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The Effect of Indomethacin on Food and Water Intake, Motor Activity and Survival in Tumour-bearing Rats

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This investigation has addressed the question whether food and water intake, motor activity and tumour growth are influenced by indomethacin in experimental cancer. Growing rats implanted with a methylcholanthrene-induced sarcoma were studied in metabolic cages connected to a computer. Food intake, water consumption and motor activity were continuously recorded over 30 days following tumour implantation. Treated tumour-bearing animals received indomethacin 1.0 mg/kg per day in drinking water. Food intake declined early in untreated tumour-bearing animals, but water intake was not affected. Motor activity decreased in untreated tumour-bearing animals from days 16–17 onward. Indomethacin treatment prolonged survival and 40% of these tumour-bearers were 'complete responders'. In some animals tumour growth was only marginally affected, but survival was still significantly improved ('partial responders'). Food intake was significantly improved in complete responders. Thus this positive effect seen in complete responders was secondary to less active tumour growth. Motor activity was also significantly higher in responders compared with non-responders.
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INTRODUCTION

ANOREXIA generally underlies the major part of cancer cachexia [1]. The biochemical alterations behind tumour-induced food aversion are not understood, but cytokines may have a role. Interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-α) may induce or promote food aversion [2–6]. We found that anti-TNF serum significantly improved appetite in tumour-bearing mice [7]. Antibody blockade of the IL-1 receptor did not improve appetite more than anti-TNF alone did, suggesting that IL-1 is not more important than TNF in explaining anorexia in experimental cancer [8]. However, it is always difficult to be sure that antibodies actually penetrate the extracellular compartment to a sufficient extent in *in vivo* experiments. Therefore we have investigated the effect of indomethacin, which is water-soluble, on anorexia, water intake, motor activity and survival in sarcoma-bearing rats, since several of the monokine effects are mediated by prostaglandins.

MATERIALS AND METHODS

Animals and treatment

Growing Sprague–Dawley rats from ALAB (Sweden) weighing 80 g at the start of the experiments were used. Rats were implanted subcutaneously in the flanks with a methylcholanthrene-induced sarcoma with a trocar. This tumour does not metastasize nor penetrate the abdominal cavity when implanted subcutaneously. Animals with ulcerated tumours were not used. Control animals were sham-implanted.

In all experiments the animals were kept in individual cages, in a temperature and humidity controlled room, with a 12 h light–dark cycle. The food was standard (Ewos, Södertälje, Sweden). Tap-water was available freely. 2 animals were studied simultaneously so that any environmental factors would have an equal influence on study and control groups. Tumour size was calculated every other day. The longest (LD) and shortest diameter (SD) of the tumour was measured by a calliper and the volume was calculated according to the formula: volume = $\pi/6 \times LD \times SD^2$, where LD is the longest diameter and SD the shortest diameter. In some experiments tumour-bearing rats were divided randomly into study and control groups. The study group received indomethacin 1.0 mg/kg per day (corresponding to 0.1 [S.D 0.02]%) in the drinking water.

Metabolic cage

We made a computerized metabolic cage to examine feeding and drinking behaviour and motor activity (details from K.L.). The cage allows accurate monitoring of food and water intake. The precision in the food balance system was 30 mg. Intake and activity were analysed automatically by computer.

Statistics

We used one-factor ANOVA for repeated measures. A 95% confidence interval (CI) was used. Survival was analysed by life tables [9]. Volumes are mean ± S.E.M.

RESULTS

The tumours became palpable between 5 and 10 days after implantation, and the tumour wet weight was 131 (12 g) in untreated tumour-bearing rats on day 30. The growth curve for untreated tumour-bearing rats (including the tumour) did not differ from that of control rats until 30 days, which was the period that was used for measurements of food and water intake and motor activity.

