NITROGEN MUSTARD

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Introduction

The nitrogen mustards are closely related chemically to the mustard gas notorious in chemical warfare. The former differs from its military ancestor only in that the sulphur of the latter is replaced by a nitrogen-containing group. Sulphur mustard becomes nitrogen mustard, thus :

History

Knowledge of the effects produced by bis-dichlorodiethylsulphide (mustard gas) in living tissues dates from World War I. Lynch *et al.* in 1918 realized that the intense toxicity of mustard gas was by virtue of its high lipoid solubility to enter rapidly living cells, there to interfere with intracellular processes.

In 1919 A. B. and Helen Krumbhaar demonstrated the toxic depression of bone marrow following exposure to mustard gas.

The first intimation of any anticarcinoma activity appears to have been made by Berenblum, who in 1929 found that mustard gas could prevent the development of neoplastic warts on the skin of animals treated with a highly carcinogenic tar. Berenblum also showed that this action was due neither to any local hyperaemia nor to any chemical interactions with the tar itself. Later he showed that the inhibitory effect of the mustard was strictly limited in extent and duration.

Pharmacology of the Nitrogen Mustards

At the time of World War I it was thought by Lynch *et al.* that mustard gas was hydrolysed in the tissues to HCl and dihydroxyethylsulphide and that it was the HCl which played the vital intracellular role. Evidence now available renders this view untenable (R. A. Peters, 1923). Berenblum

et al. in 1936 (I. Berenblum, 1936) found that mustard gas depressed the glycolysis of minced tumour tissue. The significance of this finding is tempered by their report that Ethylene-bis-b-chloroethylsulphide which also inhibits carcinogenesis possess no selective action on glycolysis.

The introduction of radio-active isotopes for research purposes, made possible a great advance in determining the fate of the mustards in living tissue. Most of this work and, in fact, all research work on the mustards which was in progress at the outbreak of World War II, was intensified and at once came under strict secrecy. It was not until 1946 that papers concerning the mustards began to be published once again. Much of the work that had been carried out over the intervening war years was reviewed by Gilman and Phillips in April, 1946, and by Haddow in 1947. Details of the biochemistry and pharmacology of the mustards were published by Banks *et al.* in a series of papers in 1946. In this series it is stated that as early as 1940 Berenblum reported confidentially that mustard gas reacts with and precipitates nucleoprotein. Regarding the fate of intravenously injected mustard gas (given in 5 mgm. per kilo body weight doses) it was found when using radio-active isotopes as tracers that the greater part of the material was fixed in the tissues, but that there were large differences between the different tissues. Thus the greatest amounts were fixed by the kidneys, by the lung and by the liver. This is most likely due to their excretory functions. To find that a tissue as susceptible as bone marrow should fix the lowest content of mustard gas; while two organs practically undamaged, namely, kidney and lung, retained the highest content, was a surprise. Yet another unexpected finding was that all the mustard gas had disappeared from the tissues after 12 hours; this renders unlikely (according to Banks *et al.*) though not impossible, the fixation of mustard gas by intracellular proteins. These observers later found other unlooked-for results. When the non-vesicant sulfoxide compound of mustard gas was given intravenously in a similar manner to the vesicant, it was found that even more of the sulfoxide was fixed in the tissue than when mustard gas was used. These large amounts of the sulfoxide compound pro-

duced no ill effects or histological damage. With yet larger doses they were able to produce systemic effects like those of mustard gas with damage to bone marrow, due possibly to conversion of the sulphoxide into a more reactive substance. The similarity of distribution of the two substances suggests a similar kind of reaction between them and the tissue, and that the same substance is responsible for fixation of both. Yet with sulphoxide mustard no damage results from an amount of fixation which in the case of mustard gas wrecks the bone marrow. This leads to the conclusion that the reaction by which the substance is fixed in the tissue is not, in the main, that which causes the damage.

The above facts can be explained by the hypothesis contained in the paper by Dixon and Needham. If it is assumed that damage results from the poisoning of an essential tissue constituent E and the tissue also contains varying amounts of a second substance N which is non-essential, but which has a higher competition factor for the mustards than E, then in those tissues which contain large amounts of N, a larger amount of mustard will be fixed, but at the same time it will be diverted from E, which will therefore be protected and the tissue spared. On the other hand a tissue containing but little N (such perhaps as marrow) will fix little mustard; that which is fixed, however, will be by combination with E and the tissue will be destroyed.

Action on Mitosis

The effects produced by the mustards on the mechanism of mitosis are of fundamental importance when viewed from the prospect of ultimate anticarcinoma activity.

