Some Inhibitory Effects of (—)-Emetine on Growth of Ehrlich Ascites Carcinoma

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(—)-Emetine has little or no effect on O_2 consumption of Ehrlich ascites-cell suspensions or on the viability of transplanted Ehrlich ascites-tumour cells exposed to, or incubated with, the drug *in vitro* before inoculation into new-host mice. (—)-Emetine administered as a single injection to mice bearing Ehrlich ascites-tumour cells slows the growth rate of the tumour. The subcutaneous or intraperitoneal injection of the drug depresses protein and DNA synthesis of the tumour cells *in vivo* in a reversible manner. Mice bearing ascitic sarcoma 180 or Ehrlich ascites-tumour cells when given a course of treatment with (—)-emetine or (—)-*O*-methyltubulosine have a substantially lower tumour load than untreated controls and correspondingly longer survival times.

Ipecac and (—)-emetine, its active principle, have long been recognized as specifics in the treatment of acute amoebic dysentery and amoebic hepatitis (Hunter *et al.*, 1966).

The chemotherapeutic range of (—)-emetine and some of its derivatives has also been extended to other conditions (Melchior, 1954; Grollman, 1965; Abd-Rabbo & Montasir, 1967). When (—)-emetine was screened for antitumour activity and was found to produce degenerative changes in sarcoma 37 cells *in vitro* (Hartwell *et al.*, 1946; McConnell *et al.*, 1947), this was not followed up since the drug had only marginal activity in prolonging the survival time of mice bearing various tumours (Hirschberg, 1948). The recognition that dosage schedule can profoundly influence the antitumour effectiveness of compounds (Venditti, 1971), and theories that hold that antiprotozoal drugs may be useful antitumour agents (Hartwell *et al.*, 1946; Jondorf, 1969), prompted Jondorf *et al.* (1971) to test (—)-emetine and a number of its derivatives quite comprehensively in mice bearing ascitic leukaemia L1210. This led to the recognition that (—)-emetine and some of its derivatives such as (—)-cephaeline and (—)-*O*-methyltubulosine display significant dose-dependent and schedule-dependent antitumour effectiveness.

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In this paper we set out to determine how (—)-emetine affects O_2 consumption and transplantability of ascitic-tumour-cell suspensions. We also set out to compare the effects of (—)-emetine and (—)-*O*-methyltubulosine on various parameters of tumour growth in mice inoculated with Ehrlich ascites-carcinoma or sarcoma 180 cells.

Experimental

Materials

(—)-Emetine dihydrochloride was obtained as a gift from Burroughs Wellcome Research Laboratories, Beckenham, Kent, U.K. through the courtesy of Dr H. T. Openshaw. (—)-*O*-Methyltubulosine was kindly donated by Dr. A. Popelak of Boehringer-Mannheim G.m.b.H., Mannheim, West Germany. Radioactive precursors L-[U- ^{14}C]leucine (254 mCi/mmol) and [methyl- 3H]thymidine (20 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass., U.S.A. Scintillants were supplied by Packard Instrument Corp., Downers Grove, Ill., U.S.A. Membrane filters type B-6 (pore size 0.45 μm) were obtained from Schleicher and Schuell, Keene, N.H., U.S.A.

The Ehrlich ascites carcinoma used in these studies was a gift from Dr. Richard H. Adamson, Laboratory of Chemical Pharmacology, National Cancer Institute, N.I.H., Bethesda, Md., U.S.A. The ascitic sarcoma 180 tumour used in some experiments was obtained from a line maintained in this laboratory for the past 3 years. It was originally obtained from the Department of Pharmacology, Yale University, New Haven, Conn., U.S.A.

Methods

Tumour transplantation. Both types of ascitic-tumour cells were carried by weekly intraperitoneal transplantation into new-host male ICR strain mice, 6–8 weeks of age (20–30 g body wt.) supplied by Flow Laboratories, Dublin, Va., U.S.A.

Cells were harvested from animals bearing 7-day tumours. The animals had been killed by cervical dislocation and injected intraperitoneally with 0.9% saline (2.5 ml) before laparotomy and draining of peritoneal contents into heparinized centrifuge tubes. The cells were suspended in 0.2% saline (20 ml) and were left at 4°C for a few minutes to haemolyse any erythrocytes present (Creasey & Markiw, 1965). The suspensions were subsequently adjusted to iso-osmolality with 4.5% saline (4 ml) and subjected to centrifugation at 400g (5 min). The tumour cells were resuspended in 0.9% saline and their final concentration was adjusted to approx. 2×10^7 cells/ml by using an electronic particle counter (model B; Coulter Electronics Inc., Hialeah, Fla., U.S.A.) set for monitoring cells from 10–100 μ m in diameter. This suspension was injected intraperitoneally into new hosts (0.1 ml/mouse).

