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Comparative Antitumour Activity of Vinblastine-isoleucinate and Related Vinca Alkaloids in Human Tumour Xenografts

Hans R. Hendriks, Simon Langdon, Dietmar P. Berger, Knut Breistøl, Heinz H. Fiebig, Øystein Fodstad and Gilberto Schwartsmann

The antitumour activity of the investigational agent vinblastine-isoleucinate (V-LEU) was compared with vintriptol, another investigational agent of the same series of vinblastine-23-oyl amino acid derivatives, and vinblastine, their clinically active parent compound, in a panel of nine human tumour xenografts growing subcutaneously in nude mice. Compounds were administered intravenously at equitoxic doses twice weekly. As assessed by optimal tumour growth inhibition and tumour growth delay, vinblastine, V-LEU and vintriptol exhibited antitumour activity in 8/9, 7/9 and 4/7 human tumour xenografts, respectively. When growth curves and numbers of complete remissions were compared, V-LEU was the most active agent in two malignant melanoma lines (THXO and LOX p28) and two small cell lung carcinoma lines tested (LXFS 538 and WX 322), whereas vinblastine was more active against the two colorectal carcinomas (CXF 243 and CXF 280). Notably, the non-small cell lung carcinoma (NSCLC) line AHXOL was resistant to the three agents. The results of this study suggest that V-LEU was as active as vinblastine in most tumour lines, exhibiting superior antitumour activity in malignant melanoma, SCLC and breast cancer lines. The decision to bring this compound into clinical trial shall await further confirmation of these preclinical results and the evaluation of its toxicity profile in relation to other vinca alkaloids.
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INTRODUCTION

VINCA ALKALOIDS such as vinblastine and vincristine are widely used in cancer chemotherapy, especially in the management of acute lymphocytic leukemia, Hodgkin's disease, non-Hodgkin lymphoma, germ-cell tumours and breast carcinoma [1]. Both compounds possess a dimeric structure composed of vindoline and catharanthine units linked together by a carbon–carbon bridge. Vinblastine and vincristine differ structurally only in a functional group bound to catharanthine. This minor distinction results in different antitumour spectra and toxicities [2]. Antitumour activity of vinca alkaloids is dependent upon their binding to tubulin and consequent depolymerisation of microtubules. The inhibition of microtubule formation leads to a block in mitosis, disorganisation of the cytoskeleton and changes in axonal transport [2–5].

In humans, neurotoxicity is the main dose-limiting toxicity of vincristine, whereas vinblastine produces myelosuppression in most cases [6, 7]. Thus, the development of new vinca derivatives aims usually at compounds which have either superior antitumour activity or a different toxicity pattern.

From a series of vinblastine-23-oyl aminoacid derivatives evaluated preclinically, two compounds, vinblastine-isoleucinate (V-LEU) and vintriptol have shown greater antitumour activity in P388 leukaemia *in vivo* compared with the parent compound vinblastine. V-LEU possesses an isoleucinate ethyl ester and vintriptol has a tryptophan ethyl ester linked to the amino ester of the vinblastine-23-oyl moiety [8].

In preliminary experiments, the preclinical antitumour profiles of vinblastine and vintriptol were almost identical. Both compounds were active in colon 26 and B16 melanoma, marginally active in L1210 leukaemia and inactive against Lewis lung carcinoma. In contrast, V-LEU proved to be more active than vinblastine and vintriptol in several tumour lines, such as P388 leukaemia, B16 melanoma and 6C3 Med lymphosarcoma [8, 9]. On the basis of this potentially superior preclinical antitumour activity against murine tumours, V-LEU was further investigated *in vivo* in comparison with the above-mentioned agents in a panel of nine human tumour xenografts (HTX) comprising various solid tumour types.

MATERIALS AND METHODS

Animals

Female nude mice of different background (NRMI nu/nu, Balb/c nu/nu, mixed background), dependent on the testing laboratory, were used. Mice were kept under specified pathogen free conditions. Food and water were supplied *ad libitum*.

Tumour lines

Selection of human tumour lines was based on differences in histology, growth characteristics and sensitivity to vinca alkaloids, if known (Table 1). Details on the characterisation of the tumour cell lines and the xenograft model have been reported previously [10–14].

