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Original Paper

Antitumour Activity and Schedule Dependency of 8-Chloroadenosine-3′,5′-monophosphate (8-ClcAMP) Against Human Tumour Xenografts

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8-Chloroadenosine-3',5'-monophosphate (8-ClcAMP) is a novel antitumour agent currently undergoing phase I clinical trials in several European centres. In this study, its antitumour activity against human tumour xenografts and its dependence on schedule were investigated. When administered by continuous infusion at doses of 100 or 50 mg/kg/day to nude mice bearing human tumour xenografts, 8-ClcAMP inhibited the growth of the HT 29 colorectal, ZR-75-1 breast, HOX 60 and PE04 ovarian and PANC-1 pancreatic carcinoma xenografts. However, these infusion schedules produced hypercalcaemia and severe weight loss. In an attempt to optimise antitumour activity and minimise toxicity, several other schedules were studied. In comparison with continuous administration of 8-ClcAMP at 50 mg/kg/day for 14 days which, although producing complete growth inhibition in the HOX 60 model, was associated with a marked body weight loss, schedules in which the infusion was interrupted (infusion on either days 0–4; 7–11 or days 0–2; 6–8) produced minimal weight loss but also reduced antitumour activity. However, co-administration of salmon calcitonin with continuous infusion of 8-ClcAMP prevented both hypercalcaemia and body weight loss in 3/6 animals while still producing marked inhibition of tumour growth. These data indicate that 8-ClcAMP has broad-spectrum antitumour activity and the major side-effect of hypercalcaemia may at least in part be ameliorated by the use of salmon calcitonin. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: 8-ClcAMP, xenograft, hypercalcaemia
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INTRODUCTION

8-CHLOROADENOSINE-3',5'-MONOPHOSPHATE (8-ClcAMP) is an analogue of cAMP that exhibits growth inhibitory activity against tumour cells both *in vitro* and *in vivo* [1–4]. Its mechanism of action has been proposed to involve differential modulation of the two isoforms of protein kinase A (PKA-I and PKA-II) [5]. Increased PKA-I expression is associated with cell transformation and proliferation, while increased PKA-II levels are linked with increased differentiation and growth inhibition [6]. 8-ClcAMP has been demonstrated to reduce PKA-I and increase PKA-II expression and is associated with growth inhibition in tumour cells. More recently, 8-ClcAMP has been proposed to act as a prodrug of 8-chloroadenosine (8-Cl-adenosine) which might then act either as an antimetabolite [7–11] or as a modulator of PKA isozymes [12]. 8-ClcAMP is currently undergoing phase I clinical studies [13–15] in which it is being administered by continuous infusion, and several studies have observed hypercalcaemia to be dose limiting [14, 15].

The aim of the present study was to investigate the activity of 8-ClcAMP in a spectrum of human tumour xenograft models to provide an indication of range of activity and to help identify potential phase II activities. The effects of different schedules of infusion on the antitumour activity and toxicity were compared and the possibility of using salmon calcitonin to ameliorate drug-induced hypercalcaemia was explored.

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MATERIALS AND METHODS

Reagents

8-ClcAMP (sodium salt) was supplied by Dr Y-S. Cho-Chung (NIH, Bethesda, Maryland, U.S.A.).

Xenograft studies

Xenografts were initiated from either cultured cell lines (HT 29 colorectal, ZR-75-1 breast, PE04 ovarian and PANC-1 pancreatic carcinomas) or a primary tumour (HOX 60 ovarian carcinoma) and were grown subcutaneously in the flanks of nude mice (Harlan Olac Ltd., U.K.). Animals were at least 8 weeks old at the time of experimentation and were maintained in negative pressure isolators (La Calhene, U.K.). Mice bearing the ZR-75-1 breast carcinoma xenograft were also implanted with a slow-release 17β-oestradiol pellet (0.72 mg released over 60 days, supplied by Innovative Research of America, Ohio, U.S.A.). Tumour fragments were implanted subcutaneously into the flanks of nude mice and allowed to grow to 4–6 mm in diameter (over a period of approximately 1 month). Animals were then allocated to treatment or control groups, with 6–8 animals/group. Treatment consisted of implanting (under anaesthetic) Alzet mini pumps (ALZA Corporation, Palo Alto, California, U.S.A.) delivering, for a maximum of 7 days, either 8-ClcAMP (5–100 mg/kg/day) or vehicle (water) into the flank opposite the tumour. Where the drug was given for 14 days, a new pump was implanted after 7 days. Certain animals additionally received salmon calcitonin administered subcutaneously (1 IU/mouse/day) into the flank. Tumour size was measured twice weekly using calipers and the volume calculated according to the formula: $0.5 \times \text{length} \times \text{width}^2$. Relative tumour volumes were calculated for each individual tumour by dividing the tumour volume on day t (V_t) by the tumour volume on day 0 (V_0) multiplied by 100%.

