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Vinca Alkaloids: Anti-vascular Effects in a Murine Tumour

S.A. Hill, S. J. Lonergan, J. Denekamp and D. J. Chaplin

We have investigated the blood flow modifying effects of the vinca alkaloids, vincristine and vinblastine in the murine carcinoma CaNT. Vinblastine at doses of 7.5 or 10 mg/kg induced profound and chronic reductions in tumour blood flow as measured by ^{86}RbCl extraction. Following the maximum tolerated dose of 10 mg/kg, blood flow was reduced to 10% of pretreatment values after 2 h and remained below 20% of pretreatment values 24 h after drug administration. These findings are consistent with the early induction of necrosis by vinblastine and suggest that vascular-mediated cell death may account for a large part of the 11 day growth delay induced by this drug dose. In contrast to the large reductions in tumour blood flow, in skin, kidney, liver and muscle, blood flow reductions did not, at any time examined, exceed 40%. In all the normal tissues studied, blood flow had fully recovered by 6 h after vinblastine administration. Similar results, albeit less pronounced, have been obtained with vincristine at the maximum tolerated dose of 3 mg/kg. The results clearly show that both vinblastine and vincristine can induce, with some selectivity, a dramatic and prolonged reduction in tumour blood flow and that this may contribute to the anti-tumour effects against the CaNT tumour.
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INTRODUCTION

THE CONCEPT of attacking tumours indirectly via their vascular supply is receiving increasing attention as experimental studies reveal that many therapies can produce transient or permanent changes in tumour vascular function [1]. A prolonged blood flow reduction can lead to substantial tumour cell death as a result of induced ischaemia [2]. A reduction in the supply of oxygen and other nutrients will also result from a disrupted vascular supply. The ensuing increase in the levels of hypoxia and acidity within the tumour might be exploitable by the addition of bioreductive or chemotherapeutic drugs.

Therapies already identified as mediating their action, at least in part, via damage to the tumour vasculature include hyperthermia, photodynamic therapy and the cytokines TNFα (tumour necrosis factor), and interleukin 1 [3–6]. Flavone acetic acid (FAA) has also been found to have pronounced effects on vascular function in many experimental tumours [7–10], although this agent has not been shown to have clinical activity [11]. In view of the potential benefits of selectivity damaging tumour vasculature, there is a need to identify other agents which might mediate their effects in this manner.

To date, little attention has been paid to the possibility of the conventional chemotherapeutic drugs having a vascular

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component of damage, although vascular complications are associated with many of the agents in current use [12]. In our search for agents which may have a vascular mode of anti-tumour action, we identified vincristine and vinblastine as potential candidates. Published studies had indicated that these compounds induce a rapid reduction in cell yield (i.e. the number of cells recoverable by enzymatic procedures) in the B16 melanoma 18–24 h following drug treatment [13]. Since similar reductions in cell recovery occur with other therapies which induce tumour vascular damage and thus ischaemic cell death, detailed studies of the blood flow modifying effects of the vinca alkaloids appeared to be warranted. In addition, the more recent reporting of induced haemorrhagic necrosis in the colon 38 tumour following administration of vinblastine or vincristine, provided further indication that the anti-tumour effects of these agents could include a vascular mechanism [14]. The sequential injection of fluorescent markers indicated a reduction in tumour blood flow within 4 h of vincristine injection, albeit at toxic drug levels. Such vascular effects would be additional to the mechanism of *in vitro* cytotoxicity for these agents, known to be mediated via binding to tubulin and the induction of mitotic arrest [15]. The purpose of the present study was to evaluate the blood flow changes and necrosis induced in the murine CaNT mammary tumour and selected normal tissues following administration of vinca alkaloids.

