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Short communication

Tumour profile of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide after intraperitoneal administration in the mouse

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Abstract. *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (AC) is an experimental antitumour agent that is being considered for phase I trials. After i.p. administration of 150 mg/kg [^{3}H]-AC to tumour-bearing mice, AC was absorbed rapidly into the plasma and tissues such as the heart, liver, kidney and brain but more slowly into the s.c. tumour. The maximal AC concentration (86±36 μmol/kg) in the tumour occurred at 35–60 min and was 3-fold the maximal plasma concentration, which occurred at 15 min. Although higher maximal concentrations were observed in other tissues, these concentrations fell rapidly in parallel with plasma concentrations. In contrast, AC concentrations in the tumour remained elevated, the $t_{1/2}$ value (16.3 h) and mean residence time (MRT, 9.5 h) being prolonged in comparison with those in the plasma and other tissues ($t_{1/2}$ range, 1.0–2.9 h; MRT, 1.2–1.4 h). AC concentrations were not detectable by our high-performance liquid chromatographic (HPLC) method (limit of detection, 0.02 μmol/l) in the plasma or other tissues at 24 or 48 h after administration but were measurable in the tumour (1.6±0.8 and 0.6±0.3 μmol/kg, respectively). Radioactivity concentrations in the plasma, tissues and tumour were very variable but were greater than the corresponding levels of unchanged parent AC. By 24 h, radioactivity concentrations in the plasma, tissues and tumour had fallen to similar levels with prolonged elimination profiles. Thus, the exposure of the s.c. implanted tumour to a threshold AC concentration for a prolonged time (>24 h) may partly explain the greater efficacy of AC against this tumour, whereas the shorter period of exposure of blood and other tissues may explain its low haematological toxicity.

Introduction

N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (AC; NSC 601316) is an experimental antitumour agent that was developed in the Cancer Research Campaign Laboratory, University of Auckland Medical School [1, 4]. It is being considered for phase I clinical trials by the ■ (CRC, UK). In preclinical testing, AC exhibited several interesting characteristics. When injected i.p., AC was more active against remotely implanted Lewis lung tumour than against proximally (i.p.) implanted P388 or L1210 leukaemia, although AC had similar cytotoxicity towards these cells in culture [1, 4]. AC's cytotoxicity was minimal at high drug concentrations following short periods of exposure (e.g., 12 μmol/l for 1 h) and maximal following exposure to lower concentrations for intermediate periods (e.g., 2 μmol/l for 6 h) [5]. The reduced cytotoxicity of higher concentrations for short exposure times is thought to be a consequence of self-inhibition of toxicity due to intercalation of AC with DNA, inducing a conformational change that hinders the formation of the cleavable complex of topoisomerase II with DNA [5]. These results indicate the importance of the administration protocol in maximising the antitumour efficacy of AC. We have recently reported the pharmacokinetics of AC in healthy BDF_1 mice after i.p. administration of 150 mg/kg, which is the optimal single dose for the cure of advanced Lewis lung carcinoma implanted s.c. [3]. In the latter study, plasma concentrations remained above 0.3 μmol/l for 6 h. However, the concentration-time profile obtained in a remotely implanted solid tumour may be very different from the plasma concentration-time profile. Our aim was to compare the AC (and radioactivity) concentration-time profiles obtained in the s.c. Lewis lung tumour with the corresponding profiles acquired in plasma and other tissues after i.p. administration of 150 mg/kg to tumour-bearing mice.

Materials and methods

The drug formulation, administration protocol, pharmacokinetic analysis and methods of AC and radioactivity ([^{3}H]-AC equivalents) measurement in plasma and tissues used in the present study have been described elsewhere [2, 3, 8, 10]. The same methods were used for the measurement of AC and radioactivity in the tumour. The accuracy and intra-assay

