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Comparative biodistribution of methotrexate and monoclonal antibody-methotrexate complexes in mice

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The biodistribution of radiolabelled methotrexate and immune complexes of methotrexate and a murine monoclonal anti-methotrexate antibody has been compared in mice. Complexes formed in-vitro with the antibody, but not with control immunoglobulin. The complexes were, characteristically, acid labile. In-vivo, blood levels, organ distribution and whole body catabolism of methotrexate in immune complexes were similar to those of free antibody, and markedly different from those of free drug. These findings suggest the feasibility of prolonging the survival of drugs and altering in-vivo distribution using complexes with monoclonal antibodies.

Monoclonal antibody technology has opened the way to the production of defined antibodies against many antigenic structures. Part of this development has been the production of antibodies against chemotherapeutic agents (Kato et al 1984; Zalcberg 1985). Obviously, such antibodies may be useful in drug assay or purification etc., but, in addition, complexing drugs with monoclonal antibodies might allow more prolonged in-vivo survival of drugs otherwise rapidly catabolized, with possible concomitant increase in therapeutic efficacy. In the present study a preliminary evaluation of this concept has been carried out with methotrexate (MTX), a drug widely used in a number of malignant diseases. A monoclonal antibody has been produced to MTX and the biodistribution of MTX and MTX-antibody complexes has been compared in experimental animals.

Materials and methods

The monoclonal antibody was produced, using standard hybridoma techniques, following immunization of BALB/c mice with MTX covalently conjugated to human serum albumin (HSA) (Garnett et al 1985). The antibody (IgG_1 isotype) reacted against MTX-HSA and MTX-thyroglobulin conjugates but not against HSA alone. The antibody was purified from hybridoma culture supernatant by binding to Protein A-Sepharose in phosphate buffered saline (PBS) at pH 8·0 (Ey et al 1978) and eluted using 3 M NaSCN followed by dialysis against PBS at pH 7·2. Purified antibody was labelled with ^{125}I or ^{131}I using an Iodogen method (Fraker & Speck 1978) and unreacted radioiodine removed by Sephadex G-25 gel filtration.

L-Methotrexate (Lederle, Gosport, Hampshire, UK) was labelled with ^{125}I by conjugation to ^{125}I-labelled histamine and separated from free radioiodide and histamine as described by Kamel & Gardner (1978). The labelled preparation is abbreviated here as [^{125}I]MTX.

Complexes of antibody and MTX were formed by incubation at 4 °C for 2 h of mixtures of purified antibody at 90 μg mL^{-1} in PBS (pH 7·2) with [^{125}I]MTX at 0·5 ng mL^{-1}. Unbound [^{125}I]MTX was removed by gel filtration in PBS (pH 7·2) using Sephadex G-25 in pre-packed 5 × 1·5 cm columns (PD-10 columns, Pharmacia, Uppsala, Sweden). Control complexation tests were carried out with an irrelevant mouse IgG_1 monoclonal antibody. Purified complexes formed with the anti-MTX antibody were tested for acid lability by similar gel filtration using pH 3·0 1M citrate phosphate buffer.

Labelled antibody and complexed or free [^{125}I]MTX were injected into groups of BALB/c mice. For whole body survival tests, preparations (approximately 10^5 counts min^{-1} of radioiodine) were injected intraperitoneally in 0·2 mL, and radioactivity in the animals counted immediately and at daily intervals in a 7·5 × 7·5 cm well crystal scintillation counter (John Caunt Scientific, Oxford, UK). For plasma clearance tests, mice were injected intravenously with 0·2 mL of preparations containing approximately 10^7 counts min^{-1} of radioiodine and blood samples (10 μL) taken from the tail vein into microcapillary pipettes (Drummond Microcaps, Drummond Scientific Co, Broomhall, Pa, USA). All mice were subsequently dissected and radioiodine(s) counted in a conventional gamma counter in weighed samples of blood, visceral organs and remaining carcass.

