Eur J Cancer Clin Oncol, Vol. 22, No. 6, pp. 637–645, 1986.
Printed in Great Britain.

0277–5379/86$3.00+0.00
Pergamon Journals Ltd.

A Preliminary Analysis of the Effects of Elliptinium on Immune Reactivities in Mice

E. SFREDDO GALLOTTA,* M. SIRONI, F. SPREAFICO and A. VECCHI

Laboratory of Oncology and Immunology, "Mario Negri" Institute for Pharmacological Research, via Eritrea, 62 - 20157 Milan, Italy

Abstract—*The immune effects of Elliptinium (2-methyl-9-hydroxyellipticinium, 9-HME), a chemical recently shown to possess clinical antineoplastic activity, were investigated in mice.*

Primary antibody responses to T-dependent and T-independent antigens, DTH reactivity and responsiveness to mitogens were significantly depressed only by post treatment with single drug doses of at least 5 mg/kg i.v., i.e. doses clearly above those known to exert full antitumoral effectiveness and to induce lymphoid cell depletion in the same species. Only drug doses in the LD_{50} range (i.e. 10 mg/kg) reduced the capacity of NK cells and of activated macrophages to express non-specific cytotoxicity towards tumor target cells.

When repeated dose regimens were used, significant immune depression was again seen at doses above those displaying chemotherapeutic activity.

Data obtained suggest that at chemotherapeutically effective dosages 9-HME possesses in mice a comparatively low immunodepressive potential and that immune cells mediating natural host defence mechanisms appear especially resistant to this drug.

INTRODUCTION

ELLIPTINIUM (2-methyl-9-hydroxyellipticinium 9-HME) is a recently described derivative of Ellipticine, that has been found effective not only on various types of experimental tumors [1] but also on a number of human cancers [2–6] while apparently displaying lower toxicity than the parent compound in terms of cardio and neurotoxicity and hemolitic capacity in both animals and humans [2].

It is well known that antiproliferative agents can affect the immune system and this immunological activity is widely regarded as playing a major role in a series of side-effects associated with treatment with such compounds, such as increased risk of infections and, possibly, of second malignancy [7].

However it has been conclusively demonstrated in recent years that cancer chemotherapeutic drugs, even when chemically analogous, can markedly differ among them both quantitatively as well as qualitatively in their modulation of the various immune cells [8, 9]. Furthermore experimental evidence obtained by various groups supports the conclusion that the quality of the interaction of cancer chemotherapeutic agents with host immune responsiveness can be an important determinant of the overall antineoplastic effectiveness *in vivo* of these drugs [8,10,11].

On this general background and in view of the lack of information on 9-HME immunological activity and the paucity of data on this activity for other compounds of the Ellipticine group [12,13] it was of interest to investigate in experimental conditions the effects of 9-HME on several parameters of humoral and cell-mediated immune responsiveness. A further reason for this study was the fact that this drug has been reported to have in animals a low toxicity for bone marrow stem cells and to possess a reduced leukopenic activity [1].

Data presented in this report favour the conclusion that 9-HME possesses in mice a comparatively modest immunomodifying activity, since significant immunodepression was only seen at doses clearly above those exerting anticancer effects. Among the various immune cells, NK cells and macrophages appeared to be comparatively more resistant to the drug. As these elements are currently credited with a primary role in natural host defences against infectious challenges and in the control of neoplastic progression and dissemination [14,15], these findings could have clinical interest.

Accepted 25 October 1985.

*To whom correspondence and reprint requests should be sent at: "Mario Negri" Institute for Pharmacological Research via Eritrea, 62 – 20157 Milan, Italy.

This work was supported by the CNR (National Research Council, Rome, Italy) contract no. 84.00622.44.

MATERIALS AND METHODS

Animals

C57BL/6NCrlBR (B6) and $CD2F_1$/CrlBR male mice (20 ± 3 g body wt), obtained from Charles River, Calco, Italy, were used.

Drugs

9-HME obtained from Midy S.p.A., Milan, Italy, was freshly dissolved in sterile isotonic 5% glucose solution and injected intravenously (i.v.). Corynebacterium parvum CN 6134 (*C. parvum* 0.7 mg/mouse) from Wellcome Research Labs. (Beckenham, U.K.) was injected i.p., in saline.

Assessment of antibody response

To evaluate the primary humoral response to a T-dependent antigen, B6 mice were given an i.p. injection of 4×10^8 SRBC on day 0 and spleen hemolytic plaque-forming cells (PFC) were counted as previously described (16) on day 5 unless otherwise stated. Rabbit antiserum to mouse IgG (Miles-Yeda, Israel), at a final dilution of 1 : 300 was used for development of IgG PFC.