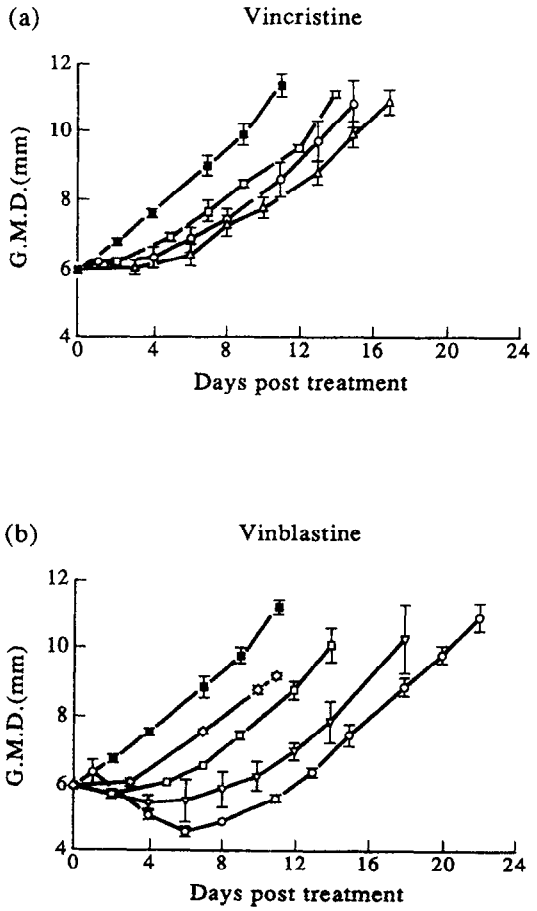

Fig. 1. Growth curves for untreated tumours (■) and tumours treated with (a) vincristine, □ 1 mg/kg; ○ 2 mg/kg; △ 3 mg/kg; or (b) vinblastine, ◇ 3 mg/kg; □ 5 mg/kg; ▽ 7.5 mg/kg; ○ 10 mg/kg. Errors are ± 1 S.E.M.

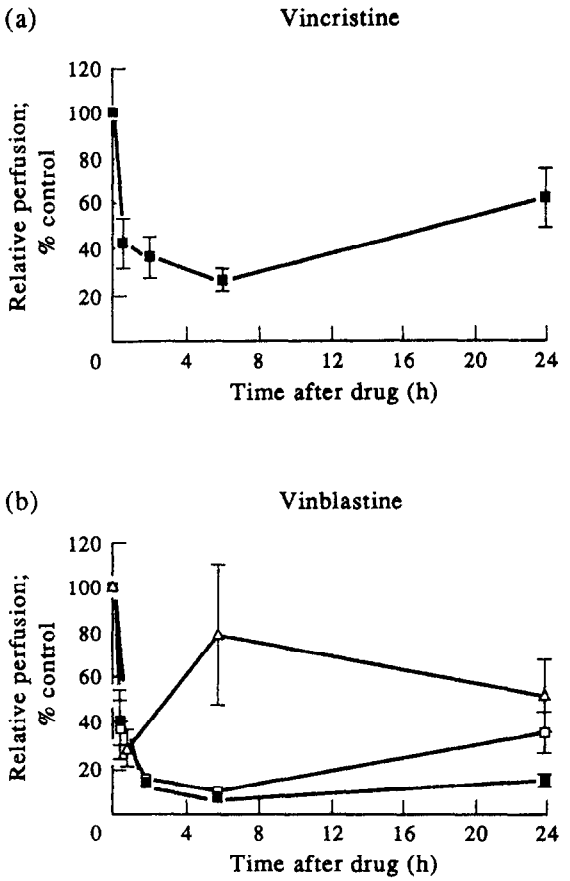

Fig. 2. Time course of blood flow changes (^{86}RbCl extraction) in CaNT tumours treated with (a) vincristine, 3 mg/kg; (b) vinblastine, △ 3 mg/kg; □ 7.5 mg/kg; ■ 10 mg/kg. All data are presented as a percentage of control values and errors are ± 1 S.E.M.

MATERIALS AND METHODS

Drugs

Vincristine and vinblastine (Eli Lilly and Co) were dissolved in bacteriocide as supplied by the manufacturers. Before administration, further dilutions were made in sterile water or 0.9% saline, respectively, allowing each dose to be given in 0.1 ml per 10 g body weight, injected intraperitoneally.