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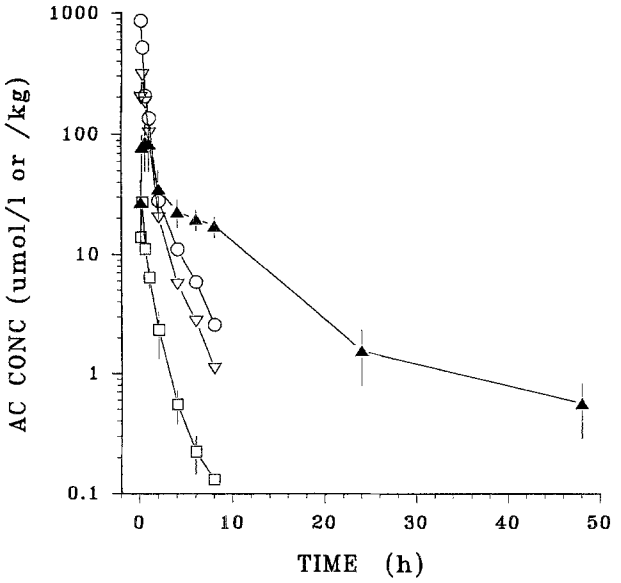

Fig. 1. Mean AC concentration-time profiles in plasma (□), tumour (▲), brain (▽) and liver (○). For the sake of clarity, only brain and liver have been included from the tissues and ± SD bars have been attached to the plasma and tumour concentrations

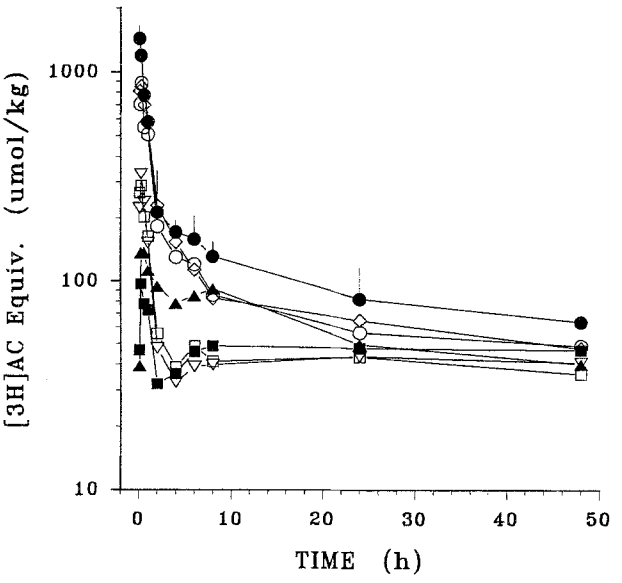

Fig. 2. Mean radioactivity concentration-time profiles in plasma (■), tumour (▲), brain (▽), liver (●), kidney (○, heart (□) and lung (◇). For the sake of clarity, + SD bars have been attached to the liver concentrations

precision of the measurement of AC added to tumour tissue was acceptable, with recoveries ranging from 97% to 107% and coefficients of variation (CV) from 1.7% to 6.8% (n= 6). Tumour tissue with known amounts of AC stored at –80°C and assayed seven times over 6 weeks gave recoveries from 102% to 108% and a CV range of 4.1–6.6%.

Female BDF_1 mice were inoculated s.c. into the right flank with 10^6 Lewis lung cells and experiments were performed when tumours had become palpable (diameter, 5–10 mm). Previously it had been shown that there was no significant difference in AC's plasma pharmacokinetics either between male and female mice or between healthy and tumour-bearing mice [2]. In the present study, tumour-bearing mice received i.p. 150 mg/kg (410 μmol/kg) [^{3}H]-AC and were killed at 5, 15 and 35 min and at 1, 2, 4, 6, 8, 24 and 48 h (n= 3 animals per time point). All animal procedures were approved by the Animal Ethics Committee, University of Auckland.