Correspondence to H.R. Hendriks.
H.R. Hendriks and G. Schwartsmann are at the EORTC New Drug Development Office, Free University Hospital, De Boelelaan 1117, NL-1081 HV Amsterdam, The Netherlands; S. Langdon is at the ICRF Medical Oncology Unit, Western General Hospital, Edinburgh, U.K.; D.P. Berger and H.H. Fiebig are at the Department of Internal Medicine I, Albert-Ludwigs Universität, Freiburg, F.R.G.; and K. Breistøl and Ø. Fodstad are at the Department of Tumor Biology, The Norwegian Radium Hospital, Oslo, Norway.
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Table 1. Characteristics of HTX

Tumour line	Histology	Tumour doubling time	Latency period (days)	Sensitivity to*: Vinblastine	Vincristine	Vindesine	Doxorubicin†	Cisplatin†	Ref.
Breast cancer									
MAXF 401	adeno. pap.	4–10	17–25		−	−	−	++++	11
MAXF 583	adeno. mod. diff.	6–12	27–34		−	++		++++	11
Colorectal cancer									
CXF 243	adeno.	5–7	18–20			−	−	−	11
CXF 280	adeno. undiff.	3–5	14–18			−	+	+++	11
Melanoma									
THXO	amelanotic	6–7	14–16	+‡			(+)	(+)	
LOX p28	amelanotic	3	8–10	+‡			+++		13
Non small cell lung cancer									
AHXOL p27	large cell	9–12	38–54				(+)	−	
Small cell lung cancer									
LXFS 538	small cell	7–13	30–45		−	+++	+++	++	11
WX 322	small cell	4–9	20–40		++‡		(+)	−	14

* Treatment results of previous experiments based on tumour growth curves:
− Progression ≥ 125% of initial tumour size; ± No change 76–125%; + Minor regression 51–75%; ++ Partial remission 20–50%; +++ Complete remission < 20%.

† Treatment results from other EORTC-NDDO studies based on the following efficacy criteria:

	T/C%		SGD
(+)	< 50	or	> 1.0
+	< 50	and	> 1.0
++	< 40	and	> 1.5
+++	< 25	and	> 2.0
++++	< 10	and	> 3.0

‡ Based on T/C% values (− T/C > 50%, + T/C < 50%, ++ T/C < 25%).

In vivo antitumour activity studies

Fragments of HTX were studied as subcutaneous implants in both flanks of nude mice. Tumour growth was assessed weekly by caliper measurements of the tumour in two dimensions. Treatment was started in a randomised fashion when tumours had reached a median tumour diameter of 5–6 mm; tumours smaller than 4 mm in diameter (or minimum volume of 30 mm^3) at the start of treatment were excluded from the final analysis. Each treatment group and control group consisted of 5–9 mice bearing in total 4–16 evaluable tumours. Prior to the start of treatment, mice were selected according to tumour volumes and assigned to groups in order to get an equal distribution of tumour volumes in the different groups. Thereafter, drug treatment and control groups were assigned randomly.

Tumour volume was calculated according to the formula $0.5 \times \text{length} \times \text{width}^2$. Relative tumour volumes (RTV) were calculated for each single tumour by dividing the tumour volume on day X by the tumour volume on day 0 at the start of treatment:

$$\text{RTV} = \frac{\text{Volume tumour day } X}{\text{Volume tumour day 0}} \times 100 .$$

Median RTV values were used for drawing growth curves and calculating treatment efficacy. Tumour doubling time (TD) of test and control groups was defined as the period required to reach a median RTV of 200% and 400%.

Treatment efficacy was assessed by three evaluation criteria used in parallel: specific growth delay (SGD), optimal growth inhibition (T/C%) and tumour growth curve. The SGD was calculated with regard to the TD of test and control groups:

$$\text{SGD} = \frac{\text{TD treated} - \text{TD control}}{\text{TD control}} .$$

Optimal growth inhibition was calculated with regard to the RTV of treated groups versus control:

$$\text{T/C\%} = \frac{\text{RTV treated}}{\text{RTV control}} \times 100\%.$$

Deaths occurring within 2 weeks after the last day of treatment were considered as toxic deaths. These mice were excluded from any evaluation in the study.

