These considerations prompted us to study the clinical pharmacology of DIC on the first and the fifth days of the five-day course. No conclusive trend was demonstrated in this study. In one of the 4 patients studied (M. D. B.), the plasma level was considerably higher on the fifth day than on the first day (Chart 4). Moreover, as was expected, cumulative excretion of the drug in this patient was exactly the reverse—much lower on the fifth day than on the first day (Chart 5). However, in another patient (H. P.), although the plasma level of DIC was higher on the fifth day, almost twice as much drug was excreted in 6 hr on that day.

DIC is not extensively bound to either human or canine plasma protein. When its concentration in human plasma solution exceeds 5 μg/ml, it is not significantly bound, presumably because of saturation of binding sites.

The mechanism of action of DIC is currently under investigation. AIC is more rapidly utilized as a precursor in purine biosynthesis by tumor cells than by normal tissues of the tumor-bearing animals (4). This suggests that as an analog of AIC, DIC may exert its antitumor activity by inhibiting the biosynthetic pathway to nucleic acids. DIC is not cross-resistant with the thiopurines in mouse L1210 leukemia (I. Wodinsky, personal communication). A related agent 5-[bis(2-chloroethyl)-triazeno]imidazole-4-carboxamide] (NSC-82196) has been shown to inhibit the growth of a strain of Escherichia coli (ATCC 11303). Partial protection of the microorganism is afforded by AIC ribonucleotide (9). The inhibition of both protein and RNA synthesis in E. coli (ATCC 9637) by another closely related analog of DIC, methyl 5-(dimethyltriazeno)-imidazole-4-carboxylate (NSC-87982), has also been reported (10).

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REFERENCES


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