Results

AC tumour and tissue profiles

Peak concentrations of AC (86 ± 36 μmol/kg) were achieved in the tumour at 35–60 min and were 3-fold those (27 ± 2 μmol/l) occurring in the plasma at 15 min (Fig. 1). Other tissues such as the brain, liver, kidney and heart had significantly higher maximal concentrations (3- to 10-fold) occurring earlier at 5–15 min, but all concentrations fell rapidly over the subsequent 8 h in a manner similar to that observed in plasma. At 8 h, tissue AC levels ranged from 1 to 3 μmol/kg in contrast to tumour levels of 17 ± 3 μmol/kg. AC was measurable in the tumour at 24 (1.6 ± 0.8 μmol/kg) and 48 h (0.6 ± 0.3 μmol/kg) but was not detectable in the plasma or tissue. The area under the concentration-time curve (AUC) in the tumour was 431 μmol h kg^{-1}, which was 20-fold that in plasma (21.6 μmol h l^{-1}) but was similar in magnitude to that in the other tissues. The most striking difference was the long elimination $t_{1/2}$ (16.3 h) and mean residence time (MRT, 9.5 h) observed in the tumour as compared with the plasma ($t_{1/2}$, 2.9 h; MRT, 1.4 h) and other tissues ($t_{1/2}$ range, 1.0–1.8 h; MRT, 1.2–1.3 h).

Radioactivity profiles

The maximal tumour radioactivity concentration (expressed as AC equivalents) was 136 ± 19 μmol/kg and occurred at 35 min. Over the initial 8 h after AC administration, the tissues appeared to fall into two groups: a group including the liver, kidney, lung and spleen with high radioactivity concentrations and a group including the plasma, red blood cells, heart and brain with relatively lower concentrations. The tumour radioactivity concentrations were intermediate (Fig. 2). By 24 h, radioactivity concentrations in the plasma and various tissues including the tumour were similar, ranging from 43 to 82 μmol/kg, with little change being noted at 48 h (40–63 μmol/kg). The AUCs for radioactivity were of similar magnitude for all tissues, ranging from 3931 μmol h kg^{-1} in the heart to 9978 μmol h kg^{-1} in the brain, with those in the tumour being intermediate (7309 μmol h kg^{-1}). The $t_{1/2}$ and MRT for radioactivity in the tumour were 77 and 104 h, respectively, being similar in magnitude to the $t_{1/2}$ (range, 33–173 h) and MRT (50–225 h) observed in the plasma and other tissues.

Discussion

In the testing of the antitumour efficacy of AC, an i.p. dose of 410 μmol/kg was the optimal single i.p. dose for the cure of advanced Lewis lung carcinoma implanted s.c. in

BDF_1 mice. Although AC is eliminated relatively swiftly from plasma with an elimination $t_{1/2}$ of 2.9 h, this study indicates that a very different profile exists in s.c. tumours. After i.p. administration, AC is rapidly absorbed into the plasma but is distributed more slowly to the tumour as compared with other tissues such as the brain, liver, kidney or heart. This discrepancy is probably due to the relatively low vascularity of the s.c. tumour as compared with other tissues such as the kidney, liver and heart. This low vascularity may also partly explain the slow efflux of AC from the tumour as compared with other well-vascularised tissues. Tumour DNA and topoisomerase II and their affinity for AC may also play an important part in the tumour retention of AC. By 24 h, AC was not detectable in the plasma or in any of the other tissues examined, but concentrations of >1 μmol/kg remained in the tumour. Thus, the exposure of the s.c. implanted tumour to moderate AC concentrations for a prolonged time (>24 h) may explain the greater efficacy of AC against this tumour, whereas the shorter period of exposure of blood and other tissues such as the bone marrow may explain the low haematopoietic toxicity that we have previously reported [3]. A comparison of the tumour/plasma AUC ratio for AC (19.9) with that (1.9) of its predecessor CI-921 showed that this ratio was 10 times greater for AC [6]. This finding indicates that AC is more efficiently taken up and retained in this tumour than is CI-921, which may partly explain its superior in vivo antitumour activity.

In the plasma and all tissues including the tumour, radioactivity concentrations were significantly higher than the corresponding AC concentrations, which is in agreement with the results of our previous i.v. and i.p. studies [3, 8]. In contrast to levels of AC, radioactivity concentrations in the tumour and the various tissues including plasma were similar at 24 h, showing similar slow rates of decline thereafter until 48 h. Evidence suggests that the retention of radioactivity is due to slow elimination of metabolite(s) of AC [9], but to date no metabolite has been shown to have significant antitumour activity [7].

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