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# A NEW METABOLIC REACTION OF NITROGEN MUSTARDS

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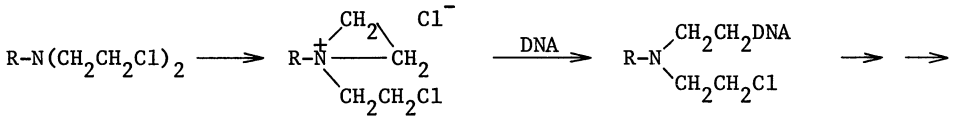
We studied the substituent effect on the metabolism of ortho-, meta- and para-alkoxyaniline mustards. Surprisingly, very high yields of N-chloroethylaniline derivatives from the dechloroethylation reaction of para-alkoxyaniline mustards were obtained. N-2-Chloroethylaniline derivatives converted spontaneously into aziridines in microsomal solution.

The order of the reaction rate of mustards in microsomal solution had a good parallel relationship with that of the antitumor activity of corresponding aniline mustards.

Nitrogen mustards are known as active alkylating reagents. In studies of their biological character in vivo, the electrophilic reaction of aziridinium ion 1, which is the first intermediate, with DNA or protein has been considered to play an important part in their antitumor activity.[1]

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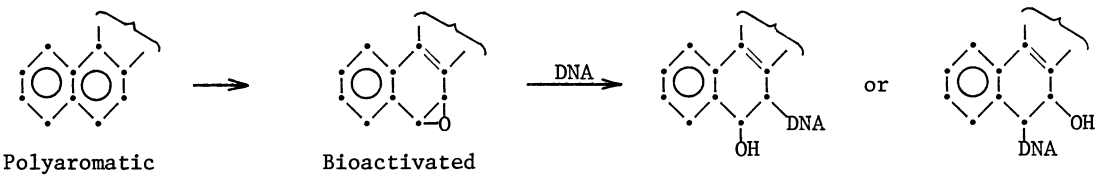
This simple concept is very fascinating and supported extensively by many biochemistry students. However, recent investigations into the mechanisms of the carcinogenic effect of polyaromatic hydrocarbons have revealed that the alkylation reaction by the arenoxide, which was thought to be a bioactivated intermediate, is clearly unrelated to the carcinogenic activity of the original aromatic compound.[2] Thus, we doubt that the alkylation reaction on DNA is an essential mechanism for the biological activity of nitrogen mustards.




Polyaromatic hydrocarbon — Bioactivated intermediate

Previously, T.A. Connors and his coworkers reported the formation of a dechloroethylation product in a study of the conversion of aniline mustard into glucuronide.[3] Although it was only a minor product of the metabolism of aniline mustard, we considered the formation of N-2-chloroethylaniline essential for elucidating the mechanism of the antitumor activity of nitrogen

mustards in connection with our fragmentation reaction of aziridines.[4] Thus, we attempted a study of the substituent effect on the metabolism of aniline mustards under the reaction conditions which had caused a fragmentation reaction of aziridines.[4]

The metabolic reactions of nitrogen mustards with liver microsomes were carried out in 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM KCl, 10 mM $MgCl_2$, 1 mM nicotinamide, 1 mM pyrophosphate, 30 mg protein-equivalent microsomes, 0.01 mM of nitrogen mustards and an NADPH-generating system (0.9 mM NADP, 10 mM glucose-6-phosphate and 2.5 units of glucose-6-phosphate dehydrogenase) in a final volume of 5 ml. Liver microsomes were isolated by generally employed differential centrifugation[5] from normal male rats and used as the enzyme source. Unless otherwise stated, incubation was carried out aerobically at 36°C for 60 min with moderate shaking. After the reaction period was over, 2N NaOH solution was added and the mixture was extracted with an appropriate amount of $CH_2Cl_2$ for vpc or HLC analysis.

Table 1 shows that N-2-chloroethyl-p-anisidine was formed when the reaction mixture contained every component required for the microsomal drug-metabolizing reaction[6] and lack of microsomes, NADPH-generating system, or molecular oxygen gave only the starting material. This clearly indicates that the dechloroethyl reaction of nitrogen mustard is enzymatic and presumed also to be a monooxygenase-type one.[6]

Table 1. Formation of N-2-chloroethyl-p-anisidine from N,N'-bis(2-chloroethyl)-p-anisidine in rat liver microsomes (37°C, 60 min incubation)

| Expt. no* | Reaction mixture | Gas phase | Product formation %† |
|---|---|---|---|
| I, II | Complete system | Air | 1.5-2.5 |
| III | Minus NADPH generating system | Air | No |
| IV | Minus microsomes | Air | No |
| V | Complete system | Argon | No |

* Experiments II and V were performed in Thunberg tubes. The complete system contained all components required for microsomal drug metabolism as described in the text. The concentration of the nitrogen mustard substrate was 2 mM.

† Corresponded to the yield of Table 2.