O₂ consumption by Ehrlich ascites-carcinoma-cell suspension in vitro. This was determined by the use of a Warburg apparatus and standard manometric techniques (Umbreit *et al.*, 1964). Tumour cells harvested as already described were suspended in 19 vol. of Robinson's (1949) medium. Portions (0.85 ml) of this suspension were added to the main compartment of micro-Warburg flasks (5 ml capacity). The sidearms of the flasks contained 0.15 ml of Robinson's (1949) medium with (–)-emetine when appropriate. The centre wells contained fluted filter-paper strips saturated with 0.1 ml of 50% (w/v) KOH. The gaseous phase was air. The flasks were equilibrated in the apparatus at 37°C for 10 min after which the stopcocks were closed and O₂ consumption was measured for 30 min. At this point the contents of the sidearms were tipped in and the O₂ consumption was monitored for a further 90 min. Manometer readings on triplicate samples were taken every 10 min throughout the incubation. Results were calculated from the pressure changes and expressed as the mean O₂ uptake in μ l/given number of cells per h.

Effects on transplantability. To determine the effects of (–)-emetine on the transplantability of the Ehrlich ascites tumour, cells were harvested and washed as already described. They were then divided into equal portions and resuspended in 10 vol. of either modified Robinson's medium (Brown *et al.*, 1963) or ice-cold 0.9% saline. (–)-Emetine (0.1 mM) was added to half of each suspension. The cells resuspended in the balanced salt–glucose–glutamine medium (Brown *et al.*, 1963) were incubated in a

shaking water-bath at 37°C for 30 min while those in 0.9% saline were kept at 0°C for this time. The various portions of tumour cells were then spun down, washed twice by resuspension in 0.9% saline to remove extracellular (–)-emetine from those cells that had been in contact with the drug. Finally, cells were resuspended in 0.9% saline, adjusted to 10^7 cells/ml by using the Coulter electronic particle counter and injected intraperitoneally into four different groups of new-host mice (0.1 ml/mouse). Animals were checked daily and the mean survival time of each group was calculated.

Tumour growth rate and life-span of tumour-bearing mice. Mice inoculated with Ehrlich ascites carcinoma (2×10^6 cells) were randomly allocated to control and (–)-emetine-treated groups, the latter receiving a single injection of drug intraperitoneally (16 mg/kg) on the third day after inoculation. Mice were killed serially in groups of four on days 1–10. Tumour cells were collected quantitatively by thorough irrigation of the peritoneal cavities and counted daily with the electronic particle counter as described.

In another type of experiment groups of ten mice were inoculated with Ehrlich ascites or sarcoma 180 cells (1.5×10^6). Control mice were then treated intraperitoneally with 0.9% saline (0.1 ml/10 g body weight) on days 1–5 after tumour transplantation. Other groups were treated daily on days 1–5 with equal volumes of (–)-emetine (8 mg/kg) or (–)-O-methyltubulosine (dissolved in a minimal volume of 0.1 M-HCl and titrated to neutrality with 0.5 M-NaOH; 16 mg/kg) by intraperitoneal injection. On the seventh day the mice were killed and tumour cells were collected. Tumour growth was estimated by measuring the volumes of packed cells after centrifugation at 400g for 5 min.

For determining the life-span of tumour-bearing mice, groups of ten animals were inoculated with Ehrlich ascites- or sarcoma 180-tumour cells as above. Groups other than saline-treated controls received (–)-emetine or (–)-O-methyltubulosine according to dosage schedules based on those found to be optimal for the treatment of mice bearing ascitic-leukaemia L1210 cells (Jondorf *et al.*, 1971). Animals found dead in the mornings were considered to have survived the previous day for the purpose of calculating mean survival times of the variously treated groups.

