Hypothesis

ALKYLATION OF COENZYMES AND THE ACUTE EFFECTS OF ALKYLATING HEPATOTOXINS

R. SCHOENTAL

Department of Pathology, Royal Veterinary College, Royal College Street, London, NW1 OTU, UK

Received 21 October 1975

Alkylating agents comprise a miscellaneous group of substances, which are able to release alkylating entities either at the site of application, or in the course of their metabolism in the liver and in other organs. Alkylating agents came into prominence when some members of this groups were found to possess anti-tumour action, often associated with carcinogenic and mutagenic activity [1,2]. Their interaction with cellular macromolecules, the nucleic acids in particular, have been extensively studied, but little attention has been paid to alkylations of coenzymes. The coenzymes in which vitamins of the B group are essential constituents, though few in number are responsible for the functioning of a great many enzymes, and accordingly are of paramount importance for cellular biochemical processes. In this paper the possibility is discussed that alkylation of coenzymes may be the biochemical basis of the acute and subacute effects of alkylating hepatotoxins.

Hepatotoxic and hepatocarcinogenic compounds (such as pyrrolizidine alkaloids, dialkylnitrosamines, etc.) are likely to undergo activation in the liver by more than one pathway, and to produce various metabolic intermediates, including bifunctional entities, as well as some alkylating species, in the form of free radicals, carbonium ions, etc.

The carcinogenic effects of such substances are probably initiated by the bifunctional metabolites, which being able to crosslink macromolecules in the chromatin could have long lasting consequences [3]. However, the more acute lesions: liver necrosis, fatty infiltration etc. appear to be consistent with inactivation of certain coenzymes and of their component B vitamins by alkylating entities, substituting the reactive C, N or O position in their molecules.

The lesions produced by a single dose of a hepatotoxin depend on its size, route of administration, the time interval after dosing and also on the species, age, sex and the nutritional status of the animal. The livers of animals which die within 2–3 days after an appropriate dose of such hepatotoxins usually present microscopically a composite pattern of changes within the liver lobules: the cells surrounding the portal triads may retain normal appearance, those in the midzonal areas show varying degrees of fatty infiltration and glycogen loss, while round the central veins there are more or less extensive areas of necrosis. Necrotic lesions appear to correlate with depletion of NAD coenzymes, while the fatty lesions may be the result of deprivation mainly of CoA and folates. Enzymes deprived of their coenzymes are less stable and are more readily inactivated than holoenzymes [4].

Attention has already been drawn to the fact that nicotinamide alkylated at its ring nitrogen to *N*-1-alkylnicotinamide, could not be used for the biosynthesis of NAD coenzymes essential for the electron transport chain and a multitude of enzymic reactions [5]. Decrease of NAD coenzymes is known to occur in the liver of animals given carbon tetrachloride [6], azaserine [7], heliotrine [8], dimethylnitrosamine [9,10], streptozotocin etc. [9,10] and to be due to a decrease of the biosynthesis of these coenzymes. Increased urinary excretion of *N*-1-methylnicotinamide has been reported after azaserine [7], dimethylnitrosamine and several other alkylating agents [12]. Large doses of nicotinamide given at the time of dosing with the alkylating hepatotoxins will prevent the lowering of NAD coenzymes [6,8,13,14] and sometimes protect against liver necrosis [6,15]; the

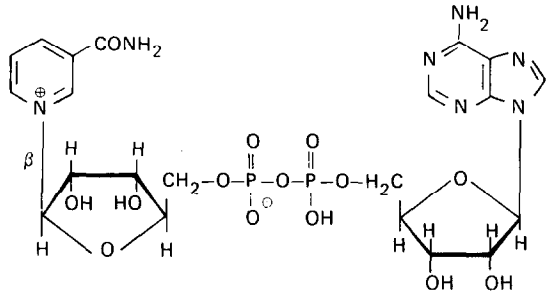

Fig.1. Nicotinamide-adenine dinucleotide coenzyme.

nicotinamide possibly acting as scavenger of the alkyl entities. Nicotinamide treatment did not prevent the development of tumours by streptozotocin [14] or heliotrine [15].

Among the components of NAD coenzymes (fig.1.), the adenine and ribose can be biosynthesised in the body, phosphoric acid moieties are abundant, but the nicotinamide (one of the vitamin B group) has to be supplied from mainly dietary sources, and would be a limiting factor when suddenly it is lost due to alkylation.

The blood entering the portal veins brings to the liver lobules the nutrients absorbed from the gastro-intestinal tract, that contain the B vitamins derived from the diet and from the metabolic activities of the intestinal microorganisms. The B vitamins would be used by the hepatocytes for the biosynthesis of the various cofactors, hence their concentration will decrease gradually as the blood passes towards the central veins. Liver cells in the centrilobular zones being less well supplied with the B vitamins will be more liable to coenzyme depletion, and more vulnerable to alkylations. This may partly explain the frequent occurrence of necrotic lesions in the centrilobular zones by the action of compounds activated in the liver. However, the localization in the liver lobules of the enzymes which activate specific compounds is obviously the determining factor.