Table 2 shows the results of enzymatic reaction of several nitrogen mustards. Surprisingly, we found a very high yield of N-chloroethyl anilines in compounds 3 and 5 of run II. In these experiments, 5.5% and 2.3% of the starting materials were consumed and 2.5% and 1.4% of products were formed, respectively, indicating that 45.5% and 60.9% of consumed starting materials were detected respectively as the corresponding metabolites. These may be minimum values because the monochloroethyl derivatives produced by the enzymatic reactions disappeared gradually from the reaction mixture due to other chemical and metabolic reactions.[7]

Although the yield depends heavily on the character of the microsomes prepared, this large product yield strongly suggests that the dechloroethyl reaction is a main metabolic pathway under suitable conditions for nitrogen mustards.

Table 2. Reaction of nitrogen mustards in the complete reaction system* (37°C)

| No. | Compd. | Run† | Incubation time (min) | X-PhNHCH$_2$CH$_2$Cl (%) or corresponding dealkylation product yield‡ | Recovery of starting‡ material (%) |
|---|---|---|---|---|---|
| 1 | (o-CH$_3$O)PhN(CH$_2$CH$_2$Cl)$_2$ | | | | |
| | | II | 60 | 0 | Quantitative |
| | | III | 10 | 0 | 97.6 |
| | | III | 60 | 0 | 90.6 |
| 2 | (m-CH$_3$O)PhN(CH$_2$CH$_2$Cl)$_2$ | | | | |
| | | II | 15 | 0 | 93.2 |
| | | II | 60 | 1.2 | 95.0 |
| | | III | 10 | 0.27 | 90.6 |
| | | III | 60 | 0.23 | 89.0 |
| 3 | (p-CH$_3$O)PhN(CH$_2$CH$_2$Cl)$_2$ | | | | |
| | | I | 60 | 1.5 | 80.2 |
| | | II | 60 | 2.5 | 94.5 |
| | | III | 10 | 1.9 | 88.5 |
| | | III | 60 | 2.2 | 79.6 |
| 4 | (m-PhCH$_2$O)PhN(CH$_2$CH$_2$Cl)$_2$ | | | | |
| | | II | 60 | 0 | Quantitative |
| | | III | 10 | 0 | Quantitative |
| | | III | 60 | 0 | Quantitative |
| 5 | (p-Ph-CH$_2$O)PhN(CH$_2$CH$_2$Cl)$_2$ | | | | |
| | | II | 60 | 1.4 | 97.7 |
| | | III | 10 | 0.4 | 95.2 |
| | | III | 60 | 0.81 | 91.2 |
| 6 | (p-Cl)PhN(CH$_2$CH$_2$Cl)$_2$ | | | | |
| | | I | 60 | 3.3 | 85.4 |
| 7 | (p-CH$_3$O)PhN(CH$_2$CH$_2$CH$_2$Cl)$_2$ | | | | |
| | | IV | 60 | approx 4 | 83.5 |
| 8 | PhN(CH$_2$CH$_2$Cl) (CH$_2$CH$_3$) | | | | |
| | | IV | 60 | 0 | Quantitative |
| 9 | PhN(CH$_2$CH$_3$)$_2$ | | | | |
| | | IV | 60 | 0 | Quantitative |

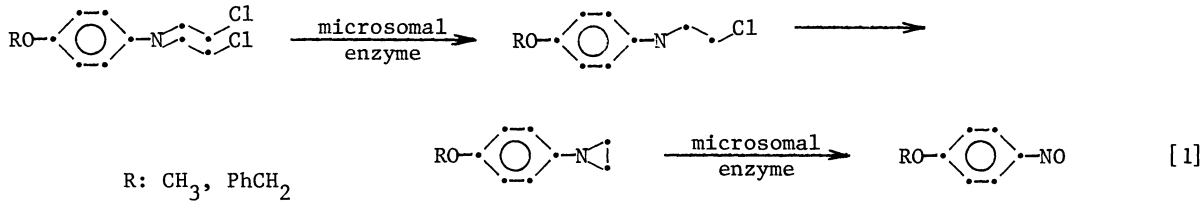
* The reactions were carried out under the conditions described for the complete system in Table 1.

† The microsomes were prepared independently for each run from normal Wistar strain male rats, weighing about 300 g, by the differential centrifugation method.

‡ These values were calculated on the basis of the amount of mustard recovered from the experiment without the NAPDH-generating system in the initial mixture.

The order of the reaction rate of mustards in microsomal solution was p-$CH_3O$ > m-$CH_3O$ > o-$CH_3O$, p-benzyl-O >> m-benzyl-O. This order had a good parallel relationship with that of the antitumor activity of the corresponding nitrogen mustards, even it was not quantitatively.[8,9]

In a solution of the complete system, the 2-chloroethylamino group was converted spontaneously into an aziridine ring. Thus, together with the results of the metabolic reaction of aziridine derivatives observed previously,[4] we propose that reaction sequence [1] is probably a main metabolic pathway of nitrogen mustards in vivo.




[1]

N,N-bis-(3-chloropropyl)para-anisidine 7, which has a longer alkyl chain than aniline mustard, also formed an N-monochloropropyl anisidine. However, substitution of the chlorine atom with hydrogen, i.e., 8 and 9 in Table 2, did not give the dechloroethylation or deethylation products and the starting material was recovered quantitatively. These results indicate that the metabolic reaction requires two chlorine atoms and a nitrogen atom in the molecule. The dechloroethyl reaction may proceed with the formation of N-oxide as an initial intermediate as reported previously.[10,11] Further details of the reaction mechanism are under study in our laboratory.

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