Mutagenicity of Cancer Chemotherapeutic Agents in the Salmonella/Microsome Test

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SUMMARY

Seventeen cancer chemotherapeutic agents were tested for their ability to mutate Salmonella typhimurium tester strains in the Salmonella/microsome mutagenicity test. There was a high correlation between the mutagenicity and carcinogenicity of a given agent. Carcinogens positive in the test were Adriamycin, daunomycin, 1-propanol-3,3'-iminodimethanesulfonate, cyclophosphamide, isophosphamide, hycanthone, chloronaphazin, nitrogen mustard, uracil mustard, melphalan, and thio-tepa. Two carcinogens, actinomycin D and bleomycin, were not detected as mutagens. The presumptive noncarcinogen, methotrexate, was negative in the test. Tilorone and 6-mercaptopurine, tentatively classified as noncarcinogens, were mutagenic. The carcinogenicity of cis-dichlorodiammineplatinum(II), which was positive in the test, has not been determined.

INTRODUCTION

A number of short-term assays for screening potential chemical carcinogens/mutagens have been developed over the last few years. Cancer chemotherapeutic drugs are one group of agents that may be particularly suited for examining the relationship among results from short-term in vitro assays and in vivo carcinogenicity studies and chemical carcinogenesis in man. Several of these agents have been tested for their in vivo carcinogenicity, and some are suspected of being carcinogenic in man. The continued clinical use of these agents may lead to further documentation of their carcinogenic potential. Recent studies on the ability of various cancer chemotherapeutic agents to produce morphological transformation in the C3H/10T½ clone 8 mouse cell line and rapid chromosomal damage in the A(T1)C1-3 hamster cell line (6) have demonstrated that there is a high correlation between the ability of an agent to produce transformation or chromosomal damage and its capacity to be carcinogenic in vivo.

This study examines several of these agents in the Salmonella/microsome mutagenesis test system (3-5, 24, 27). This in vitro test makes use of a set of histidine mutants of S. typhimurium for detecting mutagens and a mammalian liver microsomal preparation for converting carcinogens to their active, mutagenic forms. The test system has been validated in several recent studies (23-25, 32, 43).

MATERIALS AND METHODS

Chemicals. The following drugs were obtained from Harry B. Wood, Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.: actinomycin D, Adriamycin, bleomycin, chloronaphazin, cyclophosphamide, isophosphamide, 6-mercaptopurine, methotrexate, 1-propanol-3,3'-iminodimethanesulfonate, thio-tepa [tris(1-aziridinyl)phosphine sulfide], and uracil mustard. Melphalan and hycanthone were obtained from Richard Adamson, Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Md., and tilorone was received from Gerald Mayer, Merrell National Laboratories, Cincinnati, Ohio. Daunomycin-HCl was the gift of N. Bachur, National Institutes of Health, Baltimore, Md. cis-Dichlorodiammineplatinum(II) was purchased from Strem Chemicals, Danvers, Maine, and nitrogen mustard was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis.

Mutagenesis Assay. Assays were performed as previously described (3-5). S. typhimurium tester strains TA1535, TA1537, TA98, and TA100 were utilized (4, 27). The liver S-9 fraction was prepared from male Sprague-Dawley rats (Horton Animal Labs, Oakland, Calif.), given injections of Aroclor 1254 (Analabs, Inc., North Haven, Conn.) 5 days prior to sacrifice. The drugs were dissolved in water or dimethyl sulfoxide and added directly to the top agar at several concentrations ranging from nontoxic to highly cytotoxic.

RESULTS

Chemotherapeutic Drugs Mutagenic in the Salmonella Test. Chart 1 indicates those drugs found positive for mutagenesis in the Salmonella system. In cases where more than 1 strain was reverted by the drug, that strain showing greatest activity was selected. Strain TA1535, sensitive to chemicals causing base-pair substitutions, is reverted by
malphalan, thio-tepa, 1-propanol-3,3'-iminodimethanesulfonate, and isophosphamide, and (as previously shown) by nitrogen mustard (2), uracil mustard (Ref. 25; H. Rosenkranz, personal communication), 6-mercaptopurine (16, 33), and cyclophosphamide (Ref. 26 and M. Legator, personal communication). [Most of these results have been quoted in the test validation study (25)]. With the exception of 6-mercaptopurine, a purine analog, these drugs are all alkylating agents. The activity of melphalan is increased approximately 3-fold by incubating in the presence of the liver S-9 preparation, while cyclophosphamide and isophosphamide are active only in the presence of the S-9 fraction.