Results

Sephadex G-25 column calibration tests showed that radiolabelled antibody and [^{125}I]MTX were clearly separable (Fig. 1A). Following incubation of anti-MTX antibody with [^{125}I]MTX, part of the ^{125}I eluted subsequently at the position of the antibody (Fig. 1B) indicating that immune complexes had formed. [^{125}I]MTX incubated with irrelevant IgG_1 ran clearly and distinctly from the antibody (Fig. 1C). When [antibody]-[[^{125}I]MTX] complexes, purified by Sephadex G-25 filtration were re-run at pH 3·0, the label of the MTX eluted from the column in the fractions expected to contain free MTX, with no remaining complexes (Fig. 1D).

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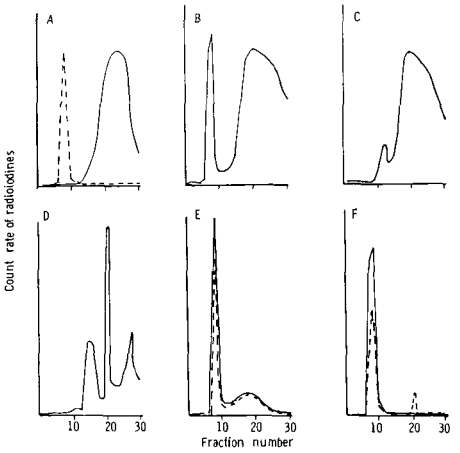

FIG. 1. Sephadex G-25 gel filtration of ^{125}I- or ^{131}I-labelled anti-MTX antibody, [^{125}I]MTX and labelled antibody-[[^{125}I]MTX] complexes. (A) Column calibrations with ^{125}I-labelled antibody (----), and free [^{125}I]MTX (——). The preparations are clearly distinguishable, antibody being excluded from the gel, while labelled MTX is retarded. (B) Elution profile of [^{125}I]MTX after pre-incubation with anti-MTX antibody. Part of the radiolabel is now excluded from the gel running in the position of antibody, i.e. as antibody-antigen complexes. (C) As (B) but using an irrelevant mouse IgG_1 monoclonal antibody, no complex formation is seen. (D) Fraction No. 8 from elution B run in pH 3·0 buffer, radiolabel now runs in the position of free [^{125}I]MTX, i.e. the complexes have been split. (E) Elution profile of complexes formed with ^{131}I-labelled antibody (——) and [^{125}I]MTX (----) (the preparation had been purified beforehand by G-25 gel filtration). The two radiolabels run co-incidentally in the antibody region. (F) as (E), but gel filtration carried out on serum taken from mice 28 h after intravenous injection of dual labelled immune complexes. The major part of both radiolabels run coincidentally. Fraction volume = 0·5 mL.

The whole body survival in mice of radiolabelled uncomplexed antibody was markedly different from that of [^{125}I]MTX (Fig. 2). Mice receiving ^{131}I-antibody complexed with [^{125}I]MTX showed virtually identical whole body survivals of both radiolabels, and these were similar to that with uncomplexed antibody (Fig. 2).

Analysis of the blood survival of radiolabelled preparations showed that [^{125}I]MTX was rapidly cleared from plasma (Fig. 3). [^{125}I]MTX as complexes with anti-methotrexate antibody showed prolonged survival, more typical of that of free antibody (Fig. 3). Organ distribution studies 24 h after injection of labelled preparations showed that blood and tissue levels of ^{131}I and ^{125}I radiolabels from [[^{131}I]antibody]-[[^{125}I]MTX] complexes were similar, with blood and lung tissue having the highest levels of injected material per gram (Fig. 4). In contrast, the radiolabel from free [^{125}I]MTX was concentrated particularly in liver and kidney (Fig. 4). Sephadex G-25 gel filtration of serum from the mice injected with the dual labelled complex showed both radiolabels predominantly co-incident at the antibody elution region of the elution profile (Fig. 1F), similar

