FATE OF 125-I LABELLED ALBUMIN-METHOTREXATE CONJUGATES AFTER INTRAVENOUS ADMINISTRATION IN THE RAT

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Delivery of macromolecular prodrugs to malignant metastases is dependent on their widespread distribution to the interstitial fluids of target tissues. We have reported the clearance and elimination of ^{125}I labeled methotrexate-bovine serum albumin conjugates (^{125}I-BSA-MTX) from the plasma after intravenous (IV) injection into rats (Pape et al, 1989). Here we present further studies of the tissue distribution and metabolic fate of ^{125}I-BSA-MTX as a function of methotrexate (MTX) content.

^{125}I-BSA-MTX prepared as described (Pape et al, 1989) was injected into the tail vein of male Wistar rats (total dose equivalent to 0.2mg BSA-MTX). Ten rats used in each study were bled by cardiac puncture under anaesthesia for assay of plasma concentration of ^{125}I. Representative rats were killed at various times after dosage to allow the tissue distribution of ^{125}I to be determined. Organs of interest were weighed and an appropriate mass used for assay using a gamma counter.

Figure 1 shows the plasma clearance during the first 5h after administration of ^{125}I-BSA and MTX conjugates containing 4.54 and 11.55 %^{w}/w MTX respectively. During this period BSA was distributed widely throughout the body fluids subsequent to elimination over several days. Tissue distribution of ^{125}I after administration of ^{125}I-BSA was representative of blood perfusion and lymph flow in each organ and consistent with the expected passive extravasation of BSA. When ^{125}I-BSA-MTXs were administered ^{125}I was cleared more rapidly from the plasma, the rate and extent of clearance being significantly greater after administration of the high-strength conjugate. Tissue distibution studies indicated that this clearance was associated initially with uptake by the liver over the first 15min. Figure 2 shows liver uptake expressed as total activity in the liver divided by activity in 1ml of plasma. This emphasises the organ uptake of ^{125}I rather than that associated with blood perfusion through the liver. The mean fractions of the total dose present in the liver after 15 min (including the blood pool) were 13.0, 23.7 and 64.3% for ^{125}I-BSA, low and high strength MTX conjugates respectively. Over the next 2h after administration of ^{125}I-BSA-MTXs high concentrations of ^{125}I appeared in the gut, presumably by way of bile, which then declined over a 24h period with little ^{125}I being excreted in the faeces over this period. After 24h tissue distributions of ^{125}I were similar for all treatments and gradually declined over approximately 100h. The nature of the liver processing of ^{125}I-MTX-BSA was not established but was not explained by removal of free ^{125}I since eventual appearance of ^{125}I in the urine was as oligopeptide derivatives. This suggested that the ^{125}I remained peptide-bound after the initial rapid pass through the liver. The dependence of liver uptake on

Figure 1: Plasma Clearance of BSA and the Conjugates

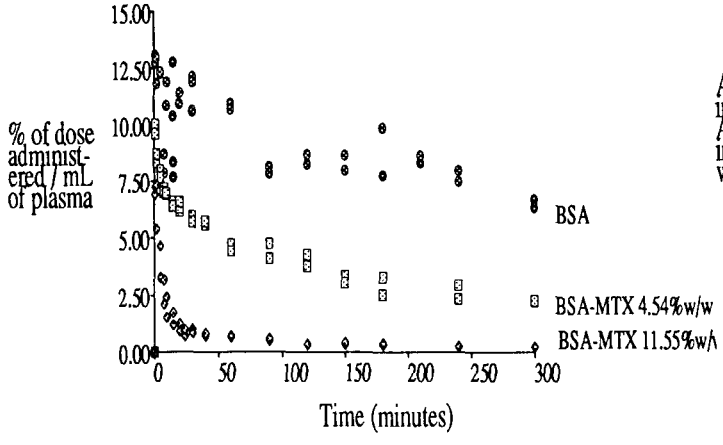
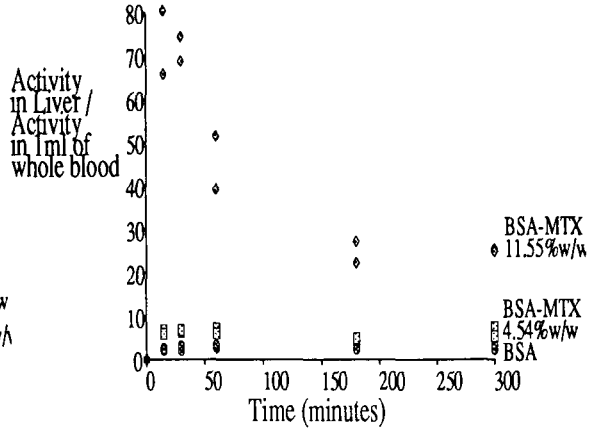


Figure 2: Liver Uptake versus Time



MTX content has clear implications for the design of macromolecular prodrugs of MTX. Whether this is a specific recognition of the folate derivative or a non-specific effect due to increased hydrophobicity is not clear from the current data.

Pape, V.E. et al (1989) J. Pharm. Pharmac. 41:49P