Strain TA100 is the same as TA1535 but contains an R-factor plasmid (27). As previously reported, this strain is reverted by cis-dichlorodiammineplatinum(II) (29) and chlornaphazin (25). Mutagenic activity with chlornaphazin was increased in the presence of S-9.

As previously shown, Adriamycin (Ref. 25; M. Legator, personal communication), daunomycin (5), and hycanthone (15, 25) revert the frameshift tester strain TA1538 and/or its R-factor derivative, strain TA98.
Tilorone at high concentrations (2 mg/plate) reverts the frameshift tester strain TA1537.

Chemotherapeutic Drugs Not Mutagenic in the Salmonella Test. Actinomycin D, bleomycin, and (as previously shown) methotrexate (16, 28, 48) were not mutagenic in Salmonella. Each drug was tested at several concentrations over a 100-fold range (actinomycin D, 0.01 to 50 μg/plate; bleomycin, 0.01 to 1.0 μg/plate; methotrexate, 10 to 1000 μg/plate), in which no plate was significantly higher than controls, and no dose response was observed. All 4 tester strains were utilized, and each compound was tested both in the presence (20 μl/plate) and absence of S-9.

Relation between Mutagenesis and Carcinogenesis. The relationship between the ability of chemotherapeutic agents to mutate the Salmonella tester strains and their known capacity to produce tumors in vivo is shown in Table 1. In general, those agents that mutate the Salmonella tester strains are also carcinogenic. Only 2 agents, actinomycin D and bleomycin, have been shown to be carcinogenic in vivo but were not mutagenic in Salmonella. Tilorone and 6-mercaptopurine, which are mutagenic in Salmonella, are tentatively classified as noncarcinogens.

**DISCUSSION**

Our studies show that 11 of 13 of the chemotherapeutic agents that are carcinogenic are also mutagenic in the *Salmonella* system. In addition to being carcinogenic in animal studies, some of the agents are suspected of being carcinogenic in man. Bladder tumors have been reported in patients receiving long-term treatment with cyclophosphamide (46) [the urine of patients treated with cyclophosphamide is mutagenic in *Salmonella* (28)] and following treatment with chloronaphazin (45). There is a marked increase in acute myelogenous leukemia in patients treated with melphalan for multiple myeloma (37), and several cases of acute myeloblastic leukemia have been reported in patients receiving thio-tepa for the treatment of solid tumors (39). It will be important to determine whether the other chemotherapeutic agents that have been shown to be mutagenic in the *Salmonella* system also yield a high risk of primary or secondary cancers following exposure to these drugs.

A few of the carcinogenic cancer chemotherapeutic agents are not detected in the *Salmonella* test. Actinomycin D and bleomycin [and also Natulan (Procarbazine) (25)] have not shown any mutagenic activity in the *Salmonella* tester strains thus far. Actinomycin D was not cytotoxic to the *Salmonella* at a concentration of 10 μg/plate. Therefore, it is likely that little actinomycin D is accumulated intracellularly in the bacteria. However, it has been shown to be mutagenic in the dominant-lethal test in the mouse, to cause chromosomal damage, to be teratogenic (see Ref. 42), and to transform cells in culture (6). We are currently examining the mutagenicity of these compounds in other *Salmonella* tester strains.

6-Mercaptopurine, tentatively classified as a noncarcinogen, is positive in the *Salmonella* test. In one study 6-mercaptopurine showed no carcinogenic activity when tested in rats (35), although, in studies with mice, lymphomas (11) and lymphosarcomas (31) were observed. It is not certain if these tumors are due to the immunosuppressive effects of the drug or to a direct carcinogenic action. As the protocols followed in these studies tend to reflect the clinical pattern of administration and dosage, rather than to provide full lifetime studies, further studies are needed to assess the full carcinogenic potential of this compound. 6-Mercaptopurine is mutagenic in the dominant-lethal assay in mice (13) and causes chromosome breakage in mouse bone marrow cells (17). However, exposure to 6-mercaptopurine in culture did not transform cells or produce chromosome damage in a recent study by one of our groups (6). Also, 6-mercaptopurine is, under certain conditions, a metabolite of azathioprine (Imuran). The latter drug is likewise used in cancer chemotherapy and is a mutagen in *Salmonella* (16, 40) and in mice, *Drosophila*, and *Neurospora* (8). It is presently unknown whether the mutagenicity of azathioprine is direct or a result of 1 or more of its metabolites.