Table 2. Efficacy of vinblastine (intravenously) in panel of HTX in nude mice

Tumour type	Dose (mg/kg)	Schedule (day)	No. of tumours	Max BW* loss (%)	Toxic death	Optimal T/C (%)	Day	SGD 1–2	SGD 1–4	CR
Breast cancer										
MAXF 401	6	0,7	10	−10.0	1/7	30.9	21	1.76	0.94	0/10
MAXF 583	6	0,14	12	− 7.4	2/8	2.5	21	17.78	NA	3/12
Colorectal cancer										
CXF 243	6	0	10	−18.8	1/6	28.1	14	2.10	1.01	0/10
CXF 280	6	0,7	9	−14.5	1/7	4.0	21	4.72	2.40	0/9
Melanoma										
THXO	6,4,4	0,7,8	10	− 6.4	0/8	6.5	12	9.23	4.52	0/10
LOX p28	4	0,7,8	11	−12.7	0/6	14.5	8	2.12	5.06	0/11
Non small cell lung cancer										
AHXOL p27	6	0,7,8	16	− 2.5	0/8	84.5	14	0.20	0.02	0/16
Small cell lung cancer										
LXFS 538	4	0,7,8	4	− 7.9	3/7	5.8	22	NA	NA	0/4
WX 322	6	0,7	7	− 8.4	2/7	0.0	18	NA	NA	6/7

* Maximum body weight loss.
CR = complete remission; NA = not applicable.

Drugs

V-LEU (CT 02-0015), vinblastine sulphate (CT 02-0001) and vintriptol (CT 02-0017) were supplied by MEDGENIX (Fleurus, Belgium). Vials contained 5 mg V-LEU, 10 mg vinblastine and 50 mg vintriptol without excipient. Vials were stored at 2–8°C in the dark. The content of the vials was dissolved in sterile 5% glucose. Reconstituted dilutions were kept at 4°C protected from light. Fresh solutions were prepared each time immediately before use.

Determination of the maximum tolerated dose

Drugs were administered to tumour bearing nude mice at equitoxic dose levels allowing a median body weight loss of about 15% of the initial weight within 2 weeks after the first injection and toxic death in one third of the animals per treatment group.

Prior to the start of the main study, dose finding studies in non-tumour bearing nude mice were carried out by a testing laboratory in order to determine the maximum tolerated dose (MTD) for each drug (intravenously, day 0 and 7). Next the other testing laboratories performed additional dose finding studies to adjust the dose level of each drug to their own nude mouse strain.

Drug treatment

Each treatment group consisted of 5–9 tumour bearing nude mice. The following groups were studied: (1) Control, day 0 and 7, 5% glucose administered intravenously in the same volume as given in the dose group with the highest dosing volume; (2) V-LEU, day 0 and 7, 12 mg/kg/injection intravenously; (3) vinblastine, day 0 and 7, 6 mg/kg/injection (parent compound) intravenously; (4) vintriptol, day 0 and 7, 80 mg/kg/injection intravenously.

RESULTS

Dose-finding studies were carried out by a testing laboratory in order to establish the MTD for vinblastine, V-LEU and vintriptol. The compounds were injected at different dose levels intravenously into non-tumour bearing female nude mice of mixed background (4–6 mice per dose level) at days 0 and 7. After dosing body weight and toxic death were recorded. From each dose group the mean body weight was determined at each day of measurement.

On the basis of numbers of toxic deaths and body weight loss in non-tumour bearing nude mice, the following dose was selected as MTD for tumour bearing nude mice: vinblastine 6 mg/kg/injection, V-LEU 12 mg/kg/injection and vintriptol 80 mg/kg/injection. The other testing laboratories subsequently used these dose levels to adjust the MTD for each of the compounds in their own nude mouse strain.

The MTD for vinblastine and V-LEU in the tumour bearing nude mice did not significantly differ from the ones established in the dose finding studies (Tables 2 and 3). Vintriptol, however, showed a broader dose range (Table 4). Occasionally the dose or the schedule of the drugs were adjusted due to large body weight loss and general conditions of the mice after the first drug injection. The results of vintriptol in both colorectal lines were non-evaluable, because the dose levels used in those experiments appeared to be toxic.

On the basis of the values of T/C% and SGD 8/9 HTX responded to one or more study drugs being either marginally sensitive or very sensitive (Table 5). The non-small cell lung cancer AHXOL was the only resistant line to all three drugs tested. 60% of the evaluable number of HTX (4/7) responded to vintriptol, 80% (7/9) were sensitive to V-LEU and 90% (8/9) were sensitive to vinblastine.

