# THIOLYTIC CLEAVAGE AND BINDING OF THE ANTITUMOUR AGENT CI-921 IN BLOOD

P. Kestell*, J.W. Paxton**, I.G.C. Robertson**,
P.C. Evans**, R.A. Dormer** and B.C. Baguley*
**Cancer Research Laboratory, and*
***Department of Pharmacology and*
*Clinical Pharmacology,*
*University of Auckland School of Medicine*
*Auckland, New Zealand*

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*Address for correspondence:* *Dr. Philip Kestell*
*Cancer Research Laboratory*
*University of Auckland School of Medicine*
*Auckland, New Zealand*

*Abbreviations used:* *CI-921: 9-[[2-methoxy-4-[methylsulphonylamino]phenyl]-amino]-N,5-dimethyl-4-acridinecarboxamide; MSA: 4-amino-3-methoxymethanesulphonanilide.*

## SUMMARY

The antitumour agent 9-[[2-methoxy-4-[methylsulphonylamino]-phenyl]amino]-N,5-dimethyl-4-acridinecarboxamide (CI-921; NSC 343499) is currently undergoing clinical evaluation. The plasma disposition of this compound together with its ability to bind to plasma proteins has been investigated in the mouse. Five minutes after intravenous administration of [acridinyl-G-$^3$H]-CI-921 (57.7 mol/kg) to male BDF1 mice, plasma samples were taken and precipitated with acetonitrile. 17% of the total plasma radioactivity was found to be bound to plasma proteins, increasing to 31% by 30 min. To ascertain the mechanism of binding, [acridinyl-G-$^3$H]-CI-921 was incubated at 37°C in mouse blood or plasma and the radioactivity analysed after precipitation with acetonitrile. CI-921 and the cleavage product 4-amino-3-methoxy-methanesulphonanilide (MSA) were detected in the acetonitrile supernatants by HPLC using electrochemical and ultraviolet detection. After incubation for 1 h with blood, extensive association of radioactivity (80% of total) with plasma proteins, together with a rapid decrease in CI-921 concentration and a concomitant increase in MSA concentration, was observed. In blood samples from mice given CI-921, low concentrations (1 to 2 μmol/l) of MSA were detected up to 1 h after injection. The results suggest that *in vivo* at least part of the covalent binding in blood arises from the nucleophilic attack by protein thiols at the C-9 position of the acridine ring resulting in covalent protein adducts and release of MSA.


## I. INTRODUCTION

The antitumour agent CI-921 (NSC 343499), which is the 4-(N-methyl-carboxamide)-5-methyl derivative of the clinical anti-leukaemia drug amsacrine /1/, is currently undergoing clinical evaluation /2/. CI-921 was selected from several other analogues primarily because it had greater activity than amsacrine against solid tumours /3/.

Previous studies investigating the fate of amsacrine in the mouse revealed that it undergoes metabolism in the liver by the microsomal oxygenase system to a reactive quinonediimine derivative which is then conjugated with glutathione in the aniline ring /4,5/. It also

rapidly forms covalent adducts with plasma proteins, both *in vivo* and when incubated with mouse blood *in vitro* at 37°C /6,7/. It has been proposed that these adducts result from the nucleophilic attack by protein thiols at the C-9 position of the acridine ring thus releasing the sidechain, 4-amino-3-methoxy-methanesulphonanilide (MSA). As yet, the metabolic fate of CI-921 has not been established. Since it has been reported for several drugs that the formation of covalent protein adducts with drugs may contribute to toxic effects such as myelosuppression and adverse immune reactions /8,9,10/, the ability of CI-921 to form covalent adducts with plasma proteins was investigated.

## II. MATERIALS AND METHODS

### *2.1* Materials

MSA, unlabelled CI-921 (isethionate salt) and [acridinyl-G-$^3$H]-labelled CI-921 (specific radioactivity 4.09 $\mu$Ci/ mol) were generously supplied by Dr. L. Whitfield of the Parke-Davis Division of Warner-Lambert Company, Michigan, USA. The radiochemical purity of the CI-921 sample was 97% as determined by HPLC and TLC (chloroform:methanol, 4:1 v/v). The impurity was identified as the corresponding acridan-9-one derivative. CI-921 and [acridinyl-G-$^3$H]-CI-921 were formulated as 10 mM solutions in sterile water for administration to mice and for use in *in vitro* studies. All solvents used were of HPLC grade.

