STUDIES ON THE MODE OF ACTION OF THE ANTITUMOUR DRUG NITROGEN MUSTARD (MUSTINE)

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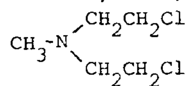
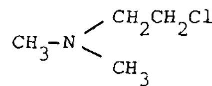
It is generally considered that the mechanism of action of antitumour difuntional alkylating agents, such as nitrogen mustard (I), involves covalent reaction with and cross-linkage of cellular DNA, an event which prevents cell replication (Connors, 1971). This hypothesis may not be able to fully explain the antitumour effects of this class of agent and other or additional targets have been suggested (Wheeler, 1967). We have investigated the mechanism of inhibition of tumour cell replication caused by nitrogen mustard by a study of its effect on cell membrane enzymeswhich control the transport of ions. Changes in ion flux, particularly those brought about by the action of $Na^{+}K^{+}$ ATPase and Mg^{2+} ATPase, are considered to regulate cell division (Sanui and Rubin, 1978; Dornand et al 1978) and we considered that the cytotoxic action of nitrogen mustard may be explained if it inhibited these enzymes. ATPases are sensitive to alkylating agents (Skou 1963), are sub-unit enzymes and so may be cross-linked (Sweadner, 1977), and have elevated activity in transformed cells when compared to normal cells (Kasorov and Friedman, 1974).



(I)



(II)

Studies were made on the ADJ/PC6 plasma tumour, a tumour sensitive to alkylating agents. The effects of nitrogen mustard on the ATPase activity of both whole cells and a membrane fraction was determined and these effects correlated with an estimate of cytotoxicity using a bioassay system. $Na^{+}K^{+}$ ATPase (E.C.3.6.1.3), Mg^{2+} ATPase, external Ca^{2+} ATPase and p-nitrophenolphosphatase (E.C.3.6.3.1) activities were characterised in the crude cell membrane preparation. Nitrogen mustard inhibited 90% of $Na^{+}K^{+}$ ATPase at 9×10^{-15}M and Mg^{2+} ATPase at 7×10^{-15}M in a time dependant manner but had less effect on either the external Ca^{2+} ATPase or p-nitrophenolphosphatase activities (ID_{90}'s > 10^{-4}M). A monofunctional analogue (II) of nitrogen mustard which has no antitumour effect and which cannot cross-link, had ID_{90} of 8×10^{-6}M on $Na^{+}K^{+}$ ATPase and of 5×10^{-6}M on Mg^{2+} ATPase.

The effect of nitrogen mustard on whole PC6 cells was determined by estimating the ability of cells given a hypotonic shock to then reduce their cell volume (as measured by a Coulter Counter Channelyser), a process dependent upon the activity of $Na^{+}K^{+}$ ATPase (Shank and Smith, 1976). Nitrogen mustard (10^{-5}M) completely inhibited the recovery from hypotonic shock and this concentration corresponds to that which, under identical incubation conditions, caused >90% cell kill in the bioassay. The monofunctional analogue (II) was inactive at 10^{-3}M

It is concluded that the inhibition of cell membrane ATPases by nitrogen mustard may be an important facet of its mechanism of antitumour action.

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