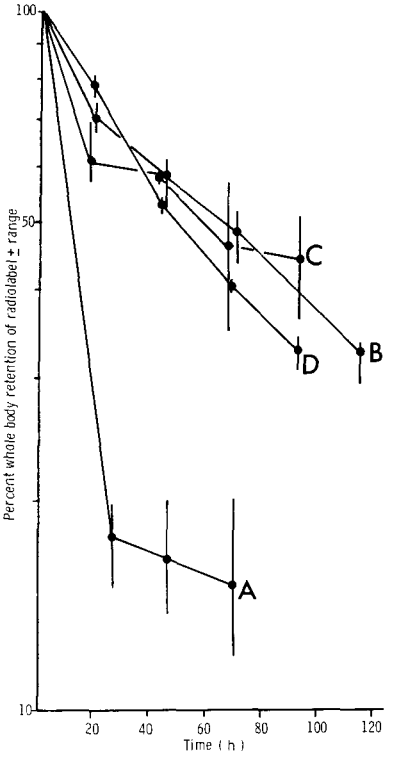

FIG. 2. Whole body retention of radioiodine in mice from (A) radiolabelled MTX (n = 4), (B) ^{125}I-labelled anti-methotrexate monoclonal antibody (n = 3), and (C) ^{125}I and (D) ^{131}I of [[^{131}I]antibody]-[[^{125}I]MTX] complexes (n = 2). The mean and range is shown for each time point.

to that seen with the preparation before injection (Fig. 1E).

Discussion

Altering the biodistribution of drugs, with controlled delivery (Pozansky & Juliano 1984) and possible site-specific targeting (Tomlinson & Davis 1986), is a major pharmaceutical objective. For example, liposomal encapsulation or nanoparticle adsorption have been evaluated but have the disadvantage of the propensity of particles to be cleared by the reticuloendothelial system. Chemical conjugation to carriers such as albumin and site-specific directed antibodies (Tomlinson & Davis 1986; Baldwin et al 1986) is also being extensively investigated but requires chemical conjugation of drug and carrier with possible concomitant loss of the drug's therapeutic activity and alteration in the biodistribution of the carrier. As an alternative, the use of antibody-drug complexes is an attractive possibility, since it requires no chemical conjugation and it may be that the in-vivo behaviour of such complexes will be dictated largely by the antibody rather than the drug. The present study was carried out to examine this possibility with methotrexate and a monoclonal anti-

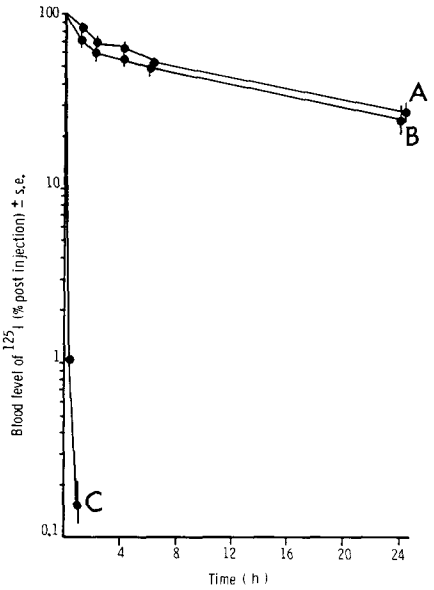

FIG. 3. Blood survival of ^{125}I in mice following intravenous injection of (A) [monoclonal antibody]-[[^{125}I]MTX] complexes, (B) [^{125}I]antibody, (C) [^{125}I]MTX. Mean ± standard error of four mice/group.

body directed against it. Optimization of complex formation and full pharmacological assessment has not been attempted at this stage, the present preliminary study with trace amounts of material being designed only to examine the feasibility of this approach. Nevertheless these present tests have shown that in-vitro-formed immune complexes of MTX and a monoclonal antibody show blood clearance, biodistribution and whole body catabolic rates similar to those of the antibody alone, and which are grossly different from those of the free drug which is known to undergo rapid clearance and excretion/catabolism (Henderson et al 1965).

There seem to be few if any similar studies on the biodistribution of antibody-drug complexes, although Reardon et al (1985) have shown prolongation of survival and altered biodistribution in mice of ^{111}In-labelled benzyl-EDTA with monoclonal antibodies specific for the radiometal-chelate complex. Whether the present findings with MTX will also apply to other drugs, and how this may alter toxicity or therapeutic efficacy, remains to be investigated.