Macromolecular biosynthesis in Ehrlich ascites cells in vivo. In this type of experiment, mice inoculated with Ehrlich ascites tumour (2×10^6 cells/mouse) were divided randomly on the seventh day after tumour transplantation into control and (–)-emetine-treated (16 mg/kg) groups, the latter then receiving a single injection of drug intraperitoneally, or subcutaneously at the intrascapular site. Control animals received corresponding volumes of 0.9%

saline by the same routes. At intervals ranging from 1 to 24 h after the injections of saline or (–)-emetine, the tumour-bearing mice were injected intraperitoneally with radioactive precursors to DNA and protein biosynthesis. Thus [*methyl*-³H]-thymidine (5 μ Ci/0.2 μ mol per 10 g body weight) and L-[U-¹⁴C]leucine (1 μ Ci/ μ mol per 10 g body weight) were administered to the mice at 40 min and 20 min before death by cervical dislocation. Laparotomies were performed, tumour cells were harvested, washed free of erythrocytes and were then uniformly suspended in 0.9% saline to a final dilution of 1:100 (v/v). Cell-suspension samples (1 ml) in triplicate were assayed for incorporation of radioactive precursors into trichloroacetic acid-insoluble macromolecular material by the membrane-filtration technique adapted from Brown *et al.* (1963).

Samples precipitated with ice-cold 10% (w/v) trichloroacetic acid (2 ml) were left for 15 min and then poured over premoistened membrane filters supported on stainless-steel grids attached to an aspirator assembly. The filters were washed twice with 10% (w/v) trichloroacetic acid (3 ml), placed in scintillation vials and dried under a heat lamp. They were then dissolved in 10 ml of Bray's scintillation solution (Bray, 1960) for double-isotopic-label counting for ³H and ¹⁴C, performed as described by Kobayashi & Maudsley (1970), on a Beckman DPM-100 liquid-scintillation spectrometer with the discriminator settings at 0–7 for ³H and 13–100 for ¹⁴C. The counting efficiencies for ³H and ¹⁴C were 25 and 80% respectively, as assessed by the external-standard method of Wang & Willis (1965).

Other samples of harvested cells were analysed in the Coulter electronic particle counter so that results could be expressed in terms of c.p.m./given number (3×10^6) of cells or as percentages of controls.

In some experiments the time-course of the inhibitory effects of (–)-emetine on protein synthesis in hepatic tissue of the tumour-bearing mice was also determined (Jondorf & Szapary, 1968).

Results

Effects of (–)-emetine on the O₂ consumption and transplantability of Ehrlich ascites-cell suspensions

These parameters were measured to give some indication of possible effects on general cellular metabolism brought about by (–)-emetine at concentrations well in excess of those required for the virtually complete inhibition of protein synthesis in suspensions of HeLa cells (Grollman, 1968). Table 1 shows that the addition of such high concentrations of (–)-emetine to Ehrlich ascites-cell suspensions produces a 15–35% inhibition in the uptake of O₂. Although statistically significant inhibition was attained, the effect of (–)-emetine

on O₂ consumption is not proportional to the concentration of the drug (nor to the duration of exposure, which is not shown).

It has been demonstrated that the survival time of inoculated animals is inversely proportional to the number of viable tumour cells transplanted (Connors & Melzack, 1971). We set out to determine whether (–)-emetine, which has been shown to produce profound inhibitory changes in DNA and protein biosynthesis of HeLa cells *in vitro* (Grollman, 1968),

Table 1. *Effect of (–)-emetine on the O₂ consumption of Ehrlich ascites cells*

Ehrlich ascites cells were suspended in Robinson's (1949) medium to a concentration of 10^7 cells/ml (0.85 ml) and added to the main compartment of micro-Warburg flasks under the general conditions outlined under 'Methods', with neutralized (–)-emetine solutions or control medium in the sidearms. Values for O₂ consumption represent means $\pm$ S.E. for three determinations.

Experiment no.	(–)-Emetine concentration (mM)	μ l of O ₂ consumed/h per 10^7 cells	% of control
I	Control	24.9 $\pm$ 0.8	—
	0.1	20.8 $\pm$ 0.5*	83
	1	20.0 $\pm$ 1.1*	81
	5	21.4 $\pm$ 1.4*	86
II	Control	23.4 $\pm$ 1.2	—
	0.1	19.9 $\pm$ 0.7	85
	1	21.2 $\pm$ 0.2	91
	5	15.5 $\pm$ 0.2*	65

* Indicates $P < 0.01$.

Table 2. *Effect of exposure to (–)-emetine in vitro on the transplantability of Ehrlich ascites cells*

Groups of 15 mice were inoculated with 10^6 tumour cells which had been: (a) kept at 0°C in saline for 30 min; (b) kept at 0°C in saline containing 0.1 mM (–)-emetine for 30 min; (c) incubated in Robinson's (1949) medium at 37°C for 30 min; (d) incubated in Robinson's medium containing 0.1 mM (–)-emetine at 37°C for 30 min. Values are expressed as mean survival time $\pm$ S.E. Values in parentheses are the range of days on which animals were found dead.