Food intake started to decline soon after tumour implantation in untreated tumour-bearing rats (Fig. 1). The tumour weight

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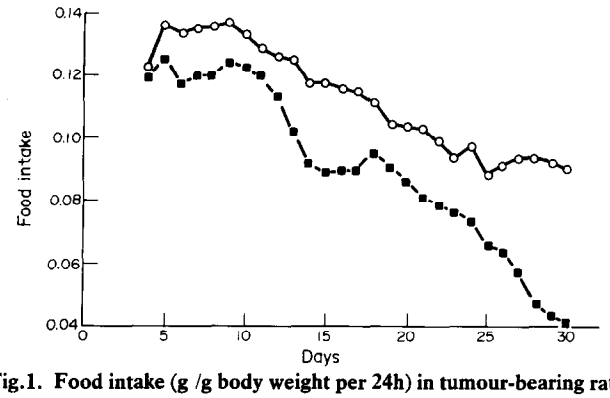

Fig.1. Food intake (g /g body weight per 24h) in tumour-bearing rats (■) compared with controls (○) (n = 12 per group; $P < 0.01$ between groups).

was than 1.4 (0.3) g corresponding to less than 1% of body weight. Water intake never differed between tumour-bearing and control rats (data not shown). Motor activity in untreated tumour-bearing rats declined from days 16–17 onwards, while it was unchanged in control rats (Fig. 2). The effect of indomethacin on improved motor activity was probably directly related to tumour size in indomethacin-treated tumour-bearing rats (results not shown).

Indomethacin treatment of tumour-bearing rats resulted in prolonged survival ($P < 0.01$) (Fig. 3). 31 tumour-bearing rats received indomethacin. Of these 13 responded with complete disappearance of tumours (Fig. 4), while 18 animals were partial responders in that survival was prolonged compared with 'controls' (Fig. 3) but the animals had less inhibition of tumour growth compared with responders (Fig. 5). Indomethacin-treatment of tumour-bearing rats resulted in less tumour ulceration and bleeding around tumours with ulceration in experiments on additional animals that were not housed in the metabolic cages. In addition, indomethacin-treated animals had a more normal lustre in coat compared with untreated tumour-bearing controls. Indomethacin treatment of non-tumour-bearing rats had no effect on any of the variables examined in tumour-bearing animals.

Food intake was significantly more preserved in the complete responders compared with partial responders and with untreated tumour-bearing rats ($P < 0.01$) (Fig. 6). Indomethacin had no significant effect on water intake in tumour-bearing rats irrespective of the animals being partial or complete responders. Motor activity was significantly higher in complete responders

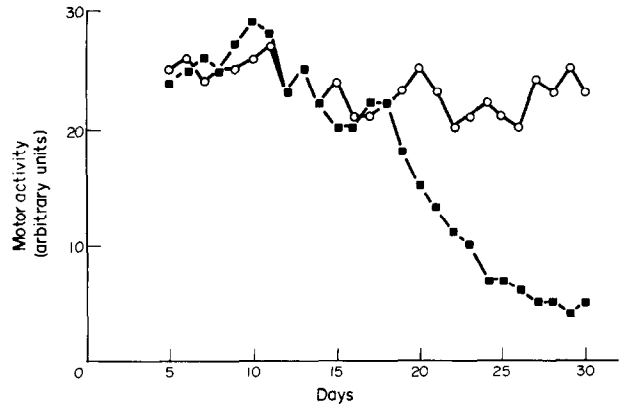

Fig.2. Motor activity after tumour implantation. Tumour-bearing rats (■) showed significant decline ($P < 0.01$) in motor activity from day 18 compared with controls (○) (n = 12 per group).

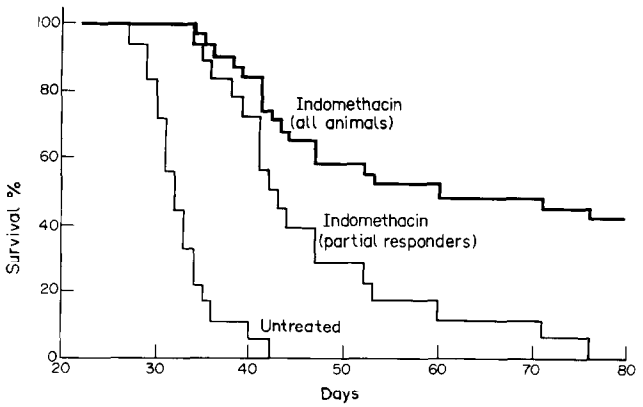

Fig.3. Survival in tumour-bearing rats treated with indomethacin compared with untreated tumour-bearing rats. Indomethacin-treated rats had significantly prolonged survival ($P < 0.01$; n = 18 per group).

compared with partial responders and untreated controls ($P < 0.05$).