Tumours

The CaNT is a poorly differentiated corded adenocarcinoma, with a volume doubling time of approximately 3.5 days. It arose spontaneously more than 10 years ago and has since been maintained by serial passage in the syngeneic strain of origin. Subcutaneous tumours were initiated by injecting 0.05 ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12–16 week-old CBA/Gy f TO mice from a specific pathogen-free inbred colony. The animals were observed at regular intervals and selected for treatment after approximately 3–4 weeks, when their tumours reached a geometric mean diameter (GMD) of 5.5–6.5 mm (150–300 mg).

Tumour response

After treatment, tumours were measured 2 or 3 times a week in three orthogonal diameters, using vernier callipers. GMDs were calculated and mean growth curves of size against time were constructed for each treatment group of at least five mice.

Growth delays were calculated as the time required for treated tumours to grow to 3 mm larger than the original size at treatment minus the time for control tumours to do the same. An additional four sample tumours per group were excised 24 h after drug injection, fixed in formalin, embedded, sectioned and stained with haematoxylin and eosin for histological evaluation. Percentage necrosis was measured using a grid eyepiece graticule at 10 × magnification.

Relative blood flow was measured at various intervals after drug injection, in tumours and normal tissues of the same mice, by the ^{86}RbCl extraction technique [16, 17]. Five mice per group were injected intravenously with 185 kBq ^{86}RbCl (Amersham International plc) and killed 1 min later, whereupon the tissues were removed, weighed and counted for radioactivity. The radioactivity in the tissue as a percentage of the total activity injected (minus that remaining in the tail) gives a measure of perfusion as a function of cardiac output and is expressed per gram.

RESULTS

Figure 1 shows the growth response of tumours treated with increasing doses of vincristine and vinblastine. Untreated tumours took approximately 7 days to grow from 6–9 mm mean diameter. An additional 6 days delay in growth was measured following treatment with the maximum tolerated dose (MTD) of vincristine (3 mg/kg in both tumour-bearing and non-tumour-bearing mice). Vinblastine, at its MTD of 10 mg/kg was more effective at delaying tumour growth; producing a growth delay of 11 days. We have also evaluated the effects of vinblastine at 7.5 and 5 mg/kg, which produced growth delays of 9 and 5 days, respectively. The lowest dose tested, 3 mg/kg, was significantly less effective than the equimolar dose of 3 mg/kg vincristine, giving a growth delay of just 2 days.

Vincristine (3 mg/kg) and vinblastine (7.5–10 mg/kg) both induced significant tumour necrosis within 24 h of drug injection. The response to vincristine was variable, with levels of necrosis ranging from approximately 20–80% in different individual tumours. All of the tumours treated with vinblastine (7.5 or 10 mg/kg) appeared almost totally necrotic when examined histologically 24 h later. Occasionally, apparently viable cells formed a narrow rim at the tumour periphery or a cord of surviving cells was supported by a single blood vessel. Signs of vascular disruption were evident at earlier times after treatment. At 6 h, in addition to the numerous mitotic figures visible throughout the tumour, blood vessels were dilated and packed with red blood cells, while extensive areas of haemorrhage were apparent.

Drug induced changes in relative tumour perfusion are illustrated in Fig. 2, expressed as a function of control values. At equitoxic doses, both agents produced a very dramatic 60% reduction in tumour blood flow within 30 min of drug injection. Further decreases were measured to at least 6 h. Some recovery was seen by 24 h, but blood flow remained significantly lower than control levels. Equimolar doses caused a similar rapid reduction in tumour blood flow, but an early recovery was measured after vinblastine. The effect of MTDs of vincristine (3 mg/kg) and vinblastine (10 mg/kg) on blood flow in kidney, liver, muscle and skin was also assessed. The results are shown

