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Biochemical Pharmacology, Vol. 18. pp. 538–541. Pergamon Press. 1969. Printed in Great Britain

The time course of covalent binding of [^{14}C]-4-*N*,*N*-di-(2′-chloroethyl) aniline (aniline mustard to mouse liver and kidney nucleic acids and proteins *in vivo*

(*Received* 7 *August* 1967; *accepted* 21 *August* 1968)

4-*N*,*N*-di-(2′-chloroethyl) aniline (aniline mustard, ¦CB1074) inhibits completely the growth of the transplanted ADJ/PC5 myeloma in mice. It causes complete regression of established tumours weighing up to 3g at a dose of 40 mg/kg. Using the tritiated (ring labelled) compound at this dose level the maximum levels of binding to myeloma DNA, RNA and cytoplasmic proteins were 0·25 μm/g, 0·23 μm/g and 0·19 μm/g respectively.[1]

In this communication the level of binding of this alkylating agent to the DNA, ribosomal RNA, and proteins of mouse liver and kidneys at various times after a similar single intraperitoneal injection are reported.

MATERIALS AND METHODS

^{14}C-4-*N*,*N*-di-(2′-chloroethyl) aniline was prepared from ^{14}C-aniline hydrogen sulphate (3 mc) (obtained from the Radiochemical Centre, Buckinghamshire, England), essentially as described before.[2] The specific radioactivity of the product was 2·2 mc/mM.

Groups of five male Bal c$^-$ mice weighing about 25 g were used to determine each point on the curves. The animals were fed rat cake and water *ad libitum*.

The labelled compound was administered intraperitoneally in arachis oil (40 mg/kg; 0·2 ml oil/mouse).

Groups of animals were killed at 2 hr, 5·5 hr, 1 hr, 48 hr, 1 week, 2 weeks and 3 weeks. DNA, ribosomal RNA, nuclear and cytoplasmic proteins were extracted from the pooled organs and purified,

as described before.[3] Radioactive contents were determined using a Packard Tri Carb Liquid Scintillation Spectrometer Model 3003.[3]

At each of the indicated times groups of animals which had been injected with the non-radioactive compound were killed and their livers and kidneys processed for histology and electron microscopy.

RESULTS AND DISCUSSION

The average levels of covalent binding of radioactivity to the DNA of mouse liver and kidney at various times after the administration of carbon-labelled aniline mustard are shown in Fig. 1. The levels of binding to ribosomal RNA are shown in Fig. 2, and to the cytoplasmic proteins in Fig. 3. The levels of binding expressed as μM/g are shown in Table 1.

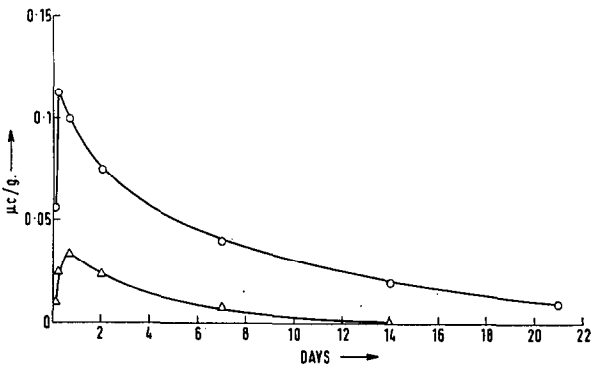

FIG. 1. The binding of radioactivity from ^{14}C-4-*N*,*N*-di(2′-chloroethyl)aniline (specific radioactivity 2·2 mc/mM) to mouse liver and kidney DNA following a single i.p. injection of 40 mg/kg. ○—○ kidney; △—△, liver.

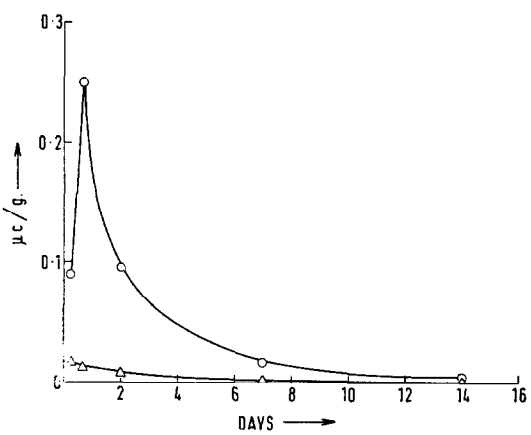

FIG. 2. The binding of radioactivity from ^{14}C-4-*N*,*N*-di(2′-chloroethyl)aniline (specific radioactivity 2·2 mc/mM) to mouse liver and kidney ribosomal RNA following a single i.p. injection of 40 mg/kg. ⊙—⊙, kidney; △—△, liver.

The maximum level of binding to kidney DNA was reached by 5–6 hr, followed by a rapid decrease in the specific radioactivity there being little radioactivity remaining at the end of 3 weeks. The half-life of the bound radioactivity was approximately 3 days.