In breast cancer MAXF 583, 11/13 tumours disappeared

Table 3. Efficacy of V-LEU (intravenously) in panel of HTX in nude mice

Tumour type	Dose (mg/kg)	Schedule (day)	No. of tumours	Max BW* loss (%)	Toxic death	Optimal T/C (%)	Day	SGD 1–2	SGD 1–4	CR
Breast cancer										
MAXF 401	12	0,7	9	−19.0	2/7	25.1	21	2.19	1.11	0/9
MAXF 583	12	0,14	9	−11.2	3/8	0.2	21	NA	NA	9/9
Colorectal cancer										
CXF 243	12	0	9	−10.9	2/7	50.5	14	1.10	0.61	0/9
CXF 280	12	0,7	8	−20.5	2/7	12.6	21	3.70	2.05	0/8
Melanoma										
THXO	8	0,7	11	− 5.4	0/8	5.1	12	NA	NA	5/11
LOX p28	8	0,7	10	− 5.2	0/6	3.6	8	11.82	5.85	0/10
Non small cell lung cancer										
AHXOL p27	12	0,7	16	− 2.6	0/8	78.8	14	0.25	−0.07	0/16
Small cell lung cancer										
LXFS 538	8	0,7	6	− 4.7	1/7	1.9	29	NA	NA	1/6
WX 322	12	0,7	11	− 9.6	0/7	0.0	16	NA	NA	11/11

* Maximum body weight loss.
CR = complete remission; NA = not applicable.

Table 4. Efficacy of vintriptol (intravenously) in panel of HTX in nude mice

Tumour type	Dose (mg/kg)	Schedule (day)	No. of tumours	Max BW* loss (%)	Toxic death	Optimal T/C (%)	Day	SGD 1–2	SGD 1–4	CR
Breast cancer										
MAXF 401	55	0,7	12	−15.2	1/7	17.5	28	2.85	1.49	0/12
MAXF 583	40	0,14	13	− 4.7	1/8	0.3	21	NA	NA	11/13
Colorectal cancer										
CXF 243	55	0	—	—	7/7	Toxic				
CXF 280	80	0	—	—	4/5	Toxic				
Melanoma										
THXO	60	0,7	12	− 6.0	0/9	23.9	12	0.50	0.45	0/12
LOX p28	60	0,7	10	− 8.6	0/7	9.5	8	3.82	3.15	0/10
Non small cell lung cancer										
AHXOL p27	80	0,7	15	—	0/8	59.9	14	0.40	−0.05	0/15
Small cell lung cancer										
LXFS 538	60	0,7	7	−15.6	2/7	4.4	18	4.44	1.91	0/7
WX 322	80	0,7	9	− 5.6	0/6	50.7	14	0.95	0.41	0/9

* Maximum body weight loss.
CR = complete remission; NA = not applicable.

Table 5. Activity rating of V-LEU, vinblastine and vintriptol in a panel of HTX in nude mice

Tumour type	V-LEU	Vinblastine	Vintriptol
Breast cancer			
MAXF 401	++/+++	++	+++
MAXF 583	++++	++++	++++
Colorectal cancer			
CXF 243	(+)	++	toxic
CXF 280	+++	++++	toxic
Melanoma			
THXO	++++	++++	(+)
LOX p28	++++	++++	++++
Non small cell lung cancer			
AHXOL p27	—	—	—
Small cell lung cancer			
LXFS 538	++++	++++	++++
WX 322	++++	++++	—
Active/total	7/9 (78%)	8/9 (89%)	4/7 (57%)

For efficacy criteria see footnote to Table 1.

completely after vintriptol administration. Regrowth did not occur in seven tumours during the rest of the observation period and four tumours relapsed. In the same tumour cell line, V-LEU produced 9/9 complete responses and no relapses were observed up to the completion of the study. Vinblastine induced only 3/12 complete responses of which one tumour relapsed (Table 6).

