The Need for Additional Alkylating Agents

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Summary

Although thousands of alkylating agents have been synthesized, their results in clinical use have proved generally disappointing. It is felt that the future of the alkylating agents lies not in the continued synthesis and routine screening of new derivatives but in their more extensive study against experimental tumor systems before their clinical application. In the past, certain alkylating agents have shown a marked selectivity for a particular animal tumor, and a detailed study of their mechanism(s) of action has revealed a previously unknown biochemical difference between the tumor and host tissues. The future role of the alkylating agents may lie in their ability to detect unique features of the biochemistry of animal tumors. A knowledge of the differences that can exist between an animal tumor and normal tissue may then lead to the design of new alkylating agents which exploit these differences, and their use in the clinic on a rational basis against human tumors showing the same properties.

If one considers the achievements of the alkylating agents in the treatment of cancer since their first application it does seem at first sight a difficult task to justify the synthesis of further derivatives.

The results obtained with the first alkylating agent to be used clinically, nitrogen mustard (HN2), were published in 1946 (10), and the authors concluded that the further synthesis and screening of the almost unlimited variants of HN2 and a detailed study of their mechanism of action might well lead to the discovery of agents highly effective in the treatment of particular cancers.

Now almost a quarter of a century later, both of these conditions appear to have been fulfilled. Many thousands of nitrogen mustards and related alkylating agents have been synthesized and screened for antitumor activity, and their mechanism of action appears to be well understood.

Nevertheless, despite this enormous effort the clinical results obtained with the alkylating agents have proved to be rather disappointing. HN2, the first nitrogen mustard tested clinically, is still well established in the clinic, an indication that the synthesis and testing of new alkylating agents has not produced any derivatives so obviously superior to HN2 that this compound has been discarded completely. The aim of successful cancer chemotherapy is the complete eradication of the disease or at least the killing of a large proportion of tumor cells so that benefit is obtained in terms of complete remission of symptoms for a long period. This is achieved in relatively few cancers; choriocarcinoma, acute lymphocytic leukemia, and Burkitt's lymphoma are three of the most notable examples. However, in the treatment of these first two cancers, highly effective drugs or drug combinations are known which do not include alkylating agents. Only in the case of Burkitt's lymphoma does the continued synthesis of new alkylating agents appear to have achieved its objective in discovering more selective agents. In the treatment of Burkitt's lymphoma, not only are the alkylating agents most effective, but some members of this class are more effective than others. The more recently synthesized o-merphalan and endoxan, for instance, give far better results in causing long-term survival than do chlorambucil or HN2 (4) (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of long-term survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen mustard</td>
<td>None</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>None</td>
</tr>
<tr>
<td>Endoxan</td>
<td>4/31</td>
</tr>
<tr>
<td>Orthomerphalan</td>
<td>6/26</td>
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Effect of alkylating agents on Burkitt's lymphoma.

However, despite the relatively poor achievements of the alkylating agents in the clinic, a case can be made for their more extensive study in the laboratory and for the synthesis of further derivatives. Although all alkylating agents probably have the same target site DNA, and although this is just as sensitive a site for some normal cells, a particular alkylating agent can theoretically be highly sensitive for a certain cell. Chart 1 shows that after administration, an alkylating agent, once it has reached the vicinity of the tumor, must be taken up by the cancer cell, traverse the cytoplasm, and react with DNA before cytotoxicity occurs. Many events can take place inside the cell which determine the eventual sensitivity of the cell to the agent. An alkylating agent of a particular chemical structure may, for instance, be much more readily taken up by a cancer cell. Some cancer cells may be less able to deactivate some forms of alkylating agent and will consequently be much more sensitive. Other alkylating agents have structures which can be acted on by intracellular enzymes to form much more toxic derivatives. A cell possessing this activating system would be much more susceptible to this type of alkylating agent than cells lacking these enzymes. Similarly, cells which have relatively few nucleophilic molecules in the vicinity of DNA to compete with DNA for the alkylating agent will be sensitive as will cells which lack any mechanism for repairing alkylated DNA.
Chart 1. An alkylating agent at physiologic dose levels must react with DNA before cell death occurs. This chart shows the various ways in which the cell can render the agent much more active or inactivate it completely. Increased activation of the agent in a cancer cell paralleled by deactivation in sensitive host tissues would lead to a highly selective antitumor agent.