The most striking of the many observations reported is that of C. Auerbach *et al.* who found a high rate of chromosome mutations in the fly, *Drosophila melanogaster*, after its exposure to the vapour of mustard gas. Chromosome breaks and spatial rearrangement of genes were also seen. An induced specific instability of the gene arrangement seemed to be transmitted from one generation to the next. This property of inducing mutation is not a function of the vesicant nature of mustard gas. Lewisite, a powerful vesicant, gave entirely negative results in mutation tests. The chemical mutagens were found to possess an unsaturated :S or :N, a type of structure associated with a tendency to intramolecular cyclization to form onium compounds (Gilman and Phillips) characteristic of the N or S mustards.

The only other agent known to produce a comparable effect is short-wave radiations. There are many other reports of the inhibitions of mitotic activity and cell proliferation in a variety of cells

from representative organisms throughout the animal and plant kingdoms.

Chemical Reactions of the Mustards

The nitrogen mustards in the form of their HCl salts are water-soluble crystalline compounds which can be readily prepared in sterile saline for intravenous use.

The basic chemical reaction which the mustards undergo is that of intramolecular cyclization in a polar solvent to form a cyclic onium cation with liberation of Cl⁻. In the case of nitrogen mustards it is ethylenimonium. This imonium cation reacts readily with anion and various uncharged nucleophilic molecules. On the great reactivity of the imonium cations does the action of the mustards on living tissue largely depend. Whether the mustards block enzyme systems or combine with certain essential amino acids is not yet settled. There is evidence that both these types of reactions are possible. (Peters, Kinsey and Grant.)

Use of Nitrogen Mustards in Neoplastic Disease

For therapeutic use the recommended dosage is 0.1 mgm. per kilo body weight given intravenously on four successive days. The upper limit for the total course should not exceed 3.0 mgm. The methyl-bis (b chloroethyl) amine is preferable to the methyl-tris (b chloroethyl) amine as the former seems less likely to cause venous thrombosis at the site of injection. (Rhoads, C.P., 1946.) In June, 1946, a statement by the Committee on Growth of the National Research Council, New York, appeared. The results of treatment in 160 patients were presented and Hodgkin's disease was stated to respond more favourably than any other neoplastic process. Goodman *et al.* (Goodman *et al.*, 1946) in a series of 67 patients were unable to detect any difference between the bis and the tris compounds. Nearly all their patients were in the terminal stages of disease, but good results were observed particularly in Hodgkin's disease, lymphosarcoma, and to a less extent, in chronic leukaemia. Radio-resistant cases responded to treatment and some regained their radio-sensitivity. Jacobson reported (Jacobson *et al.*, 1946) 59 patients treated with nitrogen mustard of which 27 were cases of Hodgkin's disease. The longest follow up period was 33 months, although 17 of the 27 cases were followed for one year or over; six of the 27 died and one was untraced. The remaining 20 cases all showed improvement; in some cases this was dramatic. In lymphosarcoma, remissions up to 18 months were recorded. One case each of acute lymphatic and acute myelogenous leukaemia failed to respond and died. Five cases of polycythaemia were

Fig. 1. Case (1). 25.3.47

Fig. 2. Case (1). 6.8.47

Fig. 3. (Case 6). 14.1.47.

Fig. 4. Case (6). 21.1.47

treated with encouraging results and remissions of over six months being produced.

In this country AptThomas and Cullumbine (AptThomas and Cullumbine, 1947) noted 'striking improvement in the patients' general condition' when they treated 25 patients with advanced neoplastic disease; of these, 21 were cases of Hodgkin's disease. Many, however, required more than one course of treatment to deal with recurrences. They compared their results with the response obtained with x-rays and found that nitrogen mustards produced a more rapid regression of tumour masses, but that recurrences occurred earlier than when x-rays were used.

Toxic Reactions to the Nitrogen Mustards

Apart from the risk of venous thrombosis at the point of injection and of acute perivenous cellulitis if any leakage occurs, the toxic reactions involve also the gastrointestinal tract. Here there is troublesome but transient nausea and vomiting occurring a few hours after administration.

It is not surprising from what has been said already that the most serious toxic reactions are those resulting from the depression of the haemopoietic and lymphatic systems. Most observers agree that leucopenia involving the polymorphonuclear leucocytes is the most marked result of treatment and is apparent within 3—21 days. It appears that the marrow can recover from depression almost amounting to agranulocytosis, providing the administration of mustard is ceased. Lymphocytes are less affected and may show a temporary increase; haemoglobin is at times reduced but not severely so.

In some of the present author's cases a marked rise in polymorphonuclear leucocytes occurred. The reason is not clear, but may possibly be due to the severe necrotic changes occurring within the tumour tissue serving as a stimulus to leucocytosis. (See case (1) where 48 hours after the first dose of mustard W.B.C.s had risen from 12,000, 84 per cent. polymorphs to 30,000, 92 per cent. polymorphs; also case (2).)

Using rabbits, G. R. Cameron *et al.* were able to detect changes in splenic auto grafts which had been transplanted to the anterior chamber of the eye for ease of observation. These changes could be seen within two to three hours. They also noted a rise in the polymorphs in the peripheral blood in the first 24 hours followed then by a neutropenia.

