Further Observations on the Efficacy of Phenylalanine Mustards against Mouse Melanoma*

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SUMMARY

Four phenylalanine nitrogen mustards were tested against the S91 Cloudman melanoma in young male mice. Two of the mustards carried the di(2-chloroethyl)amino group in the meta position but differed in respect to the location of the amino group in the alanine side chain. Likewise, two of the mustards carried the di(2-chloroethyl)amino group in the para position, again differing, however, in location of the amino group in the alanine side chain.

The most effective of the four was the meta mustard derivative of phenyl-DL-a-alanine. The two isomers containing the side chain amino group in the beta position instead of the alpha were of low toxicity and could be employed at much higher dosage levels but were of relatively low potency. In general, the observations reported confirm the findings of Gram, Mosher, and Baker on the appreciably higher potency of the alpha meta mustard as compared with the known potency of the alpha para mustard.

In previous papers we have reported on the efficacy, against mouse melanoma, of several aromatic nitrogen mustards derived from amino acids (5, 6). From these studies it was concluded that the phenylalanine mustard, p-(di[2-chloroethyl])amino-L-(or DL)-phenylalanine, was most promising for further study. The present study reports upon three isomers in which the alpha amino group was displaced to the beta position or the dichloroethyl-substituted amino group was shifted from the para to the meta position in the phenyl group of the amino acid. Trial of the beta-alanine derivatives was undertaken in the expectation that they would be less toxic than the alpha-alanine isomers. The "meta mustards," it was hoped, might be more potent than their para isomers. The place of the phenylalanine mustards in cancer chemotherapy has been discussed recently in a paper by Baker and his colleagues (3), to whom we are indebted for one of the compounds used in this present study.

MATERIALS AND METHODS

Young male mice, 6-7 weeks of age, of the strain DBA/1 were used. The animals, including a few implanted with the S91 tumor (Cloudman melanoma), were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Four mustards were used:

a) β-Phenyl-DL-a-alanine meta mustard (NSC 27381), m-(di[2-chloroethyl]amino)-β-phenyl-DL-a-alanine, kindly supplied by Dr. B. R. Baker.


c) β-Phenyl-DL-β-alanine meta mustard (CB. 3270), m-(di[2-chloroethyl]amino)-β-phenyl-DL-β-alanine, again kindly supplied by Dr. Stock.

d) β-Phenyl-DL-a-alanine para mustard, p-(di[2-chloroethyl]amino)-β-phenyl-DL-a-alanine. This was synthesized by Dr. Howard Smith by the method of Bergel and Stock (2) and was identical in melting point and infrared absorption spectrum with reference compound CB. 3007, kindly provided by Dr. Stock.

The procedures used in the synthesis of the first three compounds have been described in the following reports:

a) β-phenyl-DL-a-alanine meta mustard—Gram et al. (3), Osdene et al. (7), Vasileva et al. (8); (b) β-phenyl-DL-β-alanine para mustard—Bergel et al. (1); (c) β-phenyl-DL-β-alanine meta mustard—Johnson (4).
The substances were administered intraperitoneally as intimate suspensions in peanut oil. The toxicity of the fourth compound, β-phenyl-DL-α-alanine para mustard, was known from previous work. Its meta analog was reported by Gram et al. to be about 2-3 times as toxic. In our own experience we found the difference in toxicity to be slight, and we used them at about the same dosage levels.

The toxicity of the β-phenyl-DL-β-alanine para mustard had to be determined. Four groups of DBA/1 mice, twelve animals in each group, were given injections of increasing amounts of the mustard in peanut oil: first group, 55 mg/kg; second group, 70 mg/kg; third group, 100 mg/kg; and fourth group, 168 mg/kg. In each case we used 0.4 ml. of the mustard-peanut oil suspension per 20 gm. of mouse. Two weeks later the first group received a second injection at a dosage level of 65 mg/kg. Six weeks after the first injection the mice were sacrificed. The numbers of survivors were eleven, seven, five, and zero for groups one, two, three, and four, respectively. We concluded that the LD/50 was about 80 mg/kg. It also seemed to be probable that 100 per cent survival would result if a dose of 50 mg/kg were employed in an initial injection, even if followed 2 weeks later by a second injection of perhaps the same amount.