### *2.2* In Vivo Studies

Male BDF1 mice (20-25g) were administered [acridinyl-G-$^3$H]-CI-921 (57.7 $\mu$mol/kg) by injection into the tail vein. At least 3 animals per time point were treated and killed 5 min, 30 min, 1 h, 2 h, 3 h, 4 h, and 5 h after injection. Blood was collected in heparinised tubes by ocular extrusion under ether anaesthesia and the plasma separated by centrifugation. Deproteinisation of the plasma proteins was achieved by addition of acetonitrile (90% v/v) and centrifugation. The resulting pellet was washed once with acetonitrile (1 ml); further washes did not extract any further radioactivity. Radioactivity in the

plasma and acetonitrile supernatant was determined by liquid scintillation counting in 10 ml scintillation fluid (Aquasol-2, NEN, USA). The protein pellets were digested at 60°C for 2 h with Protosol (NEN, USA) before their radioactivity was determined.

### *2.3* In Vitro Studies

CI-921 was added to blood collected from male BDF1 mice to give final concentrations of either 60 M ([acridinyl-G-$^3$H]-CI-921) or 20 $\mu$M (unlabelled CI-921) and incubated at 37°C. Aliquots were taken at intervals and treated in the same manner as described in the *in vivo* studies.

### *2.4* Quantitation of CI-921 and MSA in Plasma and Acetonitrile Supernatants

All samples were analysed by a Waters HPLC system consisting of an automatic sample injector WISP 712, 6000A pumps, Z-module fitted with a C18 4 Novapak cartridge, model 400 UV detector and a Waters 840 data control station. The method of Jurlina and Paxton /11/ was employed to determine the concentration of CI-921 in plasma but for the quantitation in acetonitrile supernatants, samples were injected directly on the column and the CI-921 detected electrochemically at a potential of +500 mV (Model 400, EG & G Princeton Applied Research Corp., NJ, USA). The mobile phase for CI-921 was 1 M ammonium acetate (pH 5); acetonitrile; methanol (3:2:2 v/v) at a flow rate of 2 ml/min. For the analysis of MSA in the acetonitrile supernatant, samples were evaporated to dryness using a Speed Vac concentrator (Savant Instruments, NY, USA) and redissolved in mobile phase (1 M ammonium acetate, pH 5 : acetonitrile, 4:1 v/v) before aliquots were injected into the HPLC. The flow rate of the mobile phase was 1.8 ml/min and compounds were detected by UV absorption at 254 nm.

Unlabelled CI-921 or MSA were also added at concentrations of 20 $\mu$M to control mouse plasma. 99% of CI-921 and 80-90% of MSA could be recovered in the acetonitrile supernatants after deproteinisation.

## III. RESULTS

The plasma disposition of [acridinyl-G-$^3$H]-CI-921 in mice is shown in Fig. 1. A significant fraction of the total plasma radioactivity appeared to be covalently bound to protein after drug administration. This fraction was 17% after 5 min, 31% after 30 min and remained relatively constant thereafter. The covalently bound radioactivity was eliminated with a half-life of 3.8 h. The concentration of total plasma radioactivity, expressed as CI-921 equivalents, was 1.5-fold higher than that of unchanged CI-921 at 5 min and declined with a half-life of 2.8 h compared to 1.2 h for unchanged CI-921.

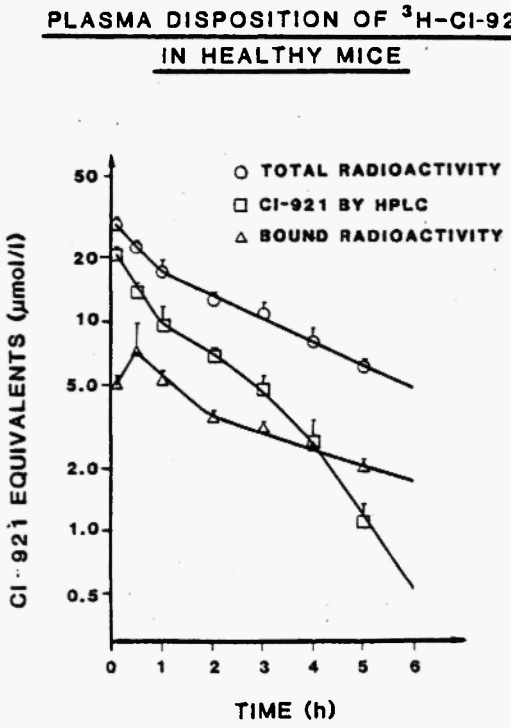


Fig. 1: Plasma disposition of [acridinyl-G-$^3$H]-CI-921 in male BDF1 mice. Values are the means ± SD.