The antitumour activities of vinblastine and V-LEU were

Table 6. Complete responses (CR) induced by V-LEU, vinblastine and vintriptol in HTX

			No relapse		Relapse	
Tumour type	Drug	No. of CR/total*	Tumour number	Follow-up days	Tumour number	After days
Breast cancer						
MAXF 583	V-LEU	9/9	9	14–56	—	—
	Vinblastine	3/12	2	28–35	1	21
	Vintriptol	11/13	7	14–56	4	14–42
Melanoma						
THXO	V-LEU	5/11	4	17–24	1	12
Small cell lung cancer						
LXFS 538	V-LEU	1/6	1	11	—	—
WX 322	V-LEU	11/11	8	39–46	3	2–39
	Vinblastine	6/7	—	—	6	2–39

* Volumes at start of treatment: 30–500 mm³ (85% of numbers of tumours > 50 mm³).

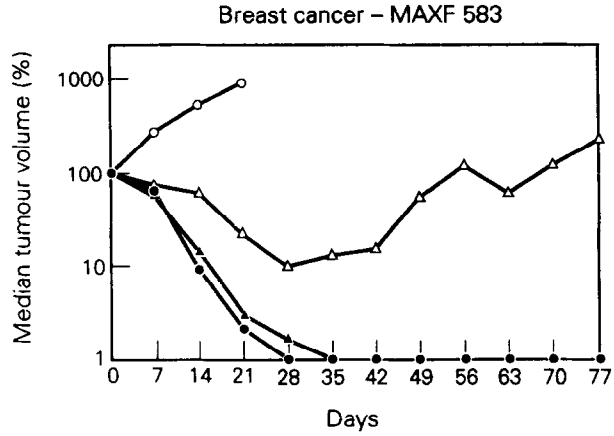

Fig. 1. Growth curve of HTX in nude mice. Tumour bearing mice were divided into groups for control (○–○) or intravenous treatment of: V-LEU 12 mg/kg, days 0 and 14 (●–●); vinblastine 6 mg/kg, days 0 and 14 (△–△); and vintriptol 40 mg/kg days 0 and 14 (▲–▲).

comparable in several tumour cell lines included in the panel (Table 5). Differences were usually marginal, because most tumour lines were sensitive to both compounds. The two colorectal lines (CXF 243 and CXF 280) responded better to vinblastine compared with V-LEU (Table 5). Of interest, however, was the observation that V-LEU was more active than vinblastine against a breast cancer line (MAXF 583), both melanomas (THXO, LOX) and the two small cell lung cancers LXFS 538 and WX 322 included in the panel. In these five lines V-LEU induced tumour regressions (Figs 1–5) and caused long-lasting complete remissions in four HTX (MAXF 583, THXO, LXFS 538, WX 322).

DISCUSSION

The search for new vinca alkaloids is mainly focused on the development of analogues with higher and/or distinct antitumour activity, lack of cross-resistance to standard agent and a favourable toxicity profile to the normal tissues. V-LEU and vintriptol are both newly synthesised vinca alkaloids, which were developed having vinblastine as the lead compound.

Vintriptol was evaluated preclinically, proving to be equally effective as vinblastine in several murine tumour lines. However, the toxicity of vintriptol seemed to be less severe compared with

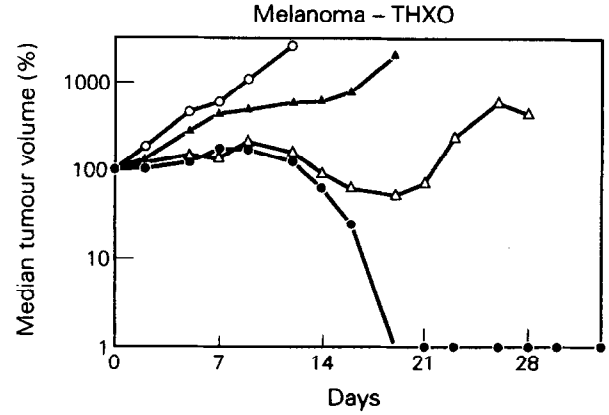

Fig. 2. Growth curve of HTX in nude mice. Tumour bearing mice were divided into groups for control (○–○) or intravenous treatment of: V-LEU 8 mg/kg on days 0 and 7 (●–●); vinblastine 6 mg/kg on days 0 and 7 (△–△); and vintriptol 60 mg/kg on days 0 and 7 (▲–▲).