Treatment	Survival time (days)
(a) Non-incubated control	14.0 $\pm$ 0.7 (7–18)
(b) Non-incubated with (–)-emetine	14.8 $\pm$ 0.6 (10–18)
(c) Incubated control	14.8 $\pm$ 0.7 (8–20)
(d) Incubated with (–)-emetine	14.9 $\pm$ 0.7 (10–18)

Fig. 1. Effect of administering (-)-emetine to Ehrlich ascites-tumour-bearing mice during the exponential phase of tumour-cell division

Mice were injected intraperitoneally with 2×10^6 Ehrlich ascites cells. Three days later the animals were randomly divided into (-)-emetine-treated [16 mg/kg, intraperitoneally (□)] and saline-treated (○) groups. Four mice from each group were killed daily and their tumour cells were collected by irrigation of the peritoneal contents. Tumour-cell load was determined with the Coulter particle counter as described under 'Methods'. Each point represents mean cell count $\pm$ S.E. of four mice.

could diminish the viability of an inoculum of Ehrlich ascites-tumour cells exposed to the drug.

Table 2 shows that exposure of Ehrlich ascites cells to (-)-emetine (0.1 mM) in culture medium for 30 min had no tangible effect on the survival of mice subsequently inoculated. This concentration of (-)-emetine is not sufficiently cytotoxic to decrease the number of viable tumour cells and thereby prolong the survival times of mice receiving such (-)-emetine-exposed transplants.

We were also interested to see whether cellular metabolism in the presence of (-)-emetine was a prerequisite for cytotoxicity in the tumour cells. However, as also shown in Table 2, the survival times of mice receiving transplants of tumour cells pre-incubated with (-)-emetine (0.1 mM) for 30 min are not prolonged.

Effects of (-)-emetine on the growth of Ehrlich ascitic-tumour cells *in vivo*

The effects of a single intraperitoneal injection of (-)-emetine during the exponential growth phase of

Fig. 2. Time-course of the inhibition by intraperitoneally administered (-)-emetine of the incorporation of L-[U-¹⁴C]-leucine into protein (●) and [methyl-³H]thymidine into DNA (○) in Ehrlich ascites cells *in vivo*

Seven days after the intraperitoneal injection of approx. 2×10^6 Ehrlich ascites cells, tumour-bearing mice were treated intraperitoneally with (-)-emetine (16 mg/kg) or with saline. At 40 min and 20 min respectively before death by cervical dislocation, all experimental animals received [methyl-³H]thymidine and L-[U-¹⁴C]leucine by intraperitoneal injection. They were then killed at the times indicated; tumour cells were harvested, washed and resuspended. At least three samples per mouse were analysed by membrane filtration as described under 'Methods' and these were simultaneously counted for ¹⁴C and ³H radioactivity by the method of Kobayashi & Maudsley (1970). Results expressed as c.p.m./ 3×10^6 cells (readily convertible into percentages of control values) were derived from analysis of further samples of cell suspensions in the Coulter particle counter. Each point represents the mean value $\pm$ S.E. of four mice.

Ehrlich ascites carcinoma are shown in Fig. 1, where groups of mice were injected with the drug (16 mg/kg) or with 0.9% saline on the third day after tumour inoculation with 2×10^6 cells and were then killed serially. The treated animals bore an average tumour load of 8.5×10^7 cells 24 h after administration of (-)-emetine, compared with 2.2×10^8 cells for the control mice. The tumour cells from treated animals, however, continued to multiply and reached the plateau phase 3 days later than the controls. It is evident that a single injection of (-)-emetine can retard the growth of Ehrlich ascites carcinoma without having a correspondingly significant effect on the plateau load of tumour.