Type 3 pneumococcal polysaccharide S III, kindly supplied by Dr. P.J. Baker (NIAID, NIH, Bethesda, MD), was used as a T-independent stimulus. $CD2F_1$ mice were given 0.5 μg S III i.p. on day 0 and the response was assessed on day 5. Splenic PFC specific for S III were detected by the technique of Cunningham and Szenberg as detailed in [16].

Data are presented as geometric means (in parentheses ± S.E.) after logarithmic transformations [16]. At least six mice per group were used in these studies.

Delayed type hypersensitivity (DTH) response

B6 mice were sensitized with a single s.c. injection of 10^8 SRBC; 4 days later the animals were challenged with 10^8 SRBC s.c. inoculated into the left hind footpad in 50 μl, the controlateral footpad receiving an equal volume of saline.

The DTH reaction was recorded 24 hr later measuring the difference in weight (△ mg) between left and right footpads [17]. At least seven mice per experimental group were used.

Mitogen stimulation

The splenocyte response to Concanavallin A (ConA, Calbiochem.), *E.coli* lipopolysaccharide B (LPS, Difco) and purified Phytohemagglutinin (PHA-16, Wellcome Research Labs.) was evaluated as previously described [18]. Briefly, 5×10^5 splenocytes from control and drug-treated B6 mice were cultured in triplicate in RPMI 1640 medium (Gibco Biocult, Glasgow, U.K.) supplemented with antibiotics and 10% fetal calf serum (FCS, Gibco Biocult) with graded mitogen concentrations in microtiter plates (Sterilin, Middlesex, U.K.) in total volumes of 0.2 ml; cells were incubated at 37°C in a humidified atmosphere with 5% CO_2. Forty-eight hours later, 0.5 μCi ^{3}H-thymidine (5Ci/mmol, Radiochemical Center, Amersham, U.K.) were added to each sample and cultures incubated for a further 16 hr. Samples were then collected with an automatic cell harvester and radioactivity was counted in a liquid scintillation spectrometer.

Macrophage-mediated cytolytic activity

The cytolytic activity of purified macrophages was evaluated as ^{3}H-thymidine release from prelabelled mKSA-TU5 (TU5) target cells, as previously described [19]. Resident peritoneal macrophages were obtained by washing the peritoneal cavity of the B6 mice with 5 ml of pH 7.4 phosphate buffered saline. After washings, differential cell counts were made on cytocentrifuge smears stained with Diff Quick (Harleco Gibbstown, NY). Graded numbers of macrophages were seeded in 0.3 ml RPMI 1640 in flat bottomed culture wells (3596, Costar,) and incubated at 37°C, 5% CO_2 for 2 hr; non-adherent cells were then removed by repeated washings with warm medium, and labelled TU5 target cells added.

Macrophages and target cells at different ratios (20 : 1 and 40 : 1) were incubated for 48 and 72 hr at 37°C, 5%CO_2. At the end of incubation time, 0.1 ml of the supernatant was collected and counted in a liquid scintillation spectrometer. Each experimental group consisted of at least five mice and cultures were in triplicate. Results presented are specific cytotoxicity values (± S.E.) [19].

NK cell activity

NK activity was evaluated using splenocytes as effectors and ^{51}Cr-labelled YAC-1 lymphoma cells as targets as previously described [20]. Briefly, 10^4 ^{51}Cr-labelled YAC-1 cells were incubated for 18 hr with graded numbers of splenocytes from individual control or drug-treated mice, so as to obtain a range of Attacker : Target (A : T) cells ratios. Spontaneous release in the absence of effector cells never exceeded 1–1.5% per hr of incubation. At least five mice per experimental group were used and cultures were in triplicates.

Statistical analysis

Statistical significance was evaluated by Dunnett's or Student's *t*-tests as detailed in figures and tables.

RESULTS

The effect of 9-HME on the primary response to SRBC

The effect of single 9-HME doses given at differ-

ent times relative to antigen challenge was firstly investigated.

As shown in Table 1 by one typical experiment, no decrease in spleen PFC numbers measured at the peak response day (i.e. day 5) was seen when a single 5 mg/kg drug dose was injected 48 hr before antigen, whereas a marked inhibition (to approx. 15% from control PFC/spleen levels) was observed when this dose was given 2 days after immunization. When 9-HME was administered on the same day of antigen, no changes in the values of PFC/10^6 splenocytes were seen and only moderate decreases in PFC/spleen values observed. The effects of 9-HME given on day +2 after SRBC were thus examined in further detail. Figure 1 shows the dose–response curve for 9-HME effect on the PFC response measured at peak day. It may be observed that statistically significant reductions in the numbers of PFC/10^6 splenocytes were observed only with drug doses of 5 mg/kg or higher whereas single administrations of 1 and 2.5 mg/kg were not significantly active in reducing the values of PFC/10^6 splenocytes. However, if the response of the whole organ is considered, PFC numbers/spleen were significantly decreased also with 2.5 mg/kg, this dose significantly decreasing the total number of splenocytes. The effects of different single 9-HME doses on spleen cellularity are presented in Fig. 1.