Measurement of plasma calcium concentration

Plasma was collected at the stated time and total calcium analysed using a Kodak Ektachem biochemical analyser.

RESULTS

Antitumour activity of 8-ClcAMP

8-ClcAMP was administered to animals bearing a range of human tumour xenografts to assess the range of its antitumour activity. When the drug was given by continuous infusion at doses of 100 and 50 mg/kg/day over a 7-day period, it significantly inhibited growth of the HT 29 colorectal, ZR-75-1 breast, PE04 and HOX 60 ovarian and PANC-1 pancreatic carcinoma xenografts (Figure 1). Inhibition was most marked towards the end of the infusion period and tumours regrew on discontinuation of the drug (data not shown). To assess effects at lower doses, doses of 5, 10 and 25 mg/kg/day were studied against the HT 29 and ZR-75-1 xenografts (Figure 2). All these doses significantly inhibited the growth of the HT 29 xenograft at day 3 but at day 7 only 25 mg/kg/day produced significant inhibition. This dose (but not lower doses) also produced significant inhibition in the ZR-75-1 model at day 7 (Figure 2b).

Toxicity of 8-ClcAMP

Animals lost weight at doses of 50 or 100 mg/kg 8-ClcAMP/day over the infusion period (see Figure 4). On discontinuation of the drug, the mice rapidly regained weight. The rapid timescale of the weight changes suggests that they may have resulted from dehydration. Since dehydration can arise from hypercalcaemia which has also been observed in phase I human studies, it was of interest to determine whether hypercalcaemia was evident in animals treated with 8-ClcAMP. Plasma calcium concentrations were therefore measured at the end of the infusion period after administration of 50 and 100 mg 8-ClcAMP/kg/day; values were significantly elevated in all drug-treated animals (Figure 3; Mann–Whitney test, 50 mg versus control $P < 0.0001$; 100 mg versus control, $P = 0.0007$).

The only lethalities were observed in mice hosting the ZR-75-1 breast carcinoma xenografts and receiving 8-ClcAMP at 100 mg/kg/day. Since only these animals received oestrogen supplementation it seems likely that co-administration of these two agents produced increased toxicity.

Schedule dependency of the antitumour activity and toxicity of 8-ClcAMP

The effect of introducing breaks into the continuous infusion on antitumour activity and toxicity was investigated