(-)-Emetine and the inhibition of protein and DNA biosynthesis of Ehrlich ascites cells *in vivo*

Fig. 2 shows the time-course of the incorporation of [methyl-³H]thymidine into DNA and L-[U-¹⁴C]-leucine into protein of Ehrlich ascites cells *in vivo*

Fig. 3. Inhibition of incorporation of L-[U- 14 C]leucine into protein (●) and [methyl- 3 H]thymidine into DNA (○) of Ehrlich ascites cells *in vivo* after subcutaneous administration of (-)-emetine to tumour-bearing mice

Except for the subcutaneous injections, conditions were exactly as given for Fig. 2.

after the intraperitoneal injection of (-)-emetine (16mg/kg) to mice bearing 7-day tumours. Both DNA and protein biosynthetic activities were depressed for 15h after administration of the drug. The inhibitory effect on protein synthesis (85–95%) is more pronounced than that on the corresponding incorporation of precursor into DNA (65–75%) in this time-period. However, at 24h after (-)-emetine administration both biosynthetic activities appear to have returned to control values indicating again (cf. Fig. 1) that the inhibitory effects of a single injection of the drug are reversible *in vivo*.

Since the intraperitoneal administration of (-)-emetine exposed the tumour cells to high concentrations of the drug injected directly into the cavity containing the tumour, it was worthwhile to compare the time-course of macromolecular biosynthesis after the subcutaneous administration of the same doses of (-)-emetine to mice bearing the tumour at the same 7-day stage of development. Fig. 3 shows that the inhibition of DNA and protein biosynthetic activities is reversed after only 10h under these circumstances. The extent of inhibition is also less pronounced. Protein synthesis is inhibited by 50–70% at various time-points up to 10h whereas DNA synthesis is inhibited during this time by 35–70%. These results are consistent with the expectancy that less drug would reach the tumour cells when it is not administered directly into the tumour area. The time-course of [U- 14 C]leucine incorporation into hepatic protein of these tumour-bearing mice receiving (-)-emetine subcutaneously

was also determined (not shown). There is no evident inhibitory selectivity of (-)-emetine for tumour-cell protein synthesis as compared with hepatic protein-biosynthetic activity.

Comparative effects of daily treatments with (-)-emetine or (-)-O-methyltubulosine on Ehrlich ascites- and sarcoma 180-tumour growth in vivo

Groups of mice inoculated with 1.5×10^6 cells of these tumours were treated daily for the first 5 days after tumour transplantation either with (-)-emetine (8mg/kg) or with (-)-O-methyltubulosine (16mg/kg). On day 7, when all animals were killed and tumour cells were harvested, untreated control mice had a packed tumour-cell volume of 2.2ml as shown in Table 3, equivalent to about 10^9 cells when estimated with the Coulter electronic particle counter. The growth of each of the tumours in the (-)-emetine-treated mice is inhibited dramatically (91%) since the packed cell volumes are only 0.2ml. (-)-O-Methyltubulosine treatment produced a similar inhibition of tumour growth (86%) with packed tumour-cell volume on day 7 averaging 0.3ml (equivalent to approx. 10^7 cells). It is clear that a course of treatment with (-)-emetine and by inference with (-)-O-methyltubulosine produces a greater differential in tumour-cell load, in comparison with saline-treated controls, than could be achieved with a single injection of drug (Fig. 1). It is also clear that treatments of tumour-bearing mice with (-)-emetine or (-)-O-methyltubulosine according to dosage schedules found effective for L1210 leukaemic mice

Table 3. Effects of daily administration of (-)-emetine or (-)-O-methyltubulosine on the growth of the Ehrlich ascites and ascitic sarcoma 180 tumours *in vivo*

Groups of 10 mice were inoculated with 1.5×10^6 cells of either tumour. They received the following treatments on days 1–5 after tumour transplantation: (a) saline 0.01ml/g body weight per day (intraperitoneally); (b) 8mg of (-)-emetine/kg per day in saline as above (intraperitoneally); (c) 16mg of O-methyltubulosine/kg per day in saline as above (intraperitoneally). Animals were killed on day 7 and tumour cells were collected. Values represent mean tumour volume after centrifugation $\pm$ S.E. Numbers in parenthesis are range of values observed.

Treatment	Packed-cell volume (ml)	
	Ehrlich ascites	Sarcoma 180
(a) Control	2.2 $\pm$ 0.2 (1.3–3.3)	2.2 $\pm$ 0.2 (1.5–2.8)
(b) (-)-Emetine	0.2 $\pm$ 0.05* (0.1–0.6)	0.2 $\pm$ 0.08* (0.1–0.9)
(c) (-)-O-Methyltubulosine	0.3 $\pm$ 0.08* (0.1–0.7)	0.3 $\pm$ 0.07* (0.1–0.7)

* $P < 0.01$.

(Jondorf *et al.*, 1971), are definitely carcinostatic, but do not completely eradicate Ehrlich ascites or sarcoma 180 cells.