Tilorone, a presumptive noncarcinogen, is a very weak mutagen in *Salmonella*. Mutagenic activity is observed at a concentration approximately 200-fold higher than that which produces over a 90% decrease in plating efficiency after a 24-hr exposure to cell culture (6). The observed activity may be due to an impurity present in the sample, although this has not been determined.

Finally, the fact that the nitrogen mustard derivatives, melphalan and chloronaphazin, show increased activity in

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**Table 1**

**Relationship between mutagenesis in Salmonella tester strains and carcinogenesis in vivo produced by chemotherapeutic agents**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Carcinogenicity in vivo</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive for mutagenesis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adriamycin</td>
<td>+</td>
<td>7, 22, 30</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>+</td>
<td>7, 22, 41</td>
</tr>
<tr>
<td>Dimethanesulfonate</td>
<td>+</td>
<td>42</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>+</td>
<td>20, 36, 42, 47</td>
</tr>
<tr>
<td>Isothiophosphate</td>
<td>+</td>
<td>42</td>
</tr>
<tr>
<td>cis-Dichlorodiammineplatinum(II)</td>
<td>ND*</td>
<td></td>
</tr>
<tr>
<td>Hycanthone</td>
<td>+</td>
<td>14; D. Clayson, Eppley Institute</td>
</tr>
<tr>
<td>Chloronaphazin</td>
<td>+</td>
<td>19, 45</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>+</td>
<td>See Ref. 38</td>
</tr>
<tr>
<td>Uracil mustard</td>
<td>+</td>
<td>1, 42, 47</td>
</tr>
<tr>
<td>Melphalan</td>
<td>+</td>
<td>36, 47</td>
</tr>
<tr>
<td>Thio-tepa</td>
<td>+</td>
<td>35, 36, 42</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>+</td>
<td>11, 31, 35</td>
</tr>
<tr>
<td>Tilorone</td>
<td>ND*</td>
<td>R. P. Adamson, NCI</td>
</tr>
</tbody>
</table>

| **Negative for mutagenesis** | | |
| Actinomycin D       | +                       | 10, 18, 21, 44, 47 |
| Bleomycin           | +                       | 21    |
| Methotrexate*       | -                       | 34, 35 |

* ND, not determined; -?, produces lymphomas and lymphosarcomas, possibly secondary to immunosuppression.
* +, unknown at high-dose exposure (see Ref. 6).
the presence of S-9 is noteworthy, since these 2 alkylating agents are not considered to need liver activation to produce their cytotoxic effects. This has previously been shown for melphalan (28). Cyclophosphamide and isophosphamide, which are biologically inactive per se, are mutagenic only in the presence of S-9. The metabolic pathway of cyclophosphamide has recently been elucidated (see Ref. 9), and several of the metabolites have been shown to be mutagenic (12). 4-Hydroxyxycyclophosphamide, the synthetic precursor of 4-hydroxycyclophosphamide (the initial product of cyclophosphamide metabolism), normitrogen mustard, and the 2 urinary metabolites, carboxyphosphamide and 4-ketocyclophosphamide, show direct mutagenic activity in Escherichia coli. It is particularly interesting that the major urinary metabolites are mutagenic, considering the reports of bladder cancer following treatment with cyclophosphamide (46).

REFERENCES

11. Doell, R. G., St. Cyr, C. deV., and Graber, P. Immune Reactivity Prior to the Presence of S-9 is noteworthy, since these 2 alkylating agents are not considered to need liver activation to produce their cytotoxic effects. This has previously been shown for melphalan (28). Cyclophosphamide and isophosphamide, which are biologically inactive per se, are mutagenic only in the presence of S-9. The metabolic pathway of cyclophosphamide has recently been elucidated (see Ref. 9), and several of the metabolites have been shown to be mutagenic (12). 4-Hydroxyxycyclophosphamide, the synthetic precursor of 4-hydroxycyclophosphamide (the initial product of cyclophosphamide metabolism), normitrogen mustard, and the 2 urinary metabolites, carboxyphosphamide and 4-ketocyclophosphamide, show direct mutagenic activity in Escherichia coli. It is particularly interesting that the major urinary metabolites are mutagenic, considering the reports of bladder cancer following treatment with cyclophosphamide (46).


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