Large doses of hepatotoxins cause liver necrosis, but when the doses are appropriately reduced to be compatible with cell survival, fatty infiltration and glycogen loss may occur. Alkylating entities could react with various cell constituents, and would probably do so according to the respective affinities of the receptor groups. Of the latter, end-thiols are

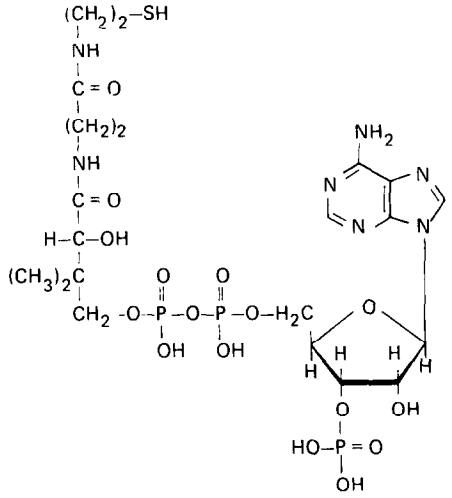

Fig.2. Coenzyme A.

known to have the highest affinities and would be preferentially substituted by alkylating entities (for references see [2]). Among compounds containing end-thiols, CoA, (fig.2) though its concentration in the liver (about 200 mg/kg fresh tissue) is only about 1 : 10 of that of glutathione, is likely to be preferentially alkylated. The thiol group in CoA is very reactive, as evidenced by its formation of thioesters with various metabolic acids. The function of CoA in the metabolism of fats and of carbohydrates, in the acetylation of choline and of aromatic amines, etc., depends on the reversible formation of thioesters [16]. However, on alkylation CoA will form thioethers; these being relatively stable will prevent the formation of thioesters, and thus inhibit the functioning of CoA.

In a careful study of the changes in the liver content of trichloroacetic acid soluble thiols in animals given azo dyes, Dijkstra [17] has shown that an immediate fall is followed by a rise beginning about 4 h and reaches a maximum at about 48 h after a single dose. The soluble thiols have been usually interpreted as representing the reduced glutathione of the liver. The possibility that the thiol-group of CoA might be included in the estimations of soluble thiols has not been considered. It is not unlikely that disregard of CoA might have been responsible for the conflicting results encountered in the course of investigations into the effects of toxic and carcin-

ogenic compounds on the acid soluble thiols [18].

It is of interest that Leaf and Neuberger [19] already in 1947 facing discrepancies in the results obtained using various methods of estimation of liver glutathione in the course of dietary deficiencies, suggested that 'a diet which may produce liver lesions, reduces considerably the glutathione content of the liver and probably also the concentration of *other low molecular sulphur-containing compounds'*. (The italics are mine).

Sulphydryl compounds, especially cysteamine, will protect animals from acute effects of alkylating hepatotoxins and prevent the fatty infiltration and glycogen loss of hepatocytes [20]. However, as in the case of nicotinamide, sulphydryl compounds do not protect from the induction of tumours [21].

Folates, which in conjunction with coenzyme B_{12} are involved in lipid metabolism, are likely to be also affected by alkylations. These cofactors function as carriers of one carbon units, and are involved in the biosynthesis, inter alia, of the lipotropes, choline and methionine. In order to exert this function, folic acid (fig.3) is reduced and via the 5,10-methylene intermediate forms 5-methyltetrahydrofolic acid, polyglutamates, etc. [22,22a]. It seems justified to expect that the alkylating entities released from hepatotoxins, even when they consist of more than one carbon unit, might alkylate folic acid. The resulting derivatives of folic acid, having bulky constituents might not be functional, but if able to function, would lead to the biosynthesis of unphysiological compounds, such as ethionine, or its higher homologues and analogues, analogues of choline, of purines etc. Whether such compounds are actually formed can at present only be surmised; appropriate studies have yet to be made.

Though the detailed mechanism of action of coenzyme B_{12} in intracellular methylations still remains to be elucidated, the role of the cobalt atom bound inside the macrocyclic tetrapyrrol structure

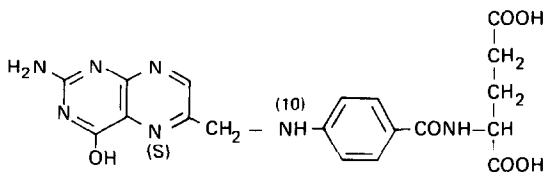

Fig.3. Folic acid.

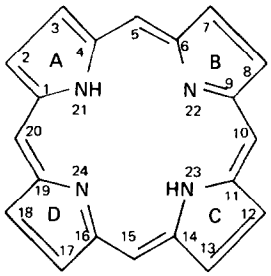

Fig.4. Porphyrin tetrapyrrol macrocycle.

[23] is known to be of paramount importance.