The complexes in the present study distributed in a manner similar to that of antibody, and the antibody has no antigen target in the mouse tissue. As a further development, the use of hybrid, bispecific, monoclonal antibodies against drug and, for example, a tumour-associated antigen may allow site-specific delivery of the drug, and the present studies indicate that, at least with MTX, the biodistribution of this complex will not be markedly perturbed by the complexed drug. Such hybrid antibodies can be produced by reassociation of enzyme-prepared fragments of two antibodies (e.g. Raso & Griffin 1981) or by the hybridization of two hybridomas (e.g. Milstein & Cuello 1983) and the feasibility of target cell killing, at least in-vitro, has been reported with ricin toxin complexed to bispecific antibody (Raso & Griffin 1981). In-vivo, in experimental systems, Corvalan et al (1987) have reported increased therapeutic effects of vinca alkaloids targeted to human tumour xenografts by a hybrid monoclonal antibody to vindesine and carcinoembryonic antigen, although here the antibody was localized first in the tumour and the drug given separately several days later. The indication from the present study is that a bispecific anti-tumour and anti-MTX antibody would specifically deliver the drug to tumour tissue, and this clearly warrants investigation, particularly in comparison with chemical conju-

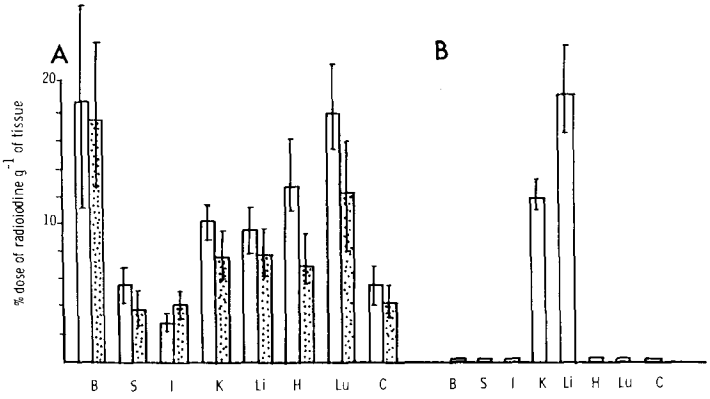

FIG. 4. A. Tissue distribution in mice of ^{131}I (open columns) and ^{125}I (dotted columns) from [[^{131}I]antibody]-[[^{125}I]MTX] complexes. B. Tissue distribution in mice of ^{125}I from [^{125}I]MTX. Mean ± standard error of four mice/group, 24 h after intravenous injection. Key: B, blood; S, spleen; I, intestine; K, kidney; Li, liver; H, heart; Lu, lungs; C, carcass.

gates of MTX and anti-tumour monoclonal antibodies (Baldwin et al 1986).

Overall, these and other related studies (Corvalan et al 1987) indicate that modification of drug biodistribution with monoclonal antibodies and/or tissue specific targeting with complexes formed with hybrid antibodies may be valid approaches to prolongation of drug survival and/or site-specific targeting. The degree of drug loading of monoclonal antibodies, with theoretical maxima of two molecules per molecule with anti-drug antibody and one molecule per molecule of hybrid antibody, might be a limitation, since the amount of antibody required to carry conventional therapeutic doses of drug would be very high. In addition the extent of extravasation of antibody is much smaller than that of drugs. But, as this study shows, the survival of drug is markedly prolonged simply with the anti-drug antibody, and the additional site targeting effect possible with hybrid antibodies might mean the possible use of greatly reduced doses of drug.

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Letter to the Editor

The influence of aromatic substituents on the binding of substituted benzamides to dopamine D-2 receptors: congruent QSAR and MEP analyses

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Substituted benzamides (orthopramides) are a group of atypical neuroleptics principally acting as selective antagonists of dopamine D-2 receptors. Besides the essential carboxamide group in position 1 and alkoxy group in position 2, these compounds exhibit various other ring substituents, mainly in positions 4 and 5. A few years ago, we demonstrated the critical role of adequate aromatic substitution at positions 4 and 5 for binding to the D-2 receptors, analogues of metoclopramide unsubstituted in these positions being fully devoid of in-vitro D-2 receptor affinity (Anker et al 1983).

In an effort to unravel the structure-activity relationships (SAR) of the 4- and 5-substituents, some of us (Testa et al 1986; Van de Waterbeemd et al 1986a,b) have calculated the molecular electrostatic potential (MEP) maps of a number of variously ring-substituted orthopramides using ab initio (STO-3G basis set) molecular orbital (MO) calculations. Consistent results were obtained which led us to propose a stereo-electro-

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