Table 1 also shows that when 9-HME was administered on days 0, 2 and 4, a significant depression in PFC numbers per spleen and per 10^6 splenocytes as well as in spleen cellularity was only obtained with the 2.5 mg/kg dose, whereas no significant reductions were associated with 1 mg/kg × 3.

The effect of 9-HME on the kinetics of the anti-SRBC response was also evaluated. Table 2 shows that a single 5 mg/kg dose injected on day 2 reduced IgM PFC/10^6 splenocytes at day 8 and 10, whereas it did not decrease IgG PFC numbers at the same times. A significant inhibition on day 8 of IgG PFC was only seen if PFC/spleen are considered. No significant changes in the levels of IgM and IgG PFC were seen on day 8 and 10, when 1 and 2.5 mg/kg were administered on days 0, 2 and 4 (data not shown).

Effect of 9-HME on the response to SIII

The effect of the drug on the immune response to the T-independent antigen, the SIII, was next

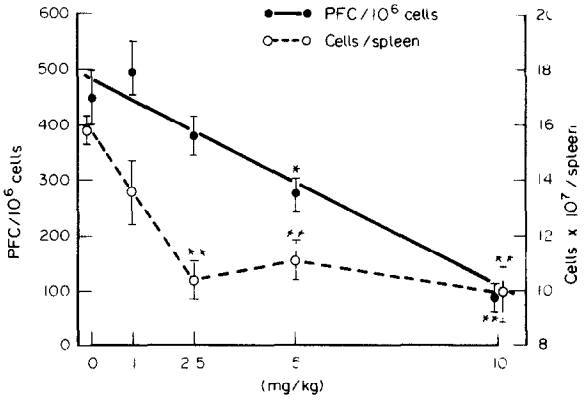

*Fig. 1. Dose-response curve of the effects of 9-HME on the primary anti-SRBC response (●—●) and on splenocyte numbers (○—○). * $P < 0.05$ by Dunnett's test; ** $P < 0.01$ by Dunnett's test.*

Table 1. Effect of 9-HME on the primary anti-SRBC response

Drug (mg/kg i.v.)	Day of treatment	Spleen cells × 10^6	PFC / 10^6 cells	PFC / spleen
Vehicle	—	100.4 ± 5.5	368 (278 – 488)	38237 (28682 – 50976)
5	− 2	72.7 ± 3.0**	284 (215 – 376)	20622 (15778 – 26952)
5	0	67.0 ± 8.0**	273 (233 – 320)	17408* (14665 – 20663)
5	+ 2	65.0 ± 3.0**	108** (91 – 128)	6908** (5772 – 8841)
Vehicle	0; +2; +4	127.8 ± 16.0	520 (505 – 534)	66456 (54149 – 81570)
1	0; +2; +4	97.0 ± 4.5	493 (457 – 531)	47821 (44185 – 51755)
2.5	0; +2; +4	75.1 ± 9.0**	259** (240 – 280)	19451** (17350 – 21806)

4×10^8 SRBC were given i.p. on day 0. Test was performed on day +5

* $P < 0.05$ by Dunnett's test

** $P < 0.01$ by Dunnett's test

Table 2. Effect of 9-HME on the kinetics of the primary anti-SRBC response

Drug (mg/kg i.v.)	Day of assay	Day of treatment	IgM PFC / 10^6 cells	IgM PFC / spleen	IgG PFC / 10^6 cells	IgG PFC / spleen
Vehicle	+8	+2	198 (176 – 222)	26890 (22876 – 31608)	854 (790 – 924)	115279 (103679 – 128176)
5	+8	+2	83* (58 – 118)	7598** (5216 – 11067)	593 (470 – 749)	54206** (43166 – 68069)
Vehicle	+10	+2	64 (58 – 71)	9240 (8294 – 10302)	770 (687 – 851)	110854 (103527 – 118700)
5	+10	+2	34* (29 – 41)	5703* (4879 – 6665)	687 (529 – 891)	112295 (91851 – 137290)

Mice were given 4×10^8 SRBC i.p. on day 0.
* $P < 0.05$ by Student's *t*-test
** $P < 0.01$ by Student's *t*-test

