Alopecia Activity of Cyclophosphamide Metabolites and Related Compounds in Sheep

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SUMMARY

Thirteen analogs of cyclophosphamide were tested for defleecing activity in sheep. Activity was found only in compounds having two chloroethyl groups and a cyclophosphamide-type ring.

INTRODUCTION

Alopecia is a frequent complication of cyclophosphamide therapy (13). This side effect has been proposed as a convenient means of defleecing sheep in areas in which mechanical shearing is not available (7). During a study of the metabolism of cyclophosphamide in sheep (3, 4), a number of related compounds were prepared as potential metabolites and as model compounds to aid interpretation of spectral data for elucidation of metabolite structures. These compounds and the known cytotoxic agents, Iphosphamide¹ and Trophosphamide, were tested for defleecing activity in sheep.

MATERIALS AND METHODS

Iphosphamide and Trophosphamide were obtained from Mead Johnson and Company, Evansville, Ind.

2-Amino-2//-l,3,2-oxazaphosphorine-2-oxide was prepared by a method similar to that used for the thio analog (10). Phosphorus oxychloride was reacted with 3-aminopropanol at −20° in the presence of triethylamine. The reaction was allowed to warm to room temperature, and the triethylamine hydrochloride was removed by precipitation with benzene. Reaction of the chlorophosphoramidate with anhydrous ammonia in methylene chloride yielded the desired amide, which was recrystallized first from hot methylene chloride and then from hot acetonitrile (m.p. 146-149°). 4-Methylcyclophosphamide reportedly melts at 72-73° (2). We were not able to obtain material with this melting point, but obtained samples with varying melting points ranging from 60-103°. One sample melted at a temperature range (71-76°) close to that reported. The dose material had a melting point of 74-95° and consisted of approximately a 3:2 mixture of isomers, as established by NMR² (see below). Repeated recrystallization yielded material of approximately a 5:1 composition, with a melting point of 80-97°. The formation of hydrates further reduced the usefulness of melting points as a measure of purity. Thus, the previous authors isolated either the lower-melting isomer or a mixture of some composition that yielded a fairly sharp melting point. An NMR study in CDCl₃ suggested the presence of isomeric methyl groups (Chart 1). Additional evidence for the presence of isomeric methyl groups was obtained by addition of Pr(FOD)₃, one of the rare-earth complexes that

1 The abbreviation used is: NMR, nuclear magnetic resonance.

² Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

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have proven valuable in resolving coincident absorbances in NMR spectra (5). This reagent caused a more rapid shifting of the upfield methyl absorption and yielded a spectrum from which chemical shifts and coupling constants could be determined (Chart 1). The methyl group may be cis or trans to the phosphate oxygen [presumably, the methyl absorption that is most responsive to the Pr(FOD)₃ is due to the cis orientation]; each of these isomers, of course, exists as a DL pair. A knowledge of the isomeric composition of 4-methylcyclophosphamide may be necessary to obtain consistent results in biological studies because the isomers may have different activities, toxicities, rates, and routes of metabolism, etc. Ideally, of course, both compounds should be isolated in pure form and studied separately.

**DISCUSSION**

Table 1 shows the structures and activities of the compounds tested. The compounds were given p.o. at doses, on a mmole/kg basis, equivalent to and in some cases twice the usual dose of cyclophosphamide; N,N-bis(2-chloroethyl)phosphorodiamidic acid was also tested i.v. At doses equivalent to that of cyclophosphamide, 4-methylcyclophosphamide was

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>Dose (mmole/kg)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>N(CH₃(CH₂CH₃))₂</td>
<td>0.115</td>
<td>+</td>
</tr>
<tr>
<td>H</td>
<td>N(CH₃)₂</td>
<td>0.115</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>N(CH₃H₂)₂</td>
<td>0.115</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>N(CH₃CH₂CH₃)₂</td>
<td>0.115</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>N(CH₃CH₂OCH₃)₂</td>
<td>0.115</td>
<td>±</td>
</tr>
<tr>
<td>H</td>
<td>N(CH₃CH₂CH₂CH₃)₂</td>
<td>0.115</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>NHCH₂CH₂Cl</td>
<td>0.115</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>NH₂</td>
<td>0.115</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>NHCH₂CH₂Cl</td>
<td>0.115</td>
<td>±</td>
</tr>
<tr>
<td>CH₂CH₂Cl</td>
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<td>+</td>
</tr>
<tr>
<td>CH₂CH₂Cl</td>
<td>N(CH₃CH₂Cl)₂</td>
<td>0.115</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.23</td>
<td>Lethal</td>
</tr>
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</table>

Sheep were dosed p.o. and were observed for defleecing activity (the last compound in the table was also given i.v.). Activity constituted a constriction of the wool fiber and thus allowed for easy removal of the fleece by hand about 7 days after treatment.

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*a* Cyclophosphamide.

*b* Iphosphamide.

*c* Trofosfamide.
highly effective as a defleecing agent, but Iphosphamide and Trophosphamide were not sufficiently active (fibers were weakened, but fleece could be removed only with some difficulty). Trophosphamide and 4-methylcyclophosphamide were lethal at twice the usual dose, whereas Iphosphamide yielded excellent defleecing with no apparent toxicity. Dose-response studies were not done.

The data suggest that 2 chloroethyl groups are necessary for defleecing activity, as is true for antitumor activity (2). The need for a higher dose of Iphosphamide for full activity is in keeping with the observations that Iphosphamide is more slowly metabolized (activated) than cyclophosphamide (1) and that higher doses are required for toxicity and antitumor activity in clinical studies (6). 4-Methylcyclophosphamide appears to be as active a defleecing agent as cyclophosphamide, which corresponds to the findings of Arnold et al. (2), who found that this compound had antitumor activity similar to that of cyclophosphamide (2).

Although \(N,N\)-bis(2-chloroethyl)phosphorodiamidic acid has been shown to have antitumor activity (11, 12), it had no defleecing activity in our study. Nathanson et al. (12) also did not observe alopecia clinically with this compound. Thus, it would appear that along with the 2 chloroethyl groups, the “cyclophosphamide ring” is also necessary for defleecing activity.

ACKNOWLEDGMENTS

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REFERENCES


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