Prolongation of life-span in tumour-bearing mice

We felt it was important to study the activity of (–)-emetine and (–)-*O*-methyltubulosine with respect to prolonging the survival times of mice bearing Ehrlich ascites- or sarcoma 180-tumour cells, since the optimal dosage schemes were solely derived from experiments with the L1210 tumour (Jondorf *et al.*, 1971) and might be inappropriate for other tumour-cell lines.

Table 4 shows the response of mice bearing the Ehrlich ascites tumour. (–)-Emetine, when given every fourth day at 16 mg/kg, is highly active, prolonging survival time by 83% relative to the untreated tumour-bearing mice. Administration of the drug daily for 5 days at 8 mg/kg, one of the schedules found to be highly effective in mice bearing leukaemia L1210 (Jondorf *et al.*, 1971), is quite toxic and resulted in only a slight prolongation of the survival time. (–)-*O*-Methyltubulosine, whether administered on every third day at 32 mg/kg or daily for 5 days at 16 mg/kg, brings about an increase in survival time of about 60%.

Table 4 shows also the antitumour activity for both drugs in mice bearing ascitic-sarcoma 180 cells. When (–)-*O*-methyltubulosine was given every fourth day at 32 mg/kg, a dosage schedule that prolonged the life-span of mice bearing leukaemia L1210 cells optimally, there were some early toxicity-associated deaths and the average survival time shows no increase. However, the drug is effective when administered daily for 5 days at 16 mg/kg.

Discussion

We have now compared some of the inhibitory effects exerted by (–)-emetine on Ehrlich ascites cells *in vitro* and *in vivo* in greater detail than hitherto (Johnson & Jondorf, 1972).

It is known that (–)-emetine is a potent inhibitor of protein biosynthesis and, to a lesser extent, of DNA synthesis in eukaryotic cells (Grollman, 1966, 1968). It has been suggested that these effects provide a biochemical explanation for the toxic and the therapeutic properties of this compound (Grollman, 1968).

We find, however, that Ehrlich ascites cells incubated *in vitro* in the presence of (–)-emetine at concentrations where, by analogy with HeLa cells, protein synthesis would be expected to be almost completely and irreversibly inhibited (Grollman, 1968), have apparently not lost their viability since cellular O₂ consumption is only slightly depressed (Table 1) and transplantability of the tumour cells is not affected (Table 2).

In contrast with the absence of any observable difference in the survival times of mice inoculated with untreated Ehrlich ascites cells, or with corresponding numbers of cells exposed to, or preincubated with, (–)-emetine (Table 2), we find that there are quite tangible drug-related inhibitory effects on tumour growth, when the drug is injected into tumour-bearing mice (Fig. 1, Table 3).

It is therefore unsatisfactory to attribute the inhibition of exponential tumour-growth *in vivo* simply to the reversible inhibitory effects of (–)-emetine on protein and DNA biosynthesis in the Ehrlich ascites cells (Fig. 2, Fig. 3), when the tumour cells preincubated with the drug *in vitro* retain

Table 4. *Effects of (–)-emetine and (–)-O-methyltubulosine on the survival times of mice bearing Ehrlich ascites tumour or ascitic sarcoma 180*

Mice were inoculated with 2×10^6 tumour cells and randomly allocated to treatment groups. Treatment was begun on the day after tumour transplantation. Animals found dead in the morning were considered to have survived throughout the previous day.

Tumour	Treatment	Dose and schedule	Survival time* (days)	% increase in survival time	Survivors†	Apparent toxicity‡
Ehrlich ascites	Untreated controls		15.0±1.0	—	0/20	—
	(–)-Emetine	16 mg/kg per day, day 1,5,9,13	27.4±4.0	83	1/10	0/10
	(–)-Emetine	8 mg/kg per day, day 1–5	17.3±1.7	15	0/10	4/10
	(–)- <i>O</i> -Methyltubulosine	32 mg/kg per day, day 1,4,7,10	23.4±3.3	58	0/10	0/10
	(–)- <i>O</i> -Methyltubulosine	16 mg/kg per day, day 1–5	24.4±2.7	63	0/10	0/10
Ascitic sarcoma 180	Untreated controls		14.3±0.5	—	0/20	—
	(–)-Emetine	16 mg/kg per day, day 1,5,9,13	18.7±1.3	31	0/10	0/10
	(–)-Emetine	8 mg/kg per day, day 1–5	20.1±1.8	41	0/10	1/10
	(–)- <i>O</i> -Methyltubulosine	32 mg/kg per day, day 1,5,9,13	14.3±1.6	0	0/10	3/10
	(–)- <i>O</i> -Methyltubulosine	16 mg/kg per day, day 1–5	21.7±2.8	52	0/10	0/10

† Animals alive after 60 days.