Table 3. Effect of 9-HME on the anti-S III *response*

Drug (mg/kg i.v.)	Day of treatment	PFC / 10^6 Cells	PFC / Spleen
Vehicle	–	103 (92 – 116)	12353 (111271 – 13540)
5	−2	107 (92 – 126)	8215 (7078 – 9536)
5	0	118 (101 – 139)	10136 (8532 – 12042)
5	+2	53** (49 – 58)	3954** (3497 – 4472)
Vehicle	–	244 (227 – 262)	27704 (25000 – 30701)
5	+2	139* (118 – 164)	11311** (8928 – 14329)
7.5	+2	127** (113 – 142)	11063** (9796 – 12494)
10	+2	107** (95 – 122)	9705** (8818 – 10682)

CD2F1 mice were given 0.5 μg/mouse S III i.p. on day 0. Test was performed on day +5.
* $P < 0.05$ by Dunnett's test.
** $P < 0.01$ by Dunnett's test.

investigated. As shown in Table 3, significant reductions in PFC levels were obtained only when 5 mg/kg 9-HME was given 2 days after antigen, treatments on day -2 or 0 being inactive in agreement with results obtained in the anti-SRBC response. In affecting this response 5 mg/kg was the lowest active dose whereas 1 or 2.5 mg/kg in spite of the reduction in spleen cellularity did not significantly modify anti-SIII PFC numbers (data not shown).

Effect on DTH response

To obtain information on 9-HME effects on cell-mediated reactivities, the anti-SRBC DTH response was investigated. Also on this reactivity 9-HME was found inhibitory only when it was administered after (i.e. 2 days) SRBC sensitization, pre or concomitant single administration being without detectable effects (Table 4). A dose–response curve was therefore performed with treatments on day 2: it may be seen that the lowest drug dose producing significant inhibition of the DTH reaction (approx. 40% from control values) was 5 mg/kg. When 9-HME was injected on days 0, 2 and 4 relative to antigen sensitization, a significant

Table 4. Effect of 9-HME on DTH response

Drug (mg/kg i.v.)	Day of treatment	Δ mg
Vehicle	–	23.6 ± 2.5
5	−2	16.6 ± 2.2
5	0	19.7 ± 3.3
5	+2	9.9 ± 1.7*
Vehicle	–	26.1 ± 4.7
2.5	+2	24.8 ± 1.8
5	+2	13.0 ± 3.0*
7	+2	10.4 ± 1.2*
10	+2	7.2 ± 2.0**
Vehicle	0; +2; +4	23.0 ± 1.7
1	0; +2; +4	18.5 ± 1.5
2.5	0; +2; +4	14.6 ± 1.9**

Mice were given 10^8 SRBC s.c. on day 0 and 10^8 s.c. in left hind footpad on day +4.
DTH was measured 24 hr after second challenge as the difference in weight (Δ mg) between left and right footpad.
* $P < 0.05$ by Dunnett's test
** $P < 0.01$ by Dunnett's test

inhibition was observed with 2.5 mg/kg, but not at the dose of 1 mg/kg (Table 4).

Effect on mitogen responsiveness

To further characterize 9-HME effect on cellular responses, its effects on the responsiveness to mitogens were assessed. Results reported in Table 5 show that treatment of mice with 5 or 10 mg/kg 2, 5 and 7 days before testing was associated with a significant inhibition in the splenocytes responsiveness to PHA and ConA only when suboptimal concentrations of these mitogens were used, whereas no significant inhibitions were detected using optimal concentrations of these stimulants. Consistently significant inhibitions of the responses to both optimal and suboptimal PHA and ConA concentrations were only observed when the toxic 10 mg/kg drug dose was given 7 days before assay. No effects were found when doses lower than 5 mg/kg were administered at the same times. The responsiveness to LPS was unaffected by 5 and 10 mg/kg 9-HME given 2 days prior to testing, whereas it was significantly reduced when these drug dosages were administered on day -5. When the 9-HME was injected on day -7, a statistically significant inhibition was only found at one LPS concentration after treatment with the toxic 10 mg/kg dose.