Cases Illustrating the Results Obtained from Nitrogen Mustard

The following cases have been treated by the

writer. It is hoped to publish full details of the series at a later date.

- (1.) Mrs. P. Hodgkin's disease—radio-resistant when seen with enlarged cervical, axillary and mediastinal glands. Good response to first course, but recurrence of symptoms from cervical and axillary glands in three months. Second course of treatment with good results. Mediastinal mass has not recurred. To date, ten months after first course, patient is still improved. See Figs. 1 and 2 of mediastinal mass.
- (2.) R.W. Acute lymphatic leukaemia in a boy of 14 years. No response; died.
- (3.) Mrs. M. Carcinoma of gall bladder with severe pain. Pain was relieved and the abdominal mass became smaller, but patient died one month after completion of course.
- (4.) Mr. J. Carcinoma of bronchus, with obstruction of superior vena cava, enlarged cervical glands and recently intractable pain down left side of neck and arm from nerve root involvement. Generalized pruritis also present. This patient had already received two courses of x-ray therapy. He received a course of nitrogen mustard which caused transient nausea and vomiting six hours after each dose. Following the course of mustard pain was markedly decreased and he was able to do without morphia. Pruritis ceased completely and appetite returned. Glands in the neck decreased in size and he became tolerably comfortable. Six weeks later he became comatose and died within 24 hours.
- (5.) Mr. P. Malignant melanoma with innumerable secondaries throughout skin and subcutaneous tissues. No response.
- (6.) Mr. F. Carcinoma of bronchus with superior vena caval obstruction of gradual onset over three weeks (Fig. 3). Nitrogen mustard commenced on 14/1/47 and repeated daily for five days. On 20/1/47 marked improvement in general condition owing to removal of obstruction to superior vena cava (Fig. 4) and patient was able to sleep comfortably, but on 30/1/47 signs of obstruction returned and death occurred on 4/2/47. Histology—oat cell carcinoma of bronchus.
- (7.) Mr. V. Carcinoma of bronchus. Three-month history of cough with dyspnoea. Examination showed evidence of obstruction to superior vena cava and glands in the neck and both axillae. Collapse of right upper lobe. Biopsy confirmed oat cell carcinoma. Nitrogen mustard commenced on 29/7/47 and given for six days, to a total of 31.2 mgm. Nausea and vomiting after first three and last injections only.

On 8/8/47 pain was less. Dyspnoea markedly diminished but physical signs unchanged.

12/8/47. Signs of obstruction less; improved air entry to right upper lobe. No dyspnoea and appetite returned.

25/8/47. Signs of obstruction returning as before.

26/8/47. Died.

- (8.) Mr. P. Polycythaemia vera with haemoglobin varying from 120—170 per cent. Red blood corpuscles 8—9,000,000 in spite of repeated courses of phenylhydrazine, venesection and radiotherapy.

On 11/8/47. Haemoglobin 170 per cent. plus red blood corpuscles 8.9 million. W.B.C.s 20.4 thousand, 75 per cent. polymorphs.

On 17/8/47 a course of nitrogen mustard 6.9 mgm. daily for five days was given—nausea and vomiting was slight.

30/8/47. Haemoglobin 154 per cent., red blood corpuscles 8.9 million.

3/9/47. Felt better; no headaches.

6/9/47. Haemoglobin 120 per cent., W.B.C.s 9,200.

He remained well and on 28/10/47 haemoglobin 124 per cent., but when seen on 3/12/47 it had risen to 138 per cent.

This is the first time this patient has responded to any kind of treatment.

- (9.) Mr. S. Adenocarcinoma of anaplastic nature with multiple secondaries in skin, subcutaneous tissues, liver, pancreas, thyroid, lung and lymph glands. Primary origin unknown. Received two courses of nitrogen mustard of 20.4 mgm. each, second course one month after the first. Nausea and vomiting occurred after first few injections of each course. W.B.C.s decreased from 8,000 to 3,000, ten days after first course, but not significantly after the second. Died two weeks after completion of second course of mustard. Site of primary not discovered at post mortem.

- (10.) Mr. D. ~~Carcinoma~~ of bronchus. Two months' history of cough with sputum followed by development of superior vena caval obstruction from a carcinoma of the right upper lobe bronchus. He received nitrogen mustard 22.4 mgm. over four days with no improvement and died 12 days later.

Conclusion

It is probable that nitrogen mustard is but a stepping stone in the chemotherapy of cancer. It is neither a cure for cancer nor is its use sufficiently free from risk to render it likely that it will become a widely-used means of therapy. Its real importance lies in its mode of action. That these substances should resemble short-wave radiation in their effects on living cells may point to the fact that they interfere with some fundamental process within the cells. Their mode of action is not fully understood but it is probable that they carry into the cells amounts of energy which are large in proportion to the mass of the agent.

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