‡ Animals dying during the first week without evidence of ascites-tumour cells at autopsy.

their viability and unimpaired transplantability, and when there is no demonstrable inhibitory selectivity for tumour-cell protein synthesis as compared with hepatic protein synthesis *in vivo*.

It could be argued that the observed (–)-emetine-related inhibition of L-[U-¹⁴C]leucine incorporation into trichloroacetic acid-insoluble protein points to some primary interference with amino acid uptake rather than protein biosynthesis. Such an inhibitory effect on uptake could have secondary effects on pyrimidine-precursor uptake and incorporation (Jones & Wild, 1973). We have examined this possibility (R. K. Johnson & W. R. Jondorf, unpublished work) by modifying the membrane-filtration technique (Brown *et al.*, 1963) in such a way that the incorporation of radioactivity into whole cells and into trichloroacetic acid-insoluble protein could be compared when Ehrlich ascites cells were appropriately incubated with L-[U-¹⁴C]leucine *in vitro*, also in the presence of varying amounts of (–)-emetine. Under these conditions, essentially all of the radioactivity taken up from the incubation medium by whole cells can be accounted for by the trichloroacetic acid-insoluble fraction, regardless of the overall concentration-dependent inhibitory effects of (–)-emetine. This tends to support the view that (–)-emetine inhibits protein synthesis rather than uptake of amino acid, particularly since it is known that L-leucine is not specifically concentrated intracellularly by Ehrlich ascites cells (Christensen *et al.*, 1952) and that active transport of amino acids into cells is very rapid and precedes incorporation into protein independently of the action of inhibitors of protein synthesis such as puromycin (Riggs & Walker, 1963). Further, (–)-emetine inhibits protein synthesis in whole-cell and in cell-free systems (Grollman, 1966).

It could also be argued that inhibition of labelled thymidine incorporation may not necessarily reflect an inhibition of DNA synthesis in Ehrlich ascites cells since phosphorylation is a prerequisite step for polynucleotide formation, and an inhibition of thymidine kinase would also explain decreased incorporation of exogenous thymidine into DNA. This is unlikely, however, since (–)-emetine has been shown to inhibit DNA synthesis in HeLa cell cultures without inhibiting thymidine kinase (Grollman, 1968). Moreover, (–)-emetine has no direct inhibitory effect on thymidine kinase in regenerating liver (Johnson *et al.*, 1971).

Although single injections of (–)-emetine have quite marked effects on the growth rate and on the macromolecular biosynthesis of Ehrlich ascites cells in tumour-bearing mice, more pronounced inhibitory effects on tumour growth *in vivo* (Table 3) and prolongation of survival times (Table 4) can be achieved by administering (–)-emetine or (–)-O-methyltubulosine according to dosage schedules previously devised for mice bearing L1210 ascites

cells (Jondorf *et al.*, 1971). It is unlikely that the effects of these drugs *in vivo* depend on formation of metabolites with greater antitumour activity in the treated mice, since both (–)-emetine (Miller *et al.*, 1970) and (–)-O-methyltubulosine (Johnson & Jondorf, 1971) decrease the activity of the drug metabolizing enzymes in a related species, the rat.

It is curious that the schedule-dependent antitumour effectiveness of (–)-emetine is not related to the maintenance of tissue concentrations of drug (Auletta *et al.*, 1971) which might conceivably overcome the observed reversibility of the blocking action on protein and DNA biosynthesis in tumour cells (Figs. 2 and 3).

In view of these considerations, it would appear that the inhibition of protein synthesis in tumour cells is undoubtedly an important factor in the antitumour effectiveness of (–)-emetine. We would not necessarily agree with Grollman (1968) that this is the only explanation for the mechanism of its wide-ranging chemotherapeutic action. It is conceivable that (–)-emetine may also exert some dose- and schedule-dependent stimulating effect on the production of host-protective interferon-like substances (Tan *et al.*, 1971). Such an indirect mechanism postulating interferon production could help to explain the more pronounced antitumour effects we have observed with (–)-emetine *in vivo* since interferon is known to inhibit transplanted (Gresser & Bourali, 1970) and carcinogen-related (Salerno *et al.*, 1972) tumours.