Effect on macrophage-mediated cytolytic activity

The effect of 9-HME on macrophage-mediated cytolytic activity was investigated using resident as well as *C.parvum*-activated macrophages as effectors. Under the experimental conditions employed, 9-HME at single doses of 5 and 10 mg/kg given 2 or 6 days before test, did not significantly modify the cytocidal activity of resident murine peritoneal macrophages (Fig. 2). A significant decrease in macrophage-mediated cytotoxic activity was only observed when 10 mg/kg 9-HME was given 2 days after injection of the macrophage-stimulant *C.parvum*, and testing macrophages after a further 6 days period. Figure 2 shows the results obtained at 40 : 1 ratio after 48 hr of incubation but similar findings were also observed at 20 : 1 ratio and after 72 hr of incubation. It should be noted however that in the same treatment conditions 5 mg/kg 9-HME was not inhibitory and that 10 mg/kg given to *C. parvum* treated mice 12 days before testing was not associated with significant changes in macrophagic cytotoxicity.

Effects on NK-activity

Table 6 shows that the levels of NK-mediated cytotoxicity expressed by fixed numbers of splenocytes were not different between controls and mice that had been treated 2 or 5 days before with 5 or 10 mg/kg 9-HME. No significant modifications in the functional activity of NK effectors were also seen when these drug doses had been injected 7 days before assay (data not shown). However, because of the cellular depletion in the spleen associated with such treatments, the total organ capacity to express NK cytotoxicity, reflected in the Lythic Units 33%, was reduced 2 and 5 days after 5 and 10 mg/kg 9-HME with a recovery to at least 80% of control values by day 7.

Table 5. Effect of 9-HME on splenocyte responsiveness to mitogen

Day of treatment	Dose (mg/kg i.v.)		ConA (μg/ml)		PHA (μg/ml)		LPS (μg/ml)	
		0	0.5	4	0.5	2.5	1	250
	Vehicle	4201§ ± 575	27855 ± 2640	218717 ± 11314	63381 ± 3285	159363 ± 5960	30281 ± 2832	61847 ± 2992
−2	5	3582 ± 510	21639* ± 1503	189444 ± 16106	30260** ± 3395	155350 ± 9488	36533 ± 5498	54916 ± 5198
	10	4179 ± 303	21654* ± 610	203354 ± 11504	19228** ± 913	129758 ± 5563	32839 ± 3185	53097 ± 2837
−5	5	5660 ± 633	17318** ± 1215	231797 ± 12542	32155** ± 993	162255 ± 12365	23721** ± 1284	52103 ± 1762
	10	1097** ± 194	19204* ± 497	254405 ± 27996	34421** ± 2759	156077 ± 14600	25525* ± 551	43995** ± 1904
−7	5	3514 ± 270	11816** ± 760	230860 ± 8141	28471** ± 2636	152652 ± 7832	30030 ± 574	49846 ± 887
	10	4927 ± 794	15108** ± 2794	61591** ± 2351	15743** ± 1181	75889* ± 5860	22591** ± 718	56616 ± 6089

§ = cpm ± S.E.
* $P < 0.05$ by Dunnett's test
** $P < 0.01$ by Dunnett's test

DISCUSSION

Results obtained in this preliminary characterization of the immunological effects of 9-HME in mice essentially indicate that although this compound can affect both humoral and cell-mediated immune reactivities, significant immune depression is only seen at relatively high drug dosages that are above those exerting full chemotherapeutic activity in the same species. It has in fact been reported [1] that for both leukemia-lymphomas and solid murine tumors optimal antineoplastic activity is seen with 9-HME in the 1–3 mg/kg dose range for both single and repeated dose regimens. Conversely, significant inhibitions of PFC numbers per 10^6 splenocytes, DTH reactivity, cytotoxicity by previously activated macrophages and lymphoid cell responsiveness to mitogens were only detected with at least 5 mg/kg × 1 or 2.5 mg/kg × 3. Lower drug doses (i.e. 2.5 mg/kg × 1) were in fact inhibitory only insofar as they reduced lymphoid organs cellularity, while not being associated with significant impairment of responsiveness on a unit cell number basis. Furthermore, a detectable functional impairment of NK effectors and of resident macrophages in their capacity to express cytotoxicity towards tumor target cells was not seen even at frankly toxic doses such as 10 mg/kg, although total spleen capacity to mediate NK activity was reduced at 5 mg/kg because of the cell depletion caused by this dose. The acute LD_{50} of 9-HME in mice has in fact been reported to be in the 12–15 mg/kg i.v. range [1].