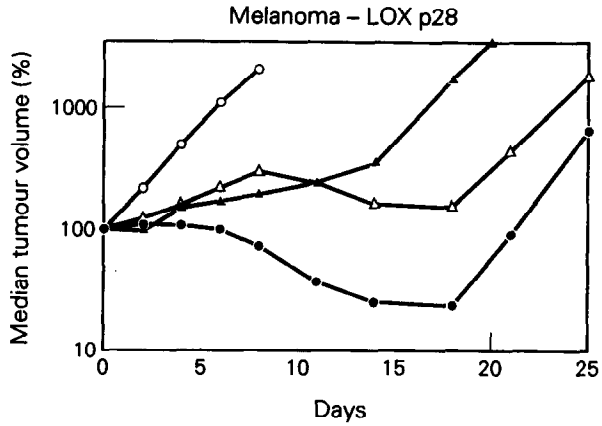

Fig. 3. Growth curve of HTX in nude mice. Tumour bearing mice were divided into groups for control (○–○) or intravenous treatment of: V-LEU 8 mg/kg on days 0 and 7 (●–●); vinblastine 4 mg/kg on days 0, 7 and 8 (△–△); and vintriptol 60 mg/kg on days 0 and 7 (▲–▲).

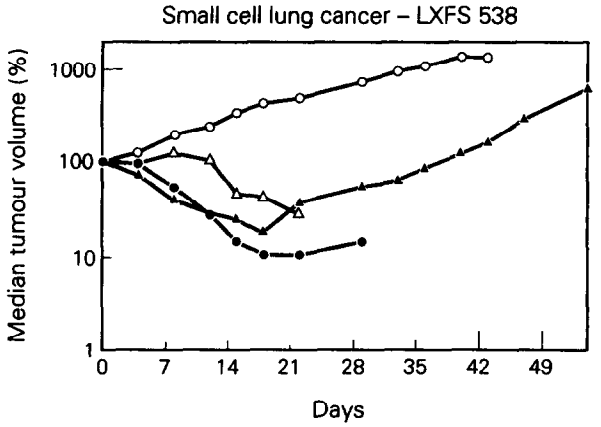

Fig. 4. Growth curve of HTX in nude mice. Drug administrations and symbols as in Fig. 3.

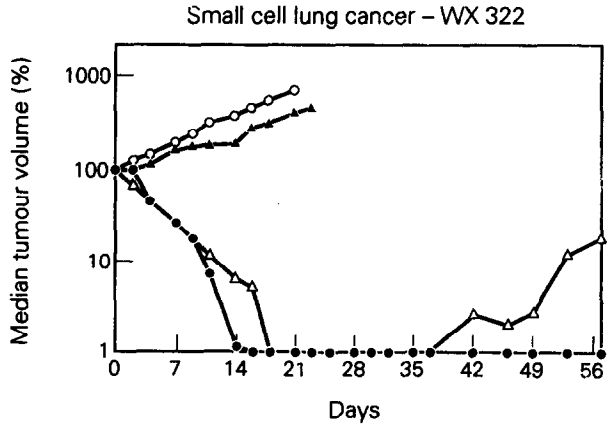

Fig. 5. Growth curve of HTX in nude mice. Tumour bearing mice were divided into groups for control (○–○) or intravenous treatment of: V-LEU 12 mg/kg on days 0 and 7 (●–●); vinblastine 6 mg/kg on days 0 and 7 (△–△); and vintriptol 80 mg/kg on days 0 and 7 (▲–▲).

vinblastine. For that reason, the compound was brought into clinical evaluation. In phase I trials, the toxicity of vintriptol is mainly associated with the bone marrow, with no significant neurological side-effects being demonstrated [15, 16]. Clinical trials of vintriptol are ongoing.

In this paper, the antitumour profile of V-LEU was evaluated in a panel of human solid tumours growing subcutaneously in athymic mice in comparison to vinblastine and vintriptol. The performance of other standard cytotoxic agents active in the clinic, such as doxorubicin and cisplatin, was previously studied in the same panel of tumour lines. Doxorubicin was active against 3/8 lines, whereas cisplatin showed activity in 4/7 of them (Table 1).