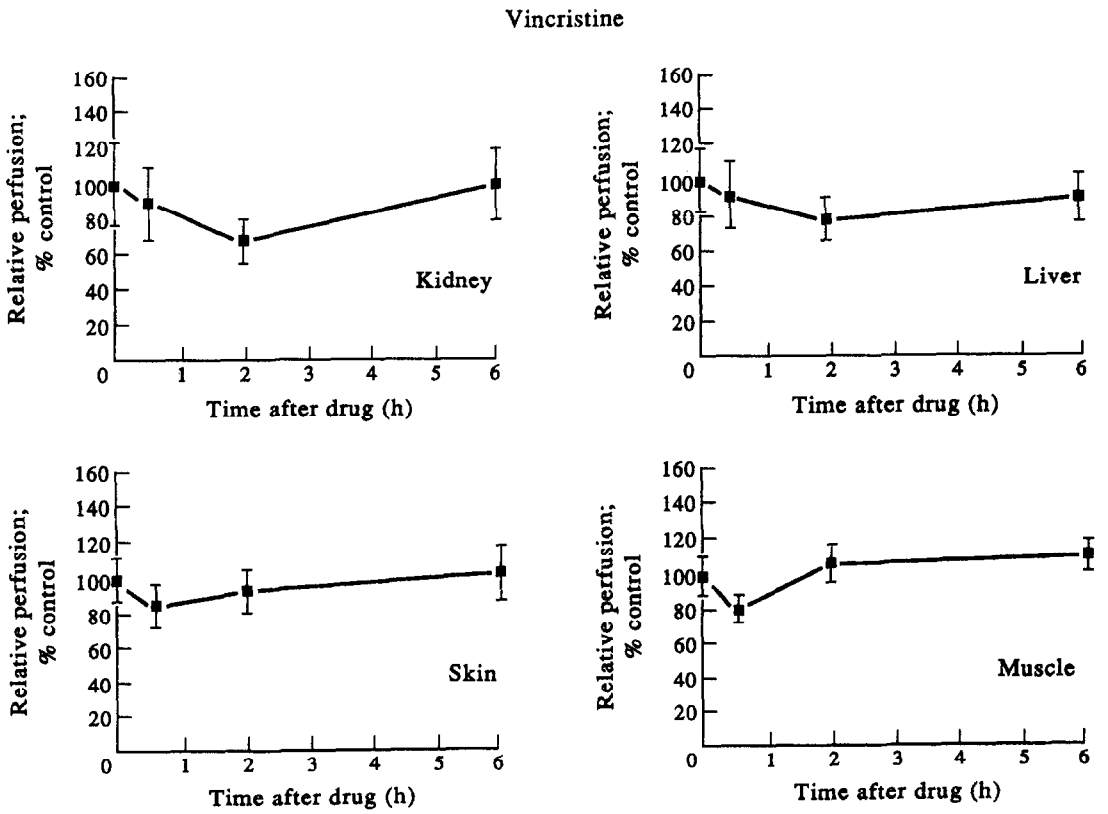

Fig. 3. The effect of 3 mg/kg vincristine on the relative perfusion of four normal tissues in CBA mice. Data are presented as a percentage of control values and errors are ± 1 S.E.M.