The relatively moderate sensitivity of lymphoid cells to 9-HME observed in these investigations is thus in general agreement with earlier data indicating that in rodents the compound was a relatively poor inhibitor of bone marrow stem cell numbers and induced only moderate leukopenia [1]. Data presented are consistent with the conclusion that T and B lymphocytes are the main cellular targets of 9-HME immunodepressive activity, whereas normal macrophages and NK effectors appear to be highly resistant to the drug. That 9-HME can inhibit T cells is directly supported by its capacity to reduce the development and expression of DTH, a typical T-dependent reactivity, and indirectly indicated by the observation of a reduction in the responsiveness of splenocytes to the T-mitogens ConA and PHA. Although macrophages are known to be involved in the response to these mitogens [21], the finding that a depressed responsiveness to these polyclonal activators was seen at 5 mg/kg whereas normal macrophages function (as judged by their capacity to mediate non-specific cytotoxicity) was not affected even at 10 mg/kg is indirect evidence in favour of the contention that this effect was dependent on inhibition by 9-HME of T and B lymphocytes. In support of the conclusion that B cells are also directly affected by this chemical is the finding that 9-HME decreased the antibody response to the T-independent antigen S-III. Although the observation that the degree of inhibition of the anti-SRBC and anti-S-III responses at peak day was comparable at 5 mg/kg may suggest that T and B lymphocytes possess equivalent sensitivity to modulation by this drug, more direct data are needed before this conclusion is formally established.

In *in vitro* studies employing various normal and tumor elements, other compounds of the Ellipticine group have been found to kill cells in all phases of the cell cycle but to be much more effective on cells in the M phase [22]. If such a differential also applies to 9-HME, such a mechanism could explain the observation that this compound, in the experimental conditions employed, inhibited anti-

Table 6. Effect of 9-HME on spleen NK activity

Drug (mg/kg i.v.)	Day of treatment	%Specific cytotoxicity 12.5 : 1*	25 : 1	50 : 1	LU 33%/ Spleen**
Vehicle	–	32.9 ± 3.0	45.7 ± 1.3	47.3 ± 3.1	287
5	−5	26.7 ± 1.3	43.8 ± 7.5	43.7 ± 2.8	181
10	−5	28.9 ± 1.7	32.8 ± 5.0	36.1 ± 1.1	152
5	−2	33.3 ± 8.0	34.7 ± 7.4	46.6 ± 4.0	186
10	−2	25.1 ± 2.8	45.8 ± 8.0	48.8 ± 2.5	179

* A : T ratios

** LU 33% : Lythic units

Test was performed on day 0. NK-activity was evaluated as per cent specific release of ^{51}Cr from prelabelled and tumor target cells (YAC/1 Lymphoma) after 18 hr of incubation.

Results are mean ± S.E.

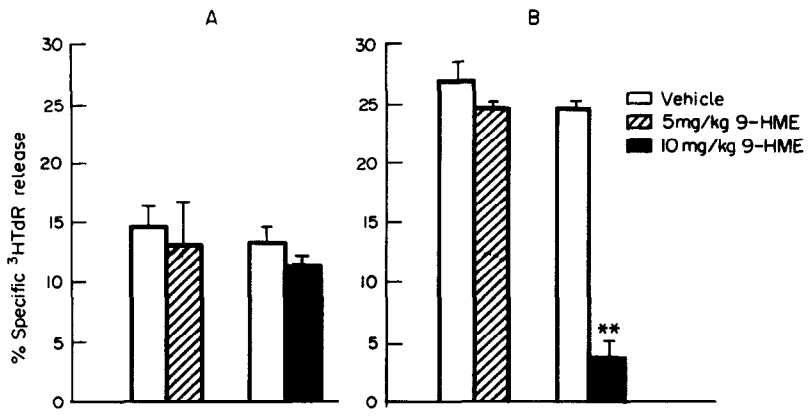

Fig. 2. Effect of 9-HME on macrophage-mediated cytolytic activity. Mice were given the drug on day –6 and test was performed on day 0. A — Resident peritoneal macrophages; B — *C. parvum* activated macrophages (0.7 mg/mouse on day – 8). A : T ratio = 40 : 1; ** P + by Student's t-test.

body production and DTH only when administered after antigen, i.e. on already proliferating cells. Such a mechanism could in principle also be invoked for explaining the resistance of resident, non proliferating macrophages to 9-HME doses inhibitory for T and B cells, and the comparatively greater sensitivity of *C. parvum* activated macrophages. *C. parvum* is not only known to activate mature macrophages but also to promote the production and differentiation of cells of the monocytic series into mature elements [23]. Young elements of the monocyte-macrophage lineage are also known to be functionally more active than fully differentiated cells and to express higher levels of non-specific cytotoxicity, spontaneously or after exposure to stimulants [24]. The finding that macrophages obtained from *C. parvum*-pretreated mice were inhibited by 9-HME doses inactive on resident macrophages could thus be sustained through destruction by the drug of the younger monocytic elements. As protein synthesis is needed for the expression of macrophage-mediated cytotoxicity [25], it may be alternatively hypothesized that previously activated cells are more sensitive to protein synthesis inhibition by 9-HME. Although Ellipticines are believed to inhibit cell proliferation via effects on DNA and RNA synthesis, protein synthesis was significantly reduced in cells exposed to this compound [22]. Since NK activity was investigated in this study in not previously stimulated hosts, the resistance of NK effectors to high 9-HME doses could additionally be attributed to the preferential inhibition by the drug of the actively multiplying viz. resting cells. Although NK cells are by many believed to belong to the T cell lineage [26], other immunosuppressants (e.g. Doxorubicin, Dacarbazine) have been shown to differentially affect T and NK cells, and dependent immune functions [16,27,28]. However further studies are needed to better resolve the mechanisms involved in the differential activity of 9-HME on the various immune cells and the relative roles played by cell growth inhibition and functional impairment of surviving cells.