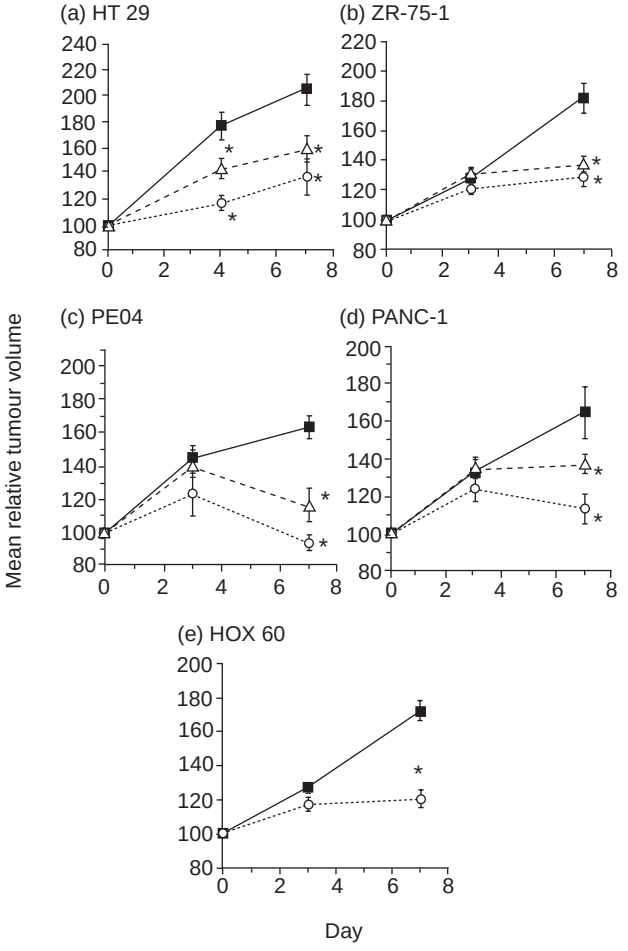

Figure 1. Activity of 8-ClcAMP against colon, breast, ovarian and pancreatic carcinoma xenografts. (a) HT 29 colon carcinoma, (b) ZR-75-1 breast carcinoma, (c) PE04 ovarian carcinoma xenograft, (d) PANC-1 pancreatic carcinoma xenograft, (e) HOX 60 ovarian carcinoma xenograft. Tumour size is expressed as a percentage of the starting volume and each point represents the mean of 6–8 xenografts. Bars represent standard errors of the mean. Statistical comparisons were made with control tumours at the same time point; *$P < 0.05$, Mann–Whitney test. ■—■, control; △---△, 50 mg/kg/day; ○----○, 100 mg/kg/day.

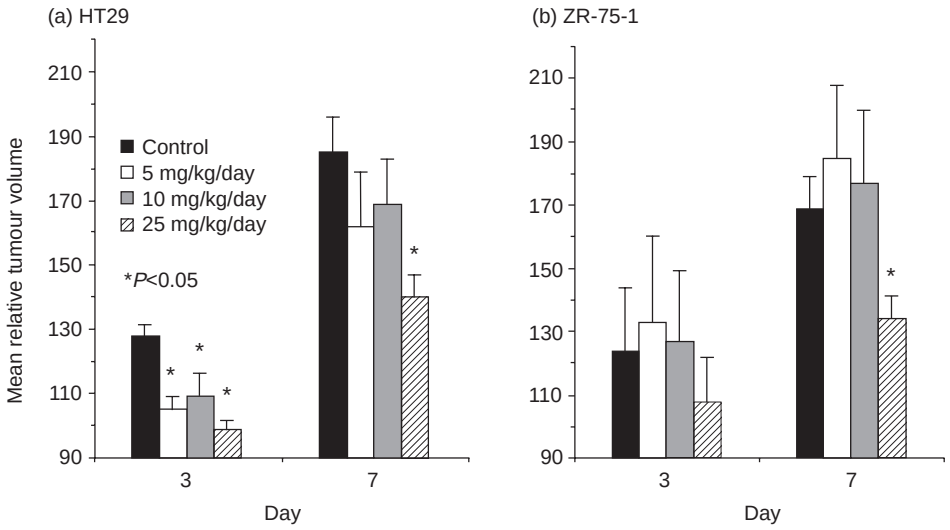

Figure 2. Activity of lower doses of 8-ClcAMP (25, 10 and 5 mg/kg) against the growth of the (a) HT 29 colorectal and (b) ZR-75-1 breast carcinoma xenograft. Bars represent standard errors of the mean. Statistical comparisons are made with control tumours at the same time point; *$P < 0.05$, Mann–Whitney test.

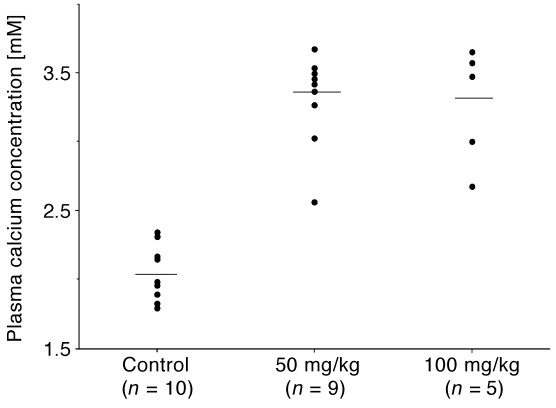

Figure 3. Effect of 8-ClcAMP on plasma calcium concentration after a 7-day infusion. Individual values are shown. Bars indicate mean values.

using the HOX 60 xenograft model. 8-ClcAMP was administered at a dose of 50 mg/kg/day. Three schedules were compared—schedule A, continuous treatment for 14 days; schedule B, treatment for 5 days, no treatment for 2 days, then resumption of treatment for 5 days and schedule C, treatment for 3 days, no treatment for 4 days, resumption of treatment for 3 days (Figure 4). Schedule A produced marked inhibition of tumour growth with little increase in size over the treatment period, although tumour growth proceeded after discontinuation of treatment (Figure 4a). This schedule was associated with major loss in body weight (approximately 30%), although recovery was rapid on discontinuation of the drug (Figure 4b). Schedules B and C produced some growth inhibition relative to controls, but markedly less than that obtained using a continuous schedule (Figure 4a), and these animals demonstrated minimal weight loss (Figure 4b).