Although some of the precise details of such a biochemical mode of action of the antitumour effects remain to be determined, the paradoxically enhanced production of interferon by (–)-emetine (Tan *et al.*, 1971) would still be compatible with a mechanism dependent on inhibition of protein biosynthesis, if it is recognized that interferon production may be related to the release of preformed precursors or the blocking of the synthesis of an inhibitor of interferon (Vilček, 1970).

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References

- Abd-Rabbo, H. & Montasir, M. (1967) *J. Trop. Med. Hyg.* **70**, 117–121

- Auletta, A. E., Gery, A. M. & Mead, J. A. R. (1971) *Pharmacologist* **13**, 261
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279-285
- Brown, F. U., Klein, M., Kandaswamy, T. S. & Mandel, H. G. (1963) *Cancer Res.* **23**, 254-259
- Christensen, H. N., Riggs, T. R., Fischer, H. & Palatine, I. M. (1952) *J. Biol. Chem.* **198**, 1-15
- Connors, T. A. & Melzack, D. H. (1971) *Int. J. Cancer* **7**, 86-92
- Creasey, W. A. & Markiw, M. E. (1965) *Biochim. Biophys. Acta* **103**, 635-645
- Gresser, I. & Bourali, C. (1970) *J. Nat. Cancer Inst.* **45**, 365-375
- Grollman, A. I. (1965) *Surg. Gynecol. Obstet.* **120**, 792-796
- Grollman, A. P. (1966) *Proc. Nat. Acad. Sci. U.S.* **56**, 1867-1874
- Grollman, A. P. (1968) *J. Biol. Chem.* **243**, 4089-4094
- Hartwell, J. L., Shear, M. J., Johnson, J. M. & Kornberg, S. R. L. (1946) *Cancer Res.* **6**, 490
- Hirschberg, E. (1948) *Cancer Res. Suppl.* **23**, 521-980
- Hunter, G. W., Frye, W. W. & Swartzwelder, J. C. (1966) *A Manual of Tropical Medicine*, 4th edn., pp. 288-308, W. B. Saunders Co., Philadelphia and London
- Johnson, R. K. & Jondorf, W. R. (1971) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **30**, 627
- Johnson, R. K. & Jondorf, W. R. (1972) *Biochem. J.* **126**, 22p-23p
- Johnson, R. K., Donahue, J. D. & Jondorf, W. R. (1971) *Xenobiotica* **1**, 131-141
- Jondorf, W. R. (1969) *Med. Ann. D.C.* **38**, 73-79
- Jondorf, W. R. & Szapary, D. (1968) *Arch. Biochem. Biophys.* **126**, 892-904
- Jondorf, W. R., Abbott, B. J., Greenberg, N. H. & Mead, J. A. R. (1971) *Chemotherapy* **16**, 109-129
- Jones, R. W. & Wild, D. G. (1973) *Biochem. J.* **134**, 617-627
- Kobayashi, Y. & Maudsley, D. V. (1970) in *Liquid Scintillation Counting* (Bansome, E. D., ed.), pp. 76-85, Stratton, New York
- McConnell, J. R., Hallett, S. R. & Shear, M. J. (1947) *Cancer Res.* **7**, 716
- Melchior, E. (1954) *Emetin seine Wirksamkeit auf schwere chirurgische Infekte*, Ferdinand Enke Verlag, Stuttgart
- Miller, H. H., Johnson, R. K., Donahue, J. D. & Jondorf, W. R. (1970) *Biochem. J.* **117**, 491-498
- Riggs, T. R. & Walker, L. M. (1963) *J. Biol. Chem.* **238**, 2663-2668
- Robinson, J. R. (1949) *Biochem. J.* **45**, 68-74
- Salerno, R. A., Whitmire, C. E., Garcia, I. M. & Huebner, R. J. (1972) *Nature (London) New Biol.* **239**, 31-32
- Tan, Y. H., Armstrong, J. A. & Ho, M. (1971) *Virology* **44**, 503-509
- Umbreit, W. W., Burris, R. H. & Stauffer, J. R. (1964) *Manometric Techniques*, 4th edn., Burgess, Minneapolis
- Venditti, J. M. (1971) *Cancer Chemother. Rep. Part 3* **2**, 35-59
- Vilček, J. (1970) *Ann. N.Y. Acad. Sci.* **173**, 390-403
- Wang, C. H. & Willis, D. L. (1965) *Radiotracer Methodology in Biological Science*, p. 134, Prentice-Hall, Englewood Cliffs, N.J.