Therefore, although basically these agents are not selective for cancer cells, one can envisage how an alkylating agent of a particular chemical structure can be highly selective in its effect on a cancer cell with the appropriate biochemical properties. This is clearly seen in the laboratory in the response of a plasma cell tumor to the alkylating agent aniline mustard. This tumor was once thought to be quite resistant to all alkylating agents, but it was then shown to be highly sensitive to aniline mustard but not to closely related analogs (13, 14). An extensive study of the mechanism of this effect showed that selectivity probably occurred as a result of metabolism as shown in Chart 2. The administered compound is converted in the liver to its O-glucuronide, a compound expected to be quite nontoxic. The intermediate p-hydroxy derivative formed is very much more toxic than the administered compound, but its rapid conversion in the liver to the glucuronide prevents it from causing any systemic toxicity. However, the plasma cell tumor has an extremely high level of O-glucuronidase [probably occurring in the cytoplasm as well as in the cellular organelles (J. A. Double, unpublished data)], with the result that any of the glucuronide entering the tumor cells is activated by conversion to the toxic p-hydroxy derivative (6, 15). In this way one alkylating agent has a highly selective effect on one particular tumor. Two facts were uncovered as a result of this study. In the first case a hitherto unknown property of the tumor was revealed; namely, its high glucuronidase level. Secondly, a particular alkylating agent could, by exploiting this property, have a highly selective action. As a consequence of this study on an animal tumor, it was suggested that the glucuronide of aniline mustard be synthesized and used in the clinic in the treatment of cancers with high levels of O-glucuronidase. The glucuronide has recently been synthesized and is at present awaiting clinical trial.
There is no doubt that so variable are the structures of alkylating agents that can be synthesized, that once a difference is revealed between a tumor and normal cells an appropriate alkylating agent can be designed which can exploit this difference and lead to high selectivity. Tumors, both animal and human, have for instance often been reported to have a lower pH than normal cells (1, 7). This difference has recently been exploited in the synthesis of a nitrogen mustard specially designed to precipitate at the low pH of tumor cells. This compound prepared for this purpose, sulphadiazine mustard, has already been shown to be more selective against animal tumor systems than clinically used alkylating agents and is at present on clinical trial (3).

Differences between a cancer cell and normal cells are often revealed by chance. The most notable example of this concerns the development of L-asparaginase in the treatment of acute leukemia. The finding that guinea pig serum caused complete regression of the Gardner lymphosarcoma (11) led to a study of the mechanism of this effect. Eventually L-asparaginase was found to be the active compound of the serum (2) and revealed for the first time that this particular tumor was unable to synthesize its own asparagine. Subsequently asparaginase has been used effectively in the clinic especially in the treatment of acute lymphocytic leukemia.

Perhaps the most important role of the alkylating agents in the future is not in their continued synthesis and screening against a limited number of tumors, but in their ability to uncover previously unsuspected differences between a cancer and normal tissues. Where an agent has a uniquely selective effect on one animal tumor, such as was the case with guinea pig serum against the Gardner tumor and aniline mustard against the plasma cell tumor, the mechanism of this effect should be studied in detail. A study in depth of the agent may reveal a biochemical feature of the tumor responsible for the selectivity observed. Once this biochemical difference is recognized, further alkylating agents may be designed to exploit this difference and give even more selective agents. It is not then unreasonable to expect that there will be human tumors with similar biochemical features (as was found with asparaginase) making them also highly sensitive to this type of alkylating agent.

One nitrogen mustard recently synthesized (Chart 3, Compound I) has proved to be one of the most selective antitumor agents ever tested in animal systems (P. Hebborn, personal communication). Agents of this structure could reveal a biochemical feature of the tumor responsible for the selectivity observed. Once this biochemical difference is recognized, further alkylating agents may be designed to exploit this difference and give even more selective agents. It is not then unreasonable to expect that there will be human tumors with similar biochemical features (as was found with asparaginase) making them also highly sensitive to this type of alkylating agent.

Chart 3. Postulated enzymatic hydrolysis of Compound I to form a highly toxic derivative, p-phenylenediamine mustard (Compound II). If this process occurred selectively in a tumor (i.e., if only the tumor had the appropriate enzyme systems), one would expect the compound to be highly selective for that tumor. LD\textsubscript{50} (μmoles/kg): I, 268; II, 15.

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Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic index</th>
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<tbody>
<tr>
<td></td>
<td>Yoshida</td>
</tr>
<tr>
<td>MePhalan</td>
<td>16</td>
</tr>
<tr>
<td>CB 1954</td>
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Effect of two alkylating agents on various animal tumors. The therapeutic index is the ratio LD50/ID90.

Table 4

<table>
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<th>Drug</th>
<th>Therapeutic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline mustard</td>
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<tr>
<td>MDMS</td>
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Effect of two alkylating agents on various animal tumors. MDMS, methylenedimethanesulphonate.

Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID50 (mg/kg)</th>
<th>LD50 (mg/kg)</th>
<th>Therapeutic index</th>
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<tr>
<td>CNH2NO2</td>
<td>0.4</td>
<td>28</td>
<td>70</td>
</tr>
<tr>
<td>(CB 1954)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CNH2NO2</td>
<td>Inactive</td>
<td>280</td>
<td></td>
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<tr>
<td>CNH2NO2</td>
<td>Inactive</td>
<td>25</td>
<td></td>
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<tr>
<td>CNH2NO2</td>
<td>30</td>
<td>60</td>
<td>2</td>
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<tr>
<td>CNH2NO2</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>


Chart 4. Postulated mechanism of action of the difunctional alkylating agents showing the cross-linking of guanine residues in DNA. Methylenedimethanesulphonate (MeSO2-CH2-O-SO2-Me) can only cross-link by a methylene bridge -CH2-, an unlikely reaction from spatial considerations.

The future of the alkylating agents lies, therefore, in their more extensive study in the laboratory before their clinical application. By detailed studies of the mechanism of action of derivatives which show a unique selectivity for a particular tumor, we may learn more about the properties of these tumors which distinguish them from other cells. One may also see how an alkylating agent of a particular structure may...

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exploit this difference to bring about a highly selective antitumor effect. A knowledge of such properties of animal tumors may then lead to the synthesis of even more selective alkylating agents, and these in turn may be used clinically on a rational basis against tumors which possess the similar property seen in the animal model.

REFERENCES

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