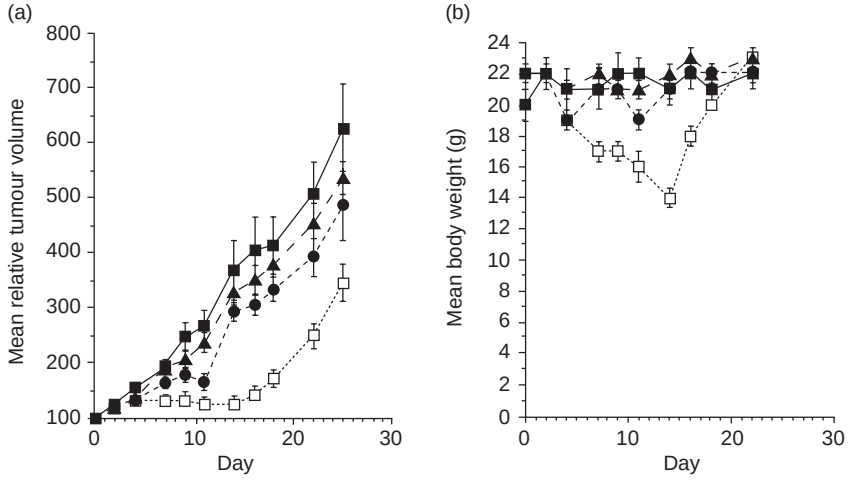

Figure 4. Effect of different schedules of administration of 50 mg/kg/day 8-ClcAMP on (a) mean relative tumour volumes and (b) mean body weights of groups of animals bearing the HOX 60 ovarian carcinoma xenograft. Three schedules were compared □- - -□ schedule A, continuous treatment tor 14 days; ●- - -● schedule B, treatment for 5 days, no treatment for 2 days, then resumption of treatment for 5 days and ▲- - - -▲ schedule C, treatment for 3 days, no treatment for 4 days, resumption of treatment tor 3 days; control pumps (■—■) contained sterile water.

Effect of calcitonin on hypercalcaemia and antitumour activity of 8-ClcAMP

Salmon calcitonin was used to prevent hypercalcaemia produced by 8-ClcAMP. Co-administration of calcitonin (1 IU/mouse/day) and 8-ClcAMP resulted in minimal (0–5%) body weight loss in 3 of 6 (50%) animals after 7 days treatment (Figure 5). Plasma calcium levels within these 3 animals (2.31, 2.38 and 2.70 mM) were within the range of those in animals receiving calcitonin alone (range 1.86–2.72 mM) consistent with calcitonin preventing this toxicity (Figure 6a). Only a single calcium value was available for 1 of the 3 animals that lost weight after this combination, but this was high (3.22 nm), consistent with a very marked (28%) loss in body weight. The antitumour activity of the combination is shown in Figure 6(b). Calcitonin alone had no effect on tumour growth, and when combined with 8-ClcAMP, the effect was equivalent to that of 8-ClcAMP alone. These data indicate that while calcitonin is able to prevent toxicity in certain animals treated with 8-ClcAMP, it does not affect antitumour activity.

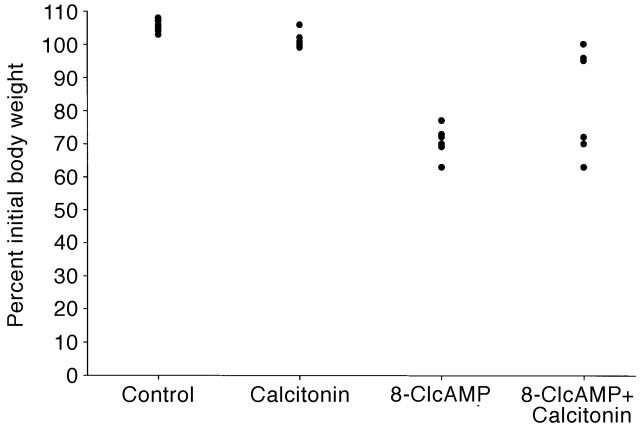

Figure 5. The effect of calcitonin and 8-ClcAMP alone or in combination on individual animal body weight after 7 days. 8-ClcAMP was administered at 100 mg/kg/day and calcitonin at 1 IU/mouse/day for 7 days.

DISCUSSION

This study demonstrates that 8-ClcAMP, when given by continuous infusion, inhibits the growth of a range of human tumour types in nude mice including carcinomas of the colon, breast, ovary and pancreas. The drug is currently undergoing several phase I clinical studies, all of which use continuous infusion-based schedules [13–15]. Initial indications of antitumour activity have already been observed with a partial response being obtained in a colorectal cancer and disease stabilisation in two colorectal and one lung carcinomas [13]. Overall results of broad-spectrum activity suggest that the compound merits phase II clinical studies in a range of disease types provided that toxicity problems can be overcome.

