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ACTIVITY OF N-(2-HYDROXYPROPYL)METHACRYLAMIDE COPOLYMERS CONTAINING DAUNOMYCIN AGAINST A RAT TUMOUR MODEL

James Cassidy 1*, Ruth Duncan 2, Gilmour J.Morrison 1, Jiri Strohalm 3, Dana Plocova 3, Jindrich Kopecek 3, Stanley B.Kaye 1.

1 CRC Department of Medical Oncology, 1 Horselethill Rd, Glasgow, G12 9LX, UK. 2 CRC Polymer Controlled Drug Delivery Group, Dept. of Biological Sciences, University of Keele, Staffs, UK. 3 Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6, Czechoslovakia.

* to whom correspondence and reprint requests should be addressed

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INTRODUCTION:

Many drug delivery or 'targeting' systems have been developed with differing degrees of success in improving therapeutic efficacy. Polymeric drug carriers based on water soluble non-immunogenic copolymers of N-(2-hydroxypropyl)methacrylamide [HPMA] have been developed in recent years by Duncan et al [1,2]. This group have shown that it is possible to covalently bind drugs to these carriers so that the polymer-drug linkage is stable in the bloodstream [3,4,5], but can be cleaved inside the cell by lysosomal cysteine proteinases [6,7]. Theoretically this could change drug effect in two fundamental ways:-

1) Modification of pharmacokinetics.

2) Delivery to specific cell populations i.e. 'targeting' to tumours (with enhanced uptake through increased endocytic activity [8]).

We present data to support both of these mechanisms using HPMA copolymers as a delivery system (for daunomycin) in a rat tumour model.

MATERIALS AND METHODS:

SYNTHESIS:

The HPMA copolymer-daunomycin conjugates were synthesised using a two step procedure [1,2]. In the first step a reactive copolymer of HPMA with methacryloylated oligopeptide p-nitrophenyl ester was synthesised (polymer precursor). To the latter, daunomycin was bound by aminolysis.

PHARMACOKINETICS,

Five mg/kg of either free daunomycin or daunomycin bound to HPMA coplymer (polymer 1 only) was administered by tail vein injection to Wistar rats bearing subcutaneous implants of Walker 256 tumour. At various times up to 24 hours thereafter animals were sacrificed by cervical dislocation. Blood was removed and plasma separated by centrifugation. Tissues of interest were dissected out immediately, blotted dry and frozen in liquid nitrogen to await analysis. The determination of free daunomycin (and its metabolites) was performed using an established HPLC method [9]. The experiment was carried out on two separate occasions using 2-4 animals per time point on each occasion.

Footnote; Abbreviations used Gly = glycine
Phe = phenylalanine
Leu = leucine
AUC = area under concentration-time curve
HPMA = N-(2-hydroxpropyl)methacrylamide

TUMOUR RESPONSE,

Five animals in each of four groups were given (by tail vein injection); saline as control, 5mg/kg of free daunomycin, the same dose of daunomycin bound to HPMA copolymer by a biodegradable spacer (Gly-Phe-Leu-Gly,POLYMER 1) or the same dose of daunomycin bound to HPMA copolymer via a non-biodegradable linkage (Gly-Gly,POLYMER 2). The injections were performed on the same day as subcutaneous implantation of 0.5 cm^3 cubes of Walker tumour. Subsequent tumour growth was measured bi-dimensionally by calipers every 2-3 days and the measurements converted to a volume assuming spherical geometry.

RESULTS:

PHARMACOKINETICS,

The results of HPLC analyses are shown for plasma (FIG. 1a), liver (FIG. 1b), tumour (FIG. 1c) and heart (FIG. 1d). The results are shown as mean values +/- standard errors from two separate experiments. In general levels of daunomycin metabolites parallel those of the parent drug (data not shown).

The results show a significant change in the pharmacokinetics of the HPMA preparation. The polymer-bound drug was found in the tumour at greater concentration than the free drug at all time points. Tumour AUC was increased approximately 4-fold at 24 hours. There was also a reduction in the cardiac concentrations with the HPMA preparation suggesting the possibility of an improved therapeutic index in this model system.

TUMOUR RESPONSE,

FIG. 2a shows the pattern of tumour growth in the four groups shown as the mean volumes +/- standard errors against time from passage. Animals were sacrificed before their tumour burdens became intolerable (>6 cm in diameter) which occurred from day 14 onwards. Thus in FIG. 2b the data are shown converted into a 'survival curve'.