Incubation of [acridinyl-G-$^3$H]-CI-921 with mouse blood at 37°C resulted in extensive binding to plasma proteins (80% of total plasma radioactivity) within 1 h (Fig. 2). In contrast, incubation of

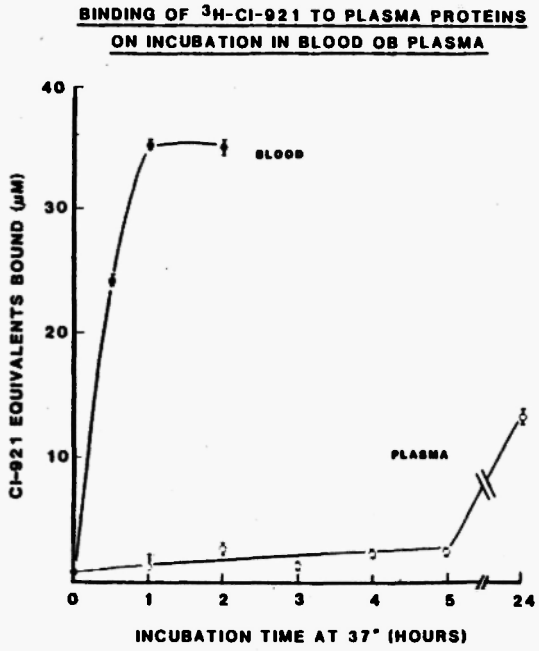


Fig. 2: Binding of [acridinyl-G-³H] to plasma proteins on incubation in blood or plasma. Values are the means of 2 determinations ± SD.

[acridinyl-G-³H]-CI-921 with mouse plasma resulted in little binding within 24 h. HPLC analysis of the plasma acetonitrile supernatants from the mouse blood CI-921 incubation indicated the presence of MSA by co-chromatography with the authentic standard (Fig. 3). Similar chromatograms were obtained upon analysis of the plasma acetonitrile supernatants from mice treated with CI-921. Concentrations of 1 to 2 μmol/l were detected up to 1 h after administration. In addition to MSA, an unknown radioactive compound with a retention time of 11.5 min was detected. This compound did not correspond to the 4-methyl-5-(N-methyl)-carboxamide derivative of either 9-aminoacridine or the acridan-9-one (data not shown). Monitoring of CI-921 and MSA during blood incubations indicated a rapid disappearance of CI-921 with a half-life of 0.6 h with the concomitant appearance of MSA eventually accounting for 57% of the parent compound (Fig. 4).

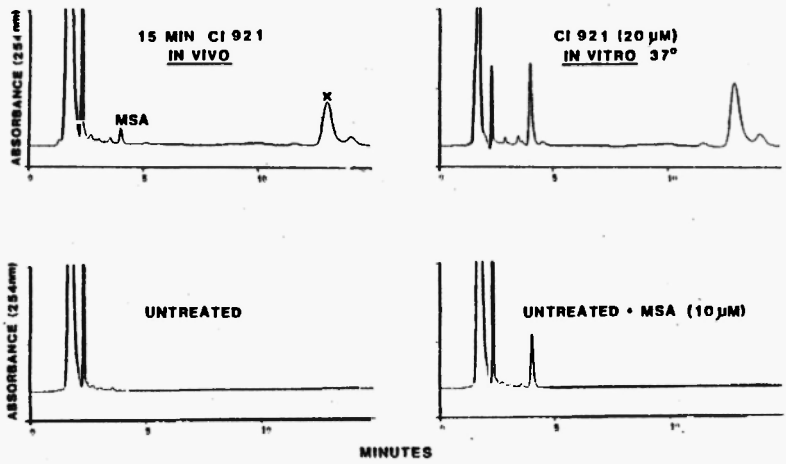


Fig. 3: High pressure liquid chromatograms of plasma acetonitrile supernatants.