With regard to their experimental immune activity, other compounds of the Ellipticine group have been investigated only for their effect on the anti-SRBC antibody response and the conclusion advanced that these drugs were highly immunodepressive. It has in fact been reported that Elliptinium and 9-hydroxy-ellipticine reduced the number of murine spleen PFC to 0.1 and 0.3% of controls, respectively, and 9-methoxy-ellipticine and 9-amino-ellipticine reduced the values to 3 and 10% respectively at drug doses that were comparably effective in prolonging the survival of L1210 leukemia-bearing mice [13]. At an equally effective dose on the same tumor (i.e. 2.5 mg/kg), 9-HME-induced depression of PFC/spleen values was of only 50% approx. If the likely assumption is made that similar differentials also apply for other types of immune reactivities, it would thus appear that 9-HME is endowed with a significantly lower immunodepressive capacity than the other members of the Ellipticine family. The exact basis for this different capacity to affect the immune system among these drugs remains at this time essentially hypothetical. Whatever the mechanism, the concept already advanced from the study of other anticancer and immunodepressive agents that chemically analogous agents may markedly differ in their immunological activity [7] appears reinforced by these findings.

The fact that at full chemotherapeutic doses 9-HME displays a relatively low immunodepressive potential in mice and that NK cells and macrophages, cells playing front line roles in natural host resistance [26] and in the control of neoplastic progression [14,15], appear to be comparatively resistant to these drugs, if confirmed

also in other species, could have practical implications. It could in fact be expected that effective antineoplastic regimens may be associated with a reduced risk of infectious complications. Moreover, such treatment would also be expected to poorly interfere with non-specific and specific host antitumoral reactivities.

Acknowledgements — The generous contribution of the Italian Association for Cancer Research, Milan, Italy, is gratefully acknowledged. E. Sfreddo Gallotta is recipient of a fellowship from A.I.D.D.A. (delegazione Lombardia, Milan, Italy).