The only group showing a statistically significant growth delay (Mann-Whitney U test) was the group given the biodegradable polymer 1. In fact 4/5 animals in this group were long term survivors (>120 days so far), with no evidence of tumour. Only one other animal from any group survived and this was in the free daunomycin group.

DISCUSSION:

In this model system the use of HPMA copolymers to deliver daunomycin has resulted in a favourable modification of the pharmacokinetic behaviour of daunomycin. More drug reached the 'target' tumour tissue, and less reached the myocardium, suggesting that cardiotoxicity of the anthracycline may be reduced. The other major site of toxicity for the anthracyclines is the bone marrow, causing dose limiting myelosuppresion [4]. We did not measure marrow uptake or monitor the haematology of the treated animals. However, in the response study,the control animals all died of tumour burden rather than drug toxicity, and in the long-term survivors from the biodegradeable polymer group there was no sign of haematological toxicity which one would expect if the bone marrow 'dosage' had been substantially increased. These pharmacokinetic changes have been shown to result in enhanced anti-tumour activity in this model system. It is interesting that the non-biodegradable polymer group also seemed to show some growth delay though this did not reach statistical significance.

The biological reason for selective delivery to tumour with this preparation is unclear. The macromolecular nature of the HPMA copolymers means that cellular uptake must be through an endocytic mechanism. It is possible that tumour cells have increased endocytic activity resulting in enhanced uptake of macromolecules [8]. It has been suggested that Walker 256 tumour contains fenestrated capillaries [10] which may allow preferential 'leakage' of the polymer in the tumour vascular bed. These preliminary

results are encouraging, but need to be reproduced in other model systems. Clinical trials using a form of HPMA copolymer targeted to hepatocellular carcinoma are planned for the near future.

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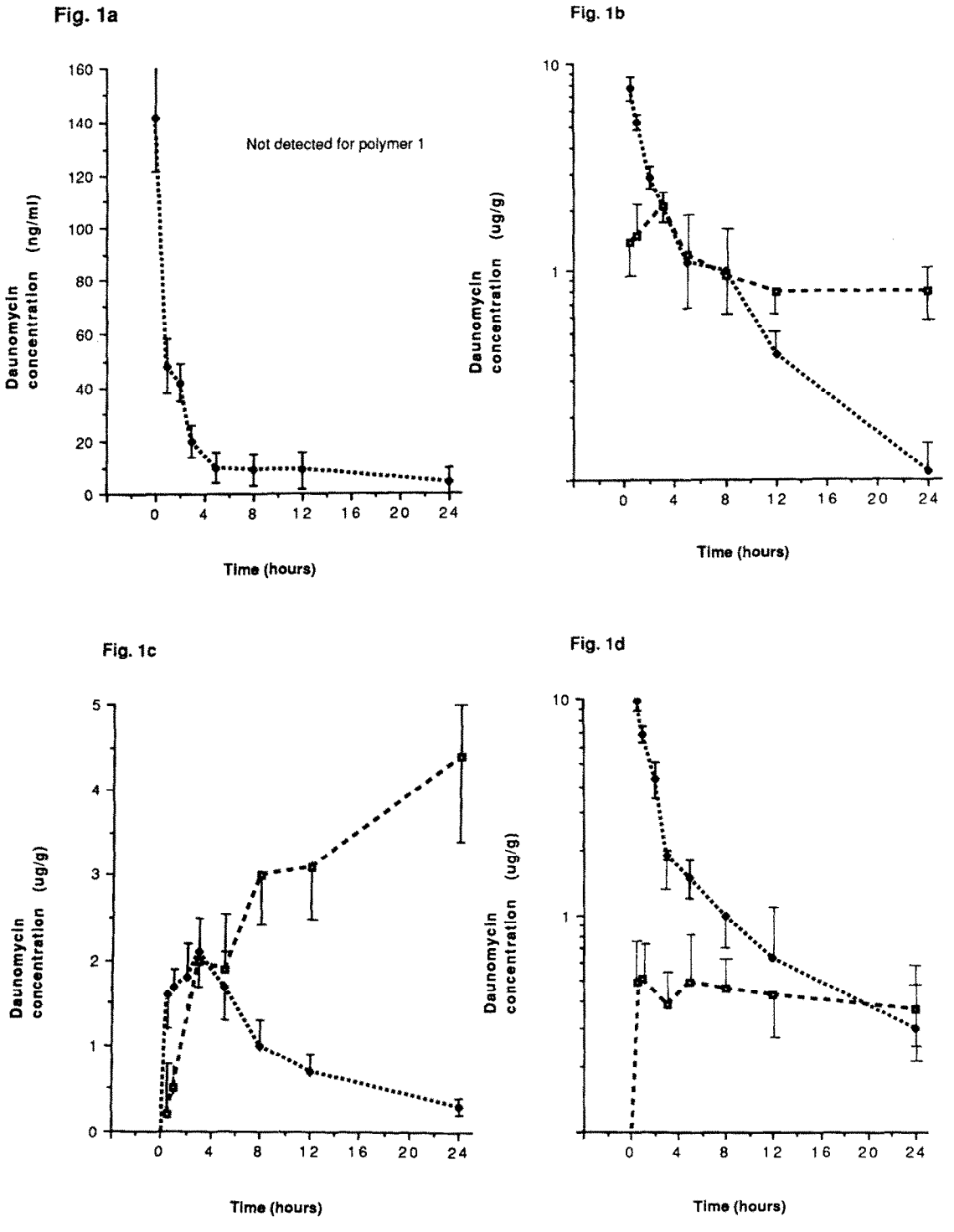