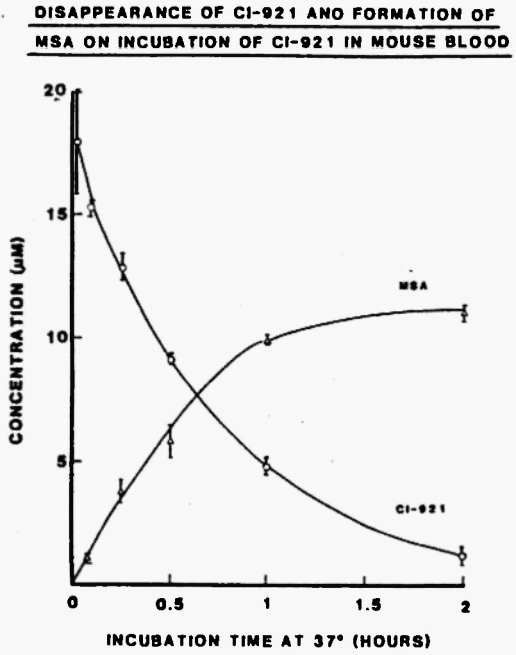


Fig. 4: Disappearance of CI-921 and formation of MSA on incubation of CI-921 in mouse blood. Values are the means of 2 determinations ± SD.

## IV. DISCUSSION

The results show that CI-921 undergoes binding to plasma proteins in the presence of mouse blood both *in vitro* and *in vivo*. It is probable that *in vitro* CI-921 forms covalent adducts with proteins via a similar mechanism to that proposed for amsacrine, whereby the acridine moiety became covalently bound to the protein after nucleophilic attack by protein thiols at the C-9 position of the acridine ring /7/. Such a process appears to be catalysed by erythrocytes, although the enzyme activity responsible is unknown. During the incubation of blood with CI-921, and also following *in vivo* exposure to CI-921, MSA was released and could be monitored by HPLC. It is likely that the binding which accompanies the release of MSA takes place by the postulated mechanism shown in Fig. 5. However, it appears that this mechanism may not solely by responsible for the binding *in vitro* as only 57% of the MSA released could account for the corresponding disappearance of the parent compound. In addition to MSA, an acridine-derived unknown compound was detected in the plasma acetonitrile supernatants following *in vitro* incubation. This compound was also detected in the blood of mice shortly after administration of CI-921, suggesting that similar mechanisms are operating for its production *in vivo* and *in vitro*.

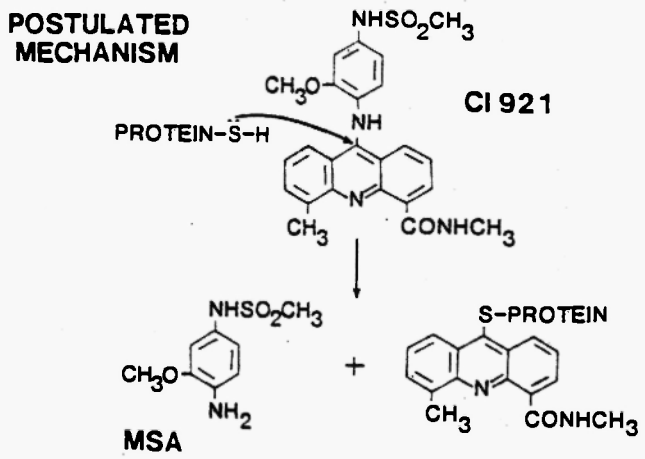


Fig. 5: Postulated mechanism by which CI-921 forms covalent protein adducts in plasma.

Hepatic metabolism, which, on analogy with amsacrine, would produce quinonoid intermediates that react rapidly with thiol-containing compounds /4,5,12/, might also apply to CI-921. Recent work in our laboratories, which has indicated that glutathione adducts of CI-921 are major metabolites in the bile of mice, suggests that a similar metabolic pathway is involved (unpublished results). The formation of protein adducts *in vivo* could well arise both from thiolysis (with the concomitant release of MSA) and from reaction of glutathione or protein thiols with quinonoid metabolites. Although MSA production *in vivo* has not been fully quantitated, the amounts observed are minor and it appears that biliary excretion rather than thiolysis is the major route by which CI-921 is eliminated from the circulation in mice.

Whatever the nature of these protein adducts might be, it is clear that they are rapidly formed and only slowly cleared from blood (Fig. 1). The ability of CI-921 to form plasma protein adducts could therefore have toxicological implications as shown for other compounds /8,9,10/. Such adducts could act as immunogens, particular in patients receiving repeated administration schedules. Further studies are underway to address the significance of this reaction in human blood. Preliminary studies indicate that the rate of adduct formation in human blood is lower than in mouse blood.

## V. ACKNOWLEDGEMENTS

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