Both vinblastine and V-LEU were highly active in the panel, producing objective tumour regression in 8/9 and 7/9 tumour lines, respectively. In contrast, vintriptol was only active against 4/7 tumour lines (Tables 2–4). Notably, V-LEU was highly active against breast cancer, melanomas and SCLC lines included in the study, producing long-lasting complete regressions in some animals (Figs 1, 2, 5).

The finding that V-LEU showed superior antitumour activity compared with vinblastine in breast tumour cell lines is of potential clinical interest, considering that vinblastine is a useful agent in the treatment of patients with breast cancer. Similarly, V-LEU showed higher antitumour activity than vinblastine in SCLC lines, which is a tumour type also sensitive to vinca alkaloids in the clinic. Considering that all three agents were evaluated at equitoxic doses, the impressive activity of V-LEU in the above-mentioned tumour types deserves further evaluation. Should the above results of V-LEU be confirmed in further experiments, toxicological studies are to be considered aiming at its future clinical evaluation.

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Cytochrome *c* Oxidase and Purine Nucleotides in Skeletal Muscle in Tumour-bearing Exercising Rats

Peter Daneryd, Ingvar Karlberg, Tore Scherstén and Bassam Soussi

We have previously shown that spontaneous physical exercise can delay the onset of experimental anorexia and cachexia and retard tumour growth and we now report the effects on the energy metabolism in skeletal muscle. Exercising tumour-bearing animals (TBE) had an increased maximal capacity for oxygen uptake expressed as V_{max} of the cytochrome *c* oxidase compared with their tumour-bearing sedentary controls (TBS) [mean (S.E.) 289.9 (30.7) vs. 141.6 (11.0); $P < 0.05$] but an unchanged K_m value. The TBS animals had a depressed V_{max} as compared with non-tumour-bearing sedentary controls (CS) [141.6 (11.0) vs. 210.1 (15.1); $P < 0.05$]. Most of the purine nucleotides in the 'glycolytic' anterior tibial muscle were significantly altered in the TBE animals compared with the TBS animals, but in the mainly 'oxidative' soleus muscle only the level of inosine monophosphate (IMP) was changed. The results indicate that physical exercise can normalise the oxidative capacity and improve the energy state in skeletal muscle in the tumour-bearing host.
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INTRODUCTION

IT HAS long been known that endurance training increases the oxidative capacity in skeletal muscle [1, 2]. It is also known that skeletal muscle metabolism is greatly altered in response to cancer anorexia and cachexia [3, 4]. Less known, however, is the effect of spontaneous physical exercise on the oxidative capacity in skeletal muscle in the tumour-bearing host. We have shown that the turnover number of cytochrome *c* oxidase (E.C.1.9.3.1), the terminal component of the mitochondrial respiratory chain, is increased in rats subjected to standardised physical exercise [5], and reduced in ischaemic and reperfused skeletal muscle tissue [6]. More recently, we have shown that spontaneous physical exercise in an experimental model with tumour-bearing rats could delay the onset of anorexia and cachexia and retard tumour growth [7]. This animal model is in contrast with most of the earlier work in the field with semi-voluntary or forced physical exercise in order to standardise the procedure [7].

Correspondence to Bassam Soussi.
The authors are at the Bioenergetics Group, Wallenberg Laboratory, Department of Surgery, University of Göteborg, Sahlgrenska Hospital, S-413 45 Göteborg, Sweden.
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The abnormality in the energy metabolism of the tumour-bearing host is characterised by an increase in energy expenditure [8–10] that results in a cumulative negative energy balance with energy depletion. The adverse effects of a reduced caloric intake are added to this metabolic derangement. Provision of extra calories in order to modify the derangement is often unsuccessful and might even result in adverse effects on the tumour host metabolism [11, 12] as well as an increase in the tumour burden [13]. The peripheral metabolic alterations that facilitate gluconeogenesis tend to parallel the increased tumour burden.

The finding that tumour-bearing animals increased their food intake in response to physical exercise without increased tumour growth and with preserved skeletal muscle mass raised the question whether this exercise could normalise tumour induced changes in oxidative capacity and energy metabolism in skeletal muscle. In this paper we present a kinetic analysis of the cytochrome *c* oxidase reaction and an evaluation of the purine nucleotides in this experimental model with freely-moving tumour-bearing rats.

MATERIALS AND METHODS

Animal and tumour model

Female Wistar Furth rats were used. All experiments were performed in growing animals and they were allocated to the