TABLE 1. THE LEVEL OF BINDING OF RADIOACTIVITY FROM ^{14}C-4-*N*,*N*-di(2′-CHLOROETHYL) ANILINE TO MOUSE LIVER AND KIDNEY DNA, RIBOSOMAL RNA AND CYTOPLASMIC PROTEINS FOLLOWING AN I.P. INJECTION OF 40 mg/kg

Time	Ribosomal RNA (μM/g)		DNA (μM/g)		Cytoplasmic protein (μM/g)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
2 hr	0·009	—	0·005	0·027	0·13	0·16
5·5 hr	0·007	0·04	0·011	0·054	0·11	0·39
16 hr	0·006	0·114	0·027	0·045	0·07	0·22
2 days	0·004	0·05	0·02	0·034	0·038	0·17
7 days	0·001	0·008	0·004	0·018	0·009	0·04
14 days	not detectable	0·001	0·0004	0·009	0·005	0·01
21 days	not detectable	not detectable	not detectabe	0·005	0·002	0·005

The maximum level of binding to liver DNA, which was about one-third that to kidney DNA was reached between 5·5 and 16 hr and again the half-life of the bound radioactivity was approximately 3 days (Fig. 1).

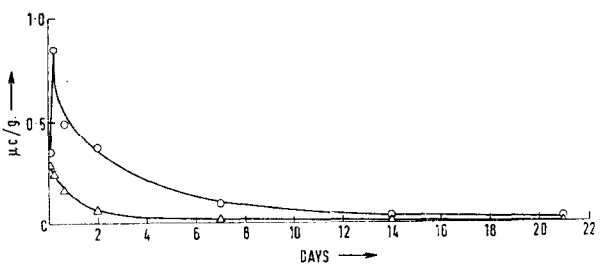

FIG. 3. The binding of radioactivity from ^{14}C-4-*N*,*N*-di(2′-chloroethyl)aniline (specific radioactivity 2·2 mc/mM) to mouse liver and kidney cytoplasmic proteins following a single i.p. injection of 40 mg/kg. ○—○, kidney; △—△, Liver.

The maximum level of binding to kidney ribosomal RNA occurred between 5·5 hr and 16 hr, the half-life of the bound radioactivity being approximately 2 days and the maximum level of binding (0·114 μM/g) was sixteen times greater than that to liver ribosomal RNA (Fig. 2).

Maximum binding to kidney cytoplasmic proteins occurred between 2 hr and 5·5 hr (0·39 μM/g). For liver cytoplasmic proteins maximum binding probably occurred before 2 hr, the maximum level recorded being one-third of that to kidney proteins. The half-life of the bound radioactivity was between 1 and 2 days (Fig. 3).

The histological appearance of the livers and kidneys at all times after administration of the unlabelled compound was nearly normal and there was no evidence of cell death or necrosis as seen following the administration of liver and kidney toxins. Electron microscopy revealed no damage to the liver endoplasmic reticulum and its associated ribosomes or to organelles such as mitochondria. There was no evidence therefore that the compound is a liver or kidney toxin.

In each case the levels of binding to the myeloma tumour[1] were greater than to either liver or kidneys, except for the cytoplasmic proteins in which case the level of drug bound to kidneys was twice that bound to the tumour. The maximum level of binding to myeloma DNA was 0·25 μm/g[1] compared with 0·027 μm/g for liver DNA and 0·054 μm/g for kidney DNA. This large difference between tumour and organ binding could be explained on the basis that aniline mustard is activated as an alkylating agent by metabolism, possibly by conversion to the 4-hydroxy derivative and it has been shown that the myeloma contains high levels of the hydroxylating enzyme.[4]

There is a remarkable similarity in the time course of covalent binding of aniline mustard to liver and kidney DNA with that observed in the case of the carcinogen dimethylnitrosamine which is a methylating agent. Thus Craddock and Magee[5] observed that maximum binding to liver DNA occurred at about 5 hr following a single injection into the rat and that this was followed by a rapid drop in specific radioactivity as observed in the case of aniline mustard. Since dimethylnitrosamine is a liver toxin this decrease in bound radioactivity may have been attributable at least to some extent to cell death, but this is unlikely in the case of aniline mustard which appeared not to damage the liver. This relatively rapid rate of loss of bound radioactive materials from DNA is in contrast to the very slow rate of removal of dye bound to DNA following a single i.p. injection of the tritiated carcinogen dimethylaminoazobenzene (butter yellow) which was still present months after a single administration.[6]

The present results demonstrate that an alkylating agent of the type used clinically is capable of reacting covalently with mouse liver and kidney DNA, ribosomal RNA, and proteins to an extent similar to a known hepatocarcinogen and liver toxin, but without having apparently similar destructive effects on liver morphology.

Acknowledgements—I thank Sir Alexander Haddow, F.R.S. and Professor L. A. Elson for their interest in the work. This work has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by Public Health Service Grant No. CA-03188-10.

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