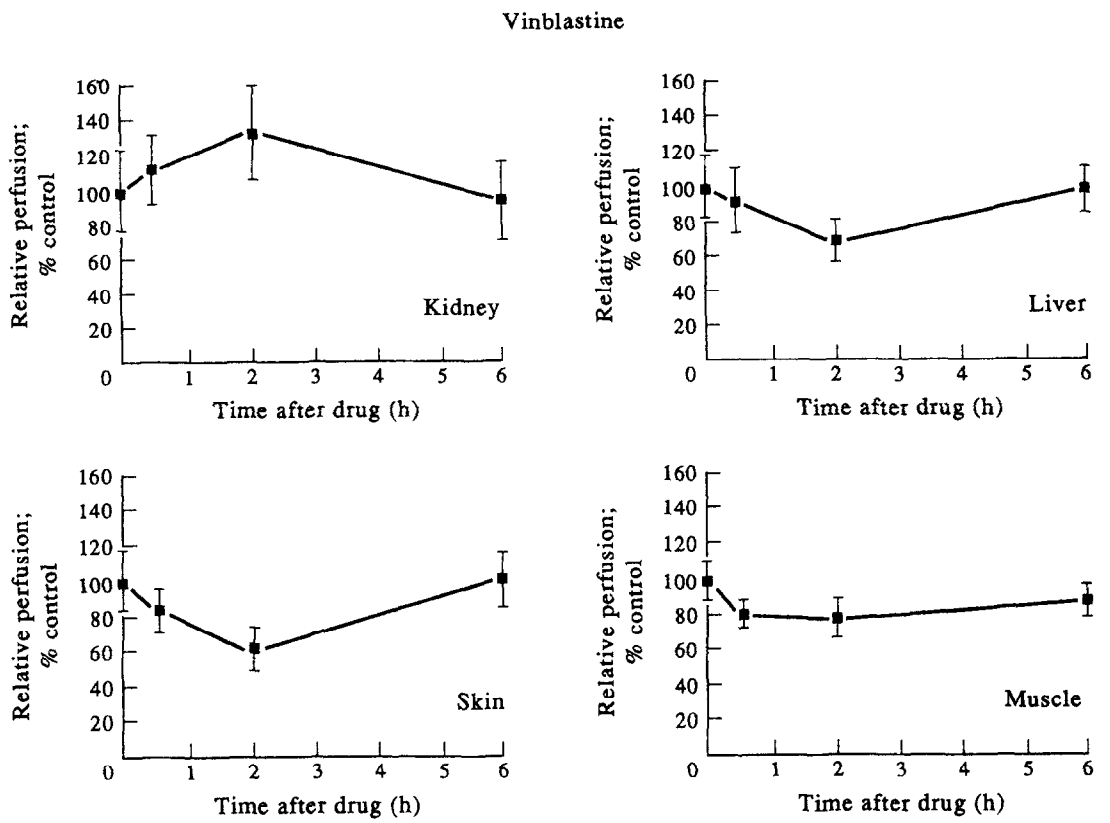

Fig. 4. The effect of 10 mg/kg vinblastine on normal tissue perfusion in CBA mice. Data are presented as a percentage of control values and errors are ± 1 S.E.M.

in Figs 3 and 4. In contrast to the tumour data, no dramatic reductions in blood flow were measured. Both drugs caused small (20–40%) transient blood flow reductions in all of the normal tissues examined, with the exception of kidney following vinblastine administration. In all cases, blood flow had returned to pretreatment values by 6 h following drug injection.

DISCUSSION

The vinca alkaloids form one of the main classes of plant alkaloids proven to have anti-cancer efficacy in mice and man. Their principal mode of action is thought to be due to their ability to bind to the intracellular protein tubulin, so inhibiting microtubule assembly and arresting the cells in mitosis. The data presented provide evidence that both vincristine and vinblastine may mediate their anti-tumour activity in part, via chronic impairment of blood flow.

The rapid induction of necrosis throughout large areas of a tumour has come to be recognised as a hallmark of vascular-mediated cell death [18]. Together with the earlier appearance of congested blood vessels and haemorrhage, this patchy, necrotic pattern has been described previously for TNFα [19] and for FAA [20], both of which were subsequently found to cause a significant and prolonged reduction in tumour blood flow [5, 7–10]. Recently, Baguley and co-workers have described a similar induction of haemorrhagic necrosis by vincristine and vinblastine [14]. This study has confirmed their observations in a further experimental solid tumour and moreover has detailed the time course of changes in tumour blood flow which may be responsible. In addition, we have evaluated normal tissue blood flow changes following administration of the vinca alkaloids.