Figure 1. Free daunomycin levels by HPLC analysis in samples from (a) plasma, (b) liver, (c) tumour, (d) heart.

Fig. 2a

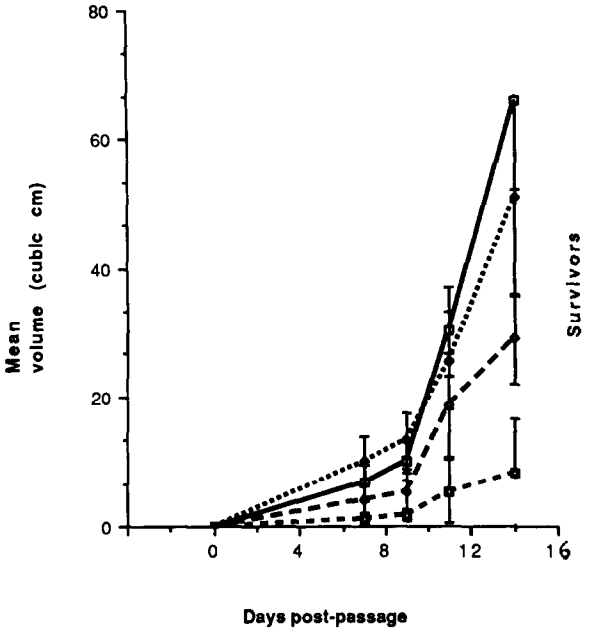

Fig. 2b

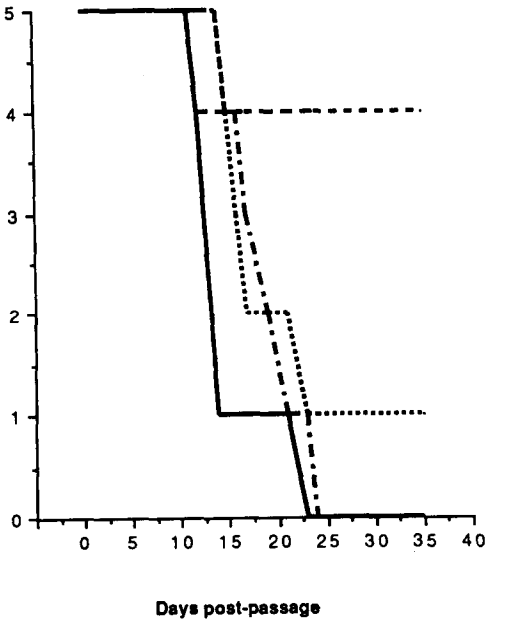

Figure 2a. Walker tumour growth in four groups of rats given saline (—⊡—), free daunomycin (····◆····), polymer 1 (- -■- -), polymer 2 (- ·◆· -).

Figure 2b. Survival of rats in groups given saline (———), free daunomycin (·····), polymer 1 (- - - - -), polymer 2 (- · - · -), following subcutaneous passage of Walker tumour.