The major toxicity observed in these nude mice studies was a persistent weight loss which reversed rapidly on cessation of the infusion (e.g. Figure 4b). The weight gain achieved by the animals was so rapid that it could only be explained by rehydration. This would be consistent with 8-ClcAMP producing dehydration as a result of hypercalcaemia (the dose-limiting toxicity observed in human phase I studies [14, 15]). Hypercalcaemia in the animals in the present study was confirmed by direct measurement of plasma calcium level. In the clinical studies, hypercalcaemia necessitated cessation of infusion to allow patient recovery. Interrupting the infusion in the xenograft study clearly allowed the weight loss to be rapidly reversed, with longer breaks in the schedule allowing more complete recovery. However, this was achieved at the expense of tumour regrowth, since only tumour stasis was maintained during the period of infusion. An alternative strategy was to co-administer an antidote such as calcitonin. Salmon calcitonin is used to treat malignancy-associated hypercalcaemia and can usually decrease serum calcium concentrations in hypercalcaemic patients [16]. Co-administration of calcitonin alongside 8-ClcAMP to animals bearing the HT29 colorectal xenograft prevented weight loss in 3 of 6 treated animals. These 3 animals had plasma calcium levels that were within the range in animals receiving calcitonin alone and approached the value of those receiving vehicle only. It may be that the inability to prevent weight loss

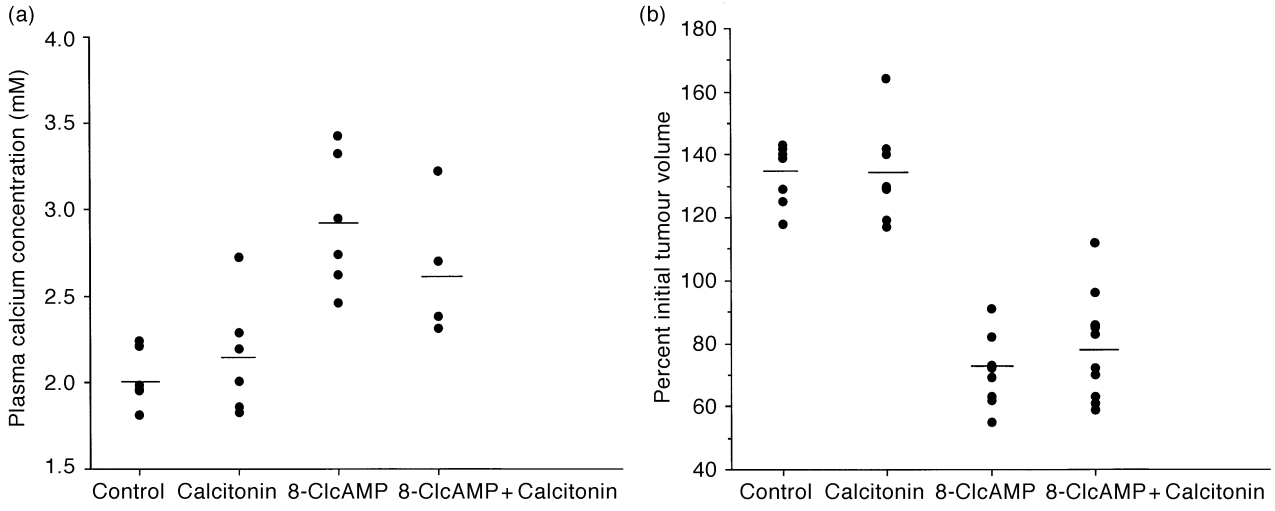

Figure 6. Effect of calcitonin and 8-ClcAMP alone or in combination on (a) plasma calcium concentrations and (b) tumour volume in mice bearing the HT 29 colorectal carcinoma xenograft. 8-ClcAMP was administered at 100 mg/kg/day and calcitonin at 1 IU/mouse/day for 7 days. Individual values are shown. Bars indicate mean values.

in certain animals is due to suboptimal scheduling or dosing of the calcitonin. Since the antitumour activity of 8-ClcAMP in this group of animals was not compromised by calcitonin, the combination of calcitonin with 8-ClcAMP is currently being investigated in further clinical studies.

In summary, these results demonstrate that 8-ClcAMP has broad-spectrum antitumour activity against a range of xenografts of different tumour types. Weight loss and hypercalcaemia observed after continuous infusion might be ameliorated by the use of calcitonin.

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