Both vincristine and vinblastine cause a dramatic and prolonged decrease in blood flow in the CaNT, significantly greater than any change measured in normal tissue perfusion. Vincristine, however, was less effective at reducing tumour blood flow than vinblastine when equitoxic doses were compared and greater recovery was seen by 24 h. This pattern is consistent with the shorter growth delay measured after vincristine. That blood flow recovery may have occurred to a greater or lesser extent in individual tumours is indicated by the increased error on the 24 h blood flow measurement, and this may explain the variable levels of necrosis measured in vincristine-treated tumours.

The observation of apparently surviving cells at the very periphery of the tumour following vinblastine treatment is in common with the pattern seen after other therapies producing prolonged vascular impairment, specifically hyperthermia and FAA. This suggests a greater resistance of the blood vessels in this region of the tumour, possibly due to a greater resemblance to the normal vessels of the subcutis. This resistance of peripheral vessels results in tumour regrowth invariably occurring from this region and accounts for the relatively short regrowth times measured after vascular-mediated therapy, since 1–2 logs of cell kill might be expected to be compensated for by 3–6 volume doublings (taking 10–20 days in this tumour model).

A further similarity with FAA is the relatively narrow effective dose window seen with these agents. The profound vascular effects reported are for treatment at or near the MTD; these are higher doses than those used clinically and doses which cause significant toxicity in terms of weight loss and a general, although short-lived, deterioration in health.

The effects of vascular mediated anti-tumour agents in mice at doses close to the toxic limit may not be exploitable in the clinic. To date, FAA and TNF have failed to reproduce the dramatic responses observed in experimental rodent tumour

systems. However, the agents may not have been used in the optimal dose range or schedule required to elicit effects in human tumours. Certainly the maximum tolerated dose of TNF in patients produces circulating levels below those associated with regression of experimental tumours in mice [21]. In addition, the clinical administration of FAA as a 1 or 6 h infusion differs from the bolus injection used for most experimental studies in rodents [22]. Because of the potential utility of agents which can selectively damage tumour vasculature, there is a clear need to derive more potent analogues of existing agents or identify new classes of agents. The current studies provide further evidence that the vinca alkaloids represent an established class of chemotherapeutic agents with potential vascular damaging properties.

In all previous studies, the vinca alkaloids have been monitored in terms of their ability to prevent chromosomal separation at metaphase, which at sufficiently high doses is irreversible and leads to cell death [15]. The anti-vascular activities may have a similar structure activity relationship or may be influenced by quite different molecular configurations. In order to investigate this, analogues would have to be tested *in vivo* as well as *in vitro*, with solid tumours being an essential part of the study. If the mechanism of anti-vascular activity is found to be different for the various classes of agents (e.g. cytokines, FAA and the vinca alkaloids) it is quite possible that combinations of drugs with synergistic anti-tumour effects but without overlapping toxicities could be developed. This, in turn, could facilitate the translation of the dramatic responses seen in mice into clinical reality.

Further efforts are now required to understand the mechanism of the anti-vascular action of the vinca alkaloids and to assess the damaging effects of both structural analogues and other tubulin binding agents. These studies will need to be carried out in solid tumour systems in rodents. Although for practical reasons transplantable tumour systems have to be used for such assessment, there is also a need to investigate the actions of potential vascular damaging agents in spontaneous tumour systems. Despite the fact that no studies with vascular damaging therapies have been performed in spontaneous rodent tumours, limited information obtained using nuclear magnetic resonance spectroscopic techniques has indicated that such tumours may respond differently to the vasoactive drug hydralazine than their transplanted counterparts [23, 24].

In conclusion, we have established that both vincristine and vinblastine, at doses close to their MTD, induce large and prolonged blood flow reductions in the CaNT tumour. These reductions are consistent with the rapid induction of necrosis in this tumour following drug treatment. Further studies with vinca alkaloids and other tubulin binding agents in both transplanted and spontaneous experimental tumour systems is now warranted. The identification of anti-vascular effects in a group of compounds structurally diverse from the flavones and xanthenones, enhances the prospects of establishing an 'armoury' of chemotherapeutic agents which mediate their anti-tumour action via the vascular system.

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