DISCUSSION

We have confirmed observations that indomethacin alone can influence experimental tumour growth rate [8, 10]. An unusual effect we saw was that 42% of the responding animals were cured. Indomethacin also prolonged survival in those tumour-bearing rats that did not have a pronounced growth inhibition effect.

Although the tumour inhibitory effect of indomethacin is recognized, the mechanisms have never been clarified. The effect may be mediated by an upregulation of cytokines in immune cells [12–14]. We have reported that the repressed IL-1 gene in macrophages from tumour-bearing mice was activated towards normal expression after indomethacin treatment [15]. Similar findings have been observed in immune compromised cancer patients [16]. Therefore, indomethacin could improve immune status in tumour-bearing hosts. However, the indomethacin effect has also been reported in both NK-cell deficient as well as in T-cell deficient tumour-bearing animals [17]. Thus it is not certain that immune effects are involved [18], although several investigators have used indomethacin as an adjunct to experimental immunotherapy [19, 20]. A second explanation may be that prostaglandin blockade affects transformed cells directly although this is not confirmed [17]. It is more likely that indomethacin acts on tumour growth *in vivo* in association

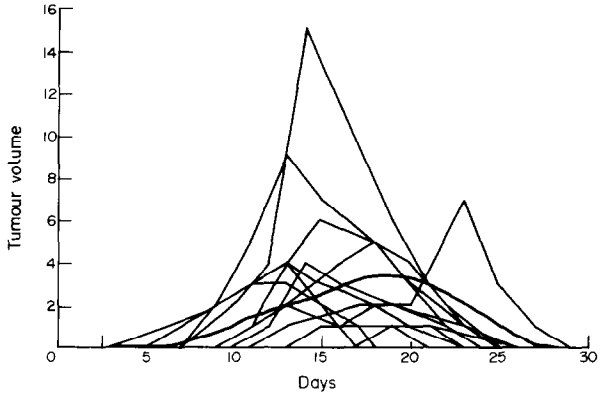

Fig.4. Change in tumour volume in tumour-bearing rats that responded to indomethacin with tumour disappearance = mean tumour volume.

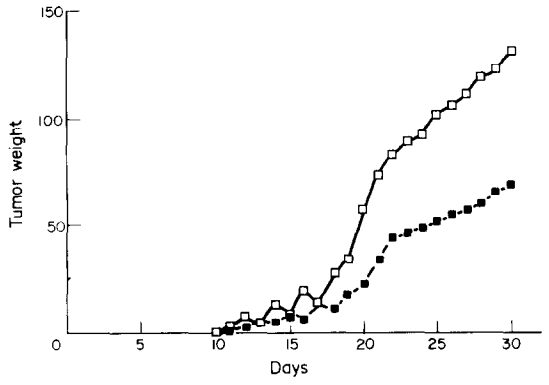

Fig.5. Change in tumour growth in all animals treated with indomethacin (■) compared with all untreated tumour-bearing rats (□). Growth curves were significantly different ($P < 0.01$). Tumour weight was calculated from tumour volume assuming that tissue density was close to 1.0.

with host factors that are not directly immune related [21]. Such factors may be the local repertoire of tumour and host produced growth factors. If so, this may represent a new area for tumour therapy [22, 23].

The presence and progressive growth of the malignant tumour caused the anorexia and decline in motor activity but had no effect on water intake. All these changes may be adaptive, to maximize survival in a tumour-bearing host. Anorexia and the subsequent decline in substrate flux may decrease DNA synthesis in rapidly proliferating transformed cells. We have found that this effect may be coupled to carbohydrates in the diet of tumour-bearing mice; an effect that is related to the energy charge of the tumour but is not necesarily the effect of insulin release after eating (Westin *et al.*, Sahlgrenska Hospital). Glucose is a limiting factor for survival in experimental cancer [24]. The decline in motor activity is sufficient to save energy in the tumour-bearing host. Others have suggested that the decline in motor activity is related to the mass of the tumour rather than to the malignancy itself [25]. This suggestion came from experiments with an expandable subcutaneous plastic device in tumour-bearing and sham-operated control rats. However, the surgical deposition of foreign material, although inert, could have elicited a local inflammatory response, which may be proportional to the extension of the subcutaneous mass. Inflammation and the local production of cytokines could influence energy expenditure and motor activity in the host. In our study, water intake did not change in tumour-bearing animals,