The toxicity of the remaining compound, β-phenyl-DL-β-alanine meta mustard, was assumed to be about the same as that of its para analog: the material was received from England too late for testing the toxicity.

Tumor tissue was implanted, by use of a trocar, in the upper right flank bordering on the axilla. The test mustards were not injected until the implant was well established and a small firm tumor, clearly palpable, was present. Such tumors were approximately 1.5-2.0 mm. in diameter, attaining this size within 2-3 weeks after implantation.

RESULTS

The effects of the four mustards on tumor size are recorded in Table 1. The intermediate effects, not shown in the table, were as follows:

a) α-Meta mustard: By the 11th day after the first injection, the tumors had seemingly disappeared from eight of the mice, and, in the ninth, a tumor could barely be detected by palpation. By the 20th day, tumors were present in all nine of the mice but had disappeared again from three of the mice by the 28th day (6 days after the second injection). A slight transitory decrease (~3 per cent) in body weight followed upon each injection.

b) β-Para mustard: The maximum effect on tumor size was observed on the 7th day, a decrease of 20 per cent in average diameter followed by a rapid increase to 6 mm. diameter by the 21st day. The second injection was without effect on tumor size. Effects on body weight were slight and transitory.

c) β-Meta mustard: Intermediate effects on tumor size were inappreciable and virtually insignificant on body weight.

d) α-Para mustard: Tumor size decreased to a minimum by the 11th day, at which time tumors could not be detected by palpation in four of the

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**TABLE 1**

 EFFECT OF CERTAIN PHENYLALANINE MUSTARDS ON THE S91 MOUSE MELANOMA

<table>
<thead>
<tr>
<th>No. Mice</th>
<th>Injection</th>
<th>Tumor wt. (av.) mg.</th>
<th>Animals sacrificed</th>
<th>Body wt. (av.) gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected</td>
<td>Survived</td>
<td>Days after implantation: dosage*</td>
<td>Substance†</td>
<td>Days after 1st inject.</td>
</tr>
<tr>
<td>0</td>
<td>9</td>
<td>18(8), 36(8)</td>
<td>a-meta</td>
<td>35</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>17(50), 40(55)</td>
<td>β-meta</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>21(10), 44(5)</td>
<td>β-para</td>
<td>470</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>14(25)</td>
<td>a-para</td>
<td>3500</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>15(10), 22(8)</td>
<td>Peanut oil</td>
<td>70</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>14(70), 25(55)</td>
<td>β-para</td>
<td>770</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>15(10), 22(8)</td>
<td>a-para</td>
<td>720</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate dosage (mg/kg body weight).
‡ When first injected.
§ Numbers in parentheses indicate days after first injection when minimum weight was reached.
mice and were very small (2–3 mm.) in the others. By the 22d day tumors could be detected in all the mice. The second injection of the mustard had no noticeable effect. Body weight decreased by a maximum of 5 per cent (5th day).

The findings recorded in Table 1 were confirmed in all essential respects in a second experiment which is entered as the lower half of Table 1. Note that the β-meta mustard was unavailable at the time and could not be used; the interval between the first and second injections was also shortened, and the β-para mustard was used at higher dosage levels than before.

These observations confirm the findings of Gram et al. (3), who reported that the α-meta mustard "shows marked inhibition of the Cloudman S-91 transplanted melanoma in preliminary trials, being about 10 times as active as the L-isomer of p-phenylalanine mustard. . . ." Preliminary observations reported by Osdene et al. (7) are also confirmed.

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REFERENCES

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