REFERENCES

1. Paoletti C, Cros S, Dat-Xuong N, Lecointe P, Moisand A. Comparative cytotoxic and antitumoral effects of ellipticine derivatives on mouse L1210 leukemia. *Chem Biol Interact* 1979, **25**, 45–58.
2. Paoletti C, Le Pecq JB, Dat-Xuong N, Juret P, Garnier H, Amiel JL, Rouesse J. Antitumor activity, pharmacology and toxicity of ellipticines, ellipticinium, and 9-Hydroxy derivatives: Preliminary clinical trials of 2-Methyl-9-Hydroxy Ellipticinium (NSC 264–137). *Recent Results Cancer Res* 1980, **74**, 107–123.
3. Amiel JL, Ben Ayed F, Tursz T, Droz JP. Chimothérapie des hépatomes par l'acétate d'elliptinium (NMHE). *Nouv Presse Med* 1981, **10**, 2118.
4. Rouesse J, Tursz T, Le Chevalier T *et al.* Intérêt de la 2-n-methyl-9-hydroxy-ellipticine (NSC 264–137) dans le traitement des cancers métastasés. *Nouv Presse Med* 1981, **10**, 1997–1999.
5. Clarysse A, Brugarolas A, Siegenthaler P *et al.* Phase II study of 9-hydroxy-methyl-ellipticinium (9-HME). *Proc Am Assoc Cancer Res* 1980, **21**, 348.
6. Juret P, Tanguy A, Girard A *et al.* Preliminary trial of 9-hydroxy-2-methyl ellipticinium (NSC 264–137) in advanced human cancers. *Eur J Cancer* 1978, **14**, 205–206.
7. Spreafico F, Mantovani A. Immunomodulation by Cancer chemotherapeutic agents and antineoplastic activity. In: Joachin HL ed. *Pathobiology Annual* New York, Raven Press, 1981, Vol. 11, 177–196.
8. Spreafico F, Vecchi A, Colotta F, Mantovani A. Cancer chemotherapeutics as immunomodulators. *Springer Semin Immunopathol*, 1985, **8**, 361–374.
9. Spreafico F. On the immunopharmacology of cancer chemotherapeutics *Behring Inst Mitt* 1984, **74**, 230–238.
10. Ozer H. Effects of alkylating agents on immunoregulatory mechanisms. In: Mihich E, Sakuraki Y, eds. *Biological Responses in Cancer*. New York, Plenum Press, 1985, Vol. 3, 95–130.
11. Spreafico F. The immunological activity of anthracyclines. In: Muggia FM, Yong CW, Carter SK, eds. *Anthracycline Antibiotics in Cancer Therapy*. The Hague, Martinus Nijhoff, 1982, 50–68.
12. Le Men J, Hayat M, Mathé G, *et al.* Methoxy-9-ellipticine lactate I: Experimental study (oncostatic and immunosuppressive actions; preclinical pharmacology). *Rev Eur Etudes Clin Biol* 1970, **XV**, 534–538.
13. Hayat M, Mathé G, Janot MM *et al.* Experimental screening of 3 forms and 19 derivatives or analogs of ellipticine: Oncostatic effect on L 1210 leukaemia and immunosuppressive effect of 4 of them. *Biomedicine* 1974, **21**, 101–106.
14. Adams DO, Snyderman R. Do macrophages destroy nascent tumors? *JNCI* 1979, **62**, 1341–1345.
15. Gerson JM. Systemic and in situ natural killer activity in tumor-bearing mice and patients with cancer. In: Herberman RB ed. *Natural Cell-Mediated Immunity Against Tumors*. New York, Academic Press 1980, 1047–1062.
16. Vecchi A, Mantovani A, Tagliabue A, Spreafico F. A characterization of the immunosuppressive activity of adriamycin and daunomycin on humoral antibody production and tumor allograft rejection. *Cancer Res* 1976, **36**, 1222–1227.
17. Miller TE, Mackness GB, Lagrange PH. Immunopotentiation with BCG II. Modulation of the response to sheep red blood cells. *JNCI* 1973, **51**, 1669–1676.
18. Vecchi A, Sironi M, Spreafico F. Preliminary characterization in mice of the effect of isoprinosine on the immune system. *Cancer Treat Rep* 1978, **62**, 1975–1979.
19. Mantovani A, Vecchi A, Luini W *et al.* Effect of 2,3,7,8,-tetrachlorodibenzo-p-dioxin on macrophage and natural killer cell-mediated cytotoxicity in mice. *Biomedicine* 1980, **32**, 200–204.
20. Luini W, Boraschi D, Alberti S, Aleotti A, Tagliabue A. Morphological characterization of a cell population responsible for natural killer activity. *Immunology* 1981, **43**, 663–668.
21. Persson V, Hammastrom L, Moller E, Moller G, Smith CIE. The role of adherent cells in B and T lymphocyte activation. *Immunol Rev* 1978, **40**, 78–101.
22. Bhuyan BK, Fraser TJ, Li LH. Cell cycle phase specificity and biochemical effects of ellipticine on mammalian cells. *Cancer Res* 1972, **32**, 2538–2544.
23. Olivotto M, Bomford R. *In vitro* inhibition of tumor cell growth and DNA synthesis by peritoneal and lung macrophages from mice injected with *Corynebacterium parvum*. *Int J Cancer* 1984, **13**, 478–488.

24. Biondi A, Bordignon C, Peri G *et al.* Regulation of the tumoricidal and suppressive activity of human macrophages. In: Hadden JW, Chedid L, Dukor P, Spreafico F, Willoughby D, eds. *Advances in Immunopharmacology* 2. New York, Pergamon Press, 1983, 251–256.
25. Sharma SD, Piessens WF. Tumor cell killing by macrophages activated *in vitro* with lymphocyte mediators II. Inhibition by inhibitors of protein synthesis. *Cell Immunol* 1978, **38**, 264–275.
26. Herberman RB, Ortaldo JR. Natural killer cells: Their role in defenses against disease. *Science* 1981, **214**, 24–30.
27. Vecchi A, Fioretti MC, Mantovani A, Barzi A, Spreafico F. The immunodepressive and hematotoxic activity of imidazole-4-carboxamide, 5-(3,3-dimethyl-1-Triazeno) in mice. *Transplantation* 1976, **22**, 619–624.
28. ,Mantovani A, Luini W, Peri G, Vecchi A, Spreafico F. Effect of chemotherapeutic agents on natural cell-mediated cytotoxicity in mice. *JNCI* 1978, **61**, 1255–1261.