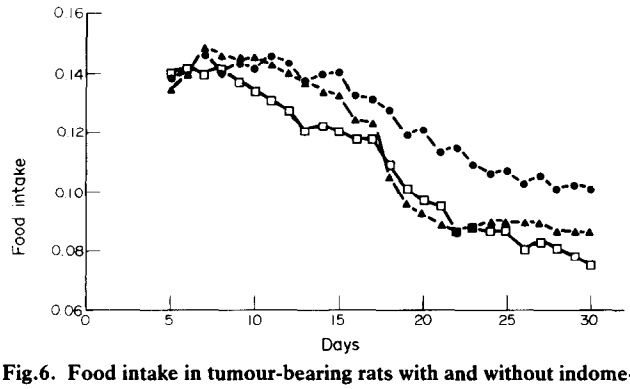

Fig.6. Food intake in tumour-bearing rats with and without indomethacin ($n = 18$ per group). Responders (●) differed from partial responders (▲) and untreated (□) tumour-bearing animals ($P < 0.02$).

which would have been rapidly critical for the host. The declines in motor activity and food intake were not 'unspecific' events due to inability or discomfort during motion, but rather a well-adapted event. Anyway, the thirst-drive overcame any such a disabling event.

Indomethacin did not prevent anorexia in tumour-bearing rats when accounting for the tumour growth inhibition in the responders. This finding may argue against the suggestion that cytokines are the major biochemical signals behind tumour-induced anorexia, or that prostaglandins of E family are the messenger secondary to cytokine activation in the central nervous system [26]. One study may support the last conclusion [27]. Cytokine receptors occur in the brain and direct infusions of recombinant molecules into the ventricular system have more effect than that after peripheral infusions [4]. In tumour-bearing mice we have not been able to document a significantly increased brain content of mRNA for IL-1 and TNF-α [28]. However, such animals have increased circulating levels of IL-6, which was not decreased by indomethacin treatment [29]. Therefore, a central effect of IL-6 may contribute to anorexia in tumour-bearing animals. IL-6 may be the factor behind the hepatic acute response in tumour-bearing animals, a response that cannot be inhibited significantly by indomethacin (unpublished results).

Our results agree with findings from similar experiments in tumour-bearing mice, a model in which the adults do not grow compared with tumour-bearing rats, which are growing hosts. Indomethacin inhibited tumour growth by mechanisms that are not understood. Secondary to the effect on tumour growth, indomethacin improved food intake and motor activity. Such actions might benefit cancer patients.

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Effect of Cyclosporin and Verapamil on the Cellular Kinetics of Daunorubicin

Xiu F. Hu, Mario de Luise, T.J. Martin and John R. Zalcberg

Both cyclosporin and verapamil modulate the multidrug-resistant (MDR) phenotype in the classical MDR cell lines, CEM/VLB100 and CEM/VLB1000. Initial studies demonstrated a significant reduction in daunorubicin accumulation in the two resistant lines compared with the drug-sensitive parent line CEM/CCRF. Both cyclosporin and verapamil increased drug accumulation in the resistant lines. This effect was dose-dependent although a plateau occurred in CEM/VLB100 cells at concentrations of cyclosporin exceeding 4.2 μmol/l. Cyclosporin 4.2 μmol/l and verapamil 10 μmol/l significantly increased daunorubicin uptake and reduced drug efflux in the CEM/VLB100 and CEM/VLB1000 lines. At low clinical concentrations of cyclosporin (0.8–1.6 μmol/l and verapamil (1–2 μmol/l), there was a synergistic increase in drug accumulation in the two resistant cell lines ($P < 0.007$). These data suggest that cyclosporin modulates the classical MDR phenotype by altering the cellular kinetics of daunorubicin. The *in vitro* synergistic action of cyclosporin and verapamil could be interesting clinically.
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INTRODUCTION

THE mutlidrug-resistant (MDR) phenotype has been described in several tumour types [1] and cell lines [2,3]. The classical MDR phenotype shows cross-resistance to several structurally unrelated cytotoxic drugs [2] and may be defined by the presence of P-glycoprotein [2,4], reduced cellular accumulation of drugs in drug-resistant variants [3] and modulation of resistance by drugs such as calcium-channel blockers [5] and calmodulin antagonists [5,6], which may increase drug accumulation. This accumulation is independent of changes in calcium metabolism