Similarly, the iron in haem in essential for electron or oxygen transport by cytochromes, haemoglobins, catalase, etc. In the absence of the metal atom, bound and coordinated with the nitrogens of the tetrapyrrole (fig.4.) macrocycles, porphyrins (and corrins) could not function. Methylation in vitro of porphyrins leads to mono-, di- and tri-methyl derivatives, in which the methyl groups substitute the pyrrole nitrogens. The methylated porphyrins appear to be able to bind metal atoms, but the biological functioning of such methylated 'pseudo-haems' is not yet known (for references see [23]). It is not unlikely, that in vivo the pyrrole nitrogens could also become alkylated by alkylating hepatotoxins. However, when bulky alkylating moieties substitute nitrogens in the tetrapyrrole macrocycles, these might prevent the insertion of the metal. Such substituted macrocyclic tetrapyrroles would obviously not be functional; they may be excreted and simulate porphyrias (as in the case of ethionine [24]), or form the respective linear tetrapyrrole oxidation products, analogous to bile pigments, and cause jaundice etc. The key role of NAD coenzymes and the interdependence of biochemical reactions is illustrated by the reported prevention of the allylisopropylacetamide induced porphyria by pretreatment with nicotinic acid [25].

In this short paper I restricted myself to a discussion of the possible effects on the liver of alkylations in vivo of cofactors (such as NAD coenzymes, CoA, folates, coenzyme B_{12} and the haems) at their most reactive positions. However, this does not mean that other cofactors may not be alkylated, nor that alkylating entities *in statu nascendi* might not substitute also other positions in the coenzymes. Additional targets for alkylations could be positions which are

involved in the binding of the respective substrates and apoenzymes; their substitution would obviously also impair the functioning of the coenzymes.

Whether and how specific alkylating compounds affect the integrity of coenzymes and of the respective B vitamin-components in relation to liver injury remains to be experimentally investigated. Such data are not yet available (compare e.g. [26,27]).

The new concept presented in this paper, that the biochemical basis of liver injury by alkylating hepatotoxins may, to some extent, be due to alkylation of coenzymes and to the in vivo formation of antivitamins, if proved correct, implies that in certain cases appropriate treatment with B vitamins might prevent the more acute liver lesions. However, the induction of tumours by these agents would not be prevented.

References

[1] Alexander, P. (1954) Adv. Cancer Res. 2, 1–72.
[2] Ross, W. C. J. (1962) Biological Effects of Alkylating Agents, Butterworth, London.
[3] Schoental, R. (1974) Proc. XIth Int. Cancer Congress, Abstracts 2, 55.
[4] Shifrin, S. and Kaplan, N. O. (1960) Adv. Enzymol. 33, 337–415.
[5] Schoental, R. (1975) Biochem. Soc. Trans. 3, 292–294.
[6] Gibb, J. W. and Brody, T. M. (1967) Biochem. Pharmacol. 16, 2047–2049.
[7] Narrod, S. A., Langan, T. A. Jr., Kaplan, N. O. and Goldin, A. (1959) Nature 183, 1674–1675.
[8] Christie, G. S. and Le Page, R. N. (1962) Biochem. J. 84, 202–212.
[9] Schein, P. S., Van Atta, G. and Davis, R. D. (1968) Fed. Proc., 27, 659.
[10] Schein, P. S. and Loftus, S. (1968) Cancer Res. 28, 1501–1506.
[11] Schein, P. S. (1969) Cancer Res. 29, 1226–1232.
[12] Chu, B. C. F. and Lawley, P. D. (1975) Chem-Buol. Interactions, 10, 333–338.
[13] Schein, P. S., Cooney, D. A. and Vernon, M. L. (1967) Cancer Res. 27, 2324–2332.
[14] Rakieten, N., Gordon, B. S., Beatty, A., Cooney, D. A., Davis, R. D. and Schein, P. S. (1971) Proc. Soc. exp. Biol. Med. 137, 283–285.
[15] Schoental, R. (1975) Cancer Res. 35, 2020–2024.
[16] Jaenicke, L. and Lynen, F. (1960) The Enzymes, Vol. 3, part B, 3, 103. Academic Press, New York and London.
[17] Dijkstra, J. (1964) Brit. J. Cancer 18, 608–617.
[18] Harington, J. S. (1967) Adv. Cancer Res. 10, 248–308.
[19] Leaf, G. and Neuberger, A. (1947) Biochem. J. 41, 280–287.
[20] Emmelot, P., Mizrahi, I. J. and Kriek, E. (1962) Nature 193, 1158–1161.
[21] Hirono, I., Laqueur, G. L. and Spatz, M. (1968) J. Nat. Cancer Inst. 40, 1003–1010.
[22] Blakley, R. L. (1969) The biochemistry of folic acid and related pteridines in: Frontiers of Biology, V. 13, North-Holland Publ. Co., Amsterdam.
[22a] Cantoni, G. L. (1975) Ann. Rev. Biochem. 44, 435–451.
[23] Johnson, A. W. (1975) Chem. Soc. Rev. 4, 1–26.
[24] Palma-Carlos, A., Palma-Carlos, L., Gajdos-Torok, M. and Gajdos, A. (1966) Nature 211, 977–978.
[25] Pinelli, A. and Favalli, L. (1972) Europ. J. Pharmacol. 20, 136–138.
[26] Judah, J. D. (1969) Brit. Med. Bull. 25, 274–277.
[27] Farber, E. (1971) Ann. Rev. Pharmacol. 11, 71–96.