Pergamon

European Journal of Cancer Vol. 31A, No. 9, pp. 1526–1530, 1995

0959-8049/95 $9.50+0.00

0959-8049(95)00246-4

Original Paper

Antitumour Activity and Retinotoxicity of Ethyldeshydroxy-sparsomycin in Mice

H.P. Hofs, D.J.Th. Wagener, D. De Vos, H.C.J. Ottenheijm, H.J. Winkens, P.H.M. Bovee and W.J. De Grip

The colony formation in agar of human tumour xenografts was used as a test system to study the cytostatic activity of ethyldeshydroxy-sparsomycin (EdSm) at the cellular level. EdSm was additionally studied *in vivo* in human tumour xenografts and murine tumour models. EdSm showed a clear dose–response effect *in vitro*. At continuous exposure with 0.01 μg/ml, 2 out of 11 of the tumours responded (a gastric and a small cell lung carcinoma). At 0.1 μ/ml EdSm, the tumour response was 5/11 tumours and at 1 μg/ml the compound was active in all tumours. The maximal tolerable doses of EdSm *in vivo* have been determined in non-tumour bearing CDF_1 mice. In the intraperitoneally (i.p.) given multiple dose schedules the respective LD_{10} doses indicated that the tolerable cumulative dose increases when lower doses are given more frequently. This also enhances the antitumour activity in L1210 leukaemia to 172% T/C. On the other hand, continuous infusion strongly diminished the tolerable dose as well as the antitumour activity. EdSm was also active against i.p. inoculated P388 leukaemia (150% T/C), B16 melanoma (156% T/C), and RC carcinoma (197% T/C), and the subcutaneously (s.c.) inoculated L1210 (139% T/C) and RC (138% T/C). Absence of tumour responses was found in the following s.c. implanted murine tumours: M5076 sarcoma, osteosarcomas C22LR and CP369, and the LL carcinoma, as well as in the human tumour xenografts: LXFG 529, a non-small cell lung carcinoma; GXF 251, a gastric carcinoma; and FMa, an ovary carcinoma. Possible long-range retinotoxic effects of EdSm were investigated in tumour-bearing mice, cured after surviving treatment with LD_{50} doses of EdSm, by assaying the protein biosynthetic capacity of the retina by assaying the ocular rhodopsin and opsin levels as parameters. In none of these cases could a significant reduction in either opsin or rhodopsin levels be measured and no changes were seen histologically.

Key words: sparsomycin, protein synthesis inhibitors, retinotoxicity
Eur J Cancer, Vol. 31A, No. 9, pp. 1526–1530, 1995

INTRODUCTION

THE NATURAL product sparsomycin (Sm) is an antibiotic produced by *Streptomyces sparsogenes* and *Streptomyces cuspidosporus*. It was discovered in 1962 by Owen and associates [1] and characterised by Argoudelis and Herr in 1962 [2]. Antitumour activity was observed towards P388 leukaemia only [3]. A clinical phase I study with Sm (NSC-59729) was initiated in 1964 by Close and McFarlane, using a daily schedule for 10–14 days [4]. This study was discontinued because 2 out of 5 patients reported blurred vision, probably caused by degeneration of the retinal pigment epithelium [4, 5].

Despite these disappointing phase I results there is still interest in Sm and its derivatives have attracted our attention. Firstly, an important aspect is that their mode of action differs from classical antitumour agents. These Sm compounds are potent inhibitors of protein synthesis by blocking the ribosomal peptidyl transferase centre [6–11]. Because of this different mode of action, Sm and several derivatives were used in combination chemotherapy studies and were shown to enhance the antitumour activity of cisplatin [12–14]. The structure–activity relationship of Sm derivatives has been explored and several were found to have a therapeutic index broader than that of Sm itself. Overall, ethyldeshydroxy-sparsomycin (EdSm) showed the best biological activities [15, 16]. Secondly, the use of high daily doses of cytotoxic drugs in phase I studies has been abandoned nowadays because of the high risk of accumulation and toxicity. Finally, intensive histopathological and biochemi-

Correspondence to H.P. Hofs at the University of Amsterdam, Laboratory of Radiobiology F0-212, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands.
H.P. Hofs and D.J.Th. Wagener are at the Department of Internal Medicine, Division of Medical Oncology, Nijmegen University Hospital; D. De Vos is at Pharmachemie B.V., Medical Division, Haarlem; H.C.J. Ottenheijm was at the Department of Organic Chemistry, University of Nijmegen, but presently at Organon International B.V., Oss; H.J. Winkens is at the Institute of Ophthalmology, Nijmegen University Hospital; and P.H.M. Bovee and W.J. De Grip are at the Department of Biochemistry, University of Nijmegen, The Netherlands.
Revised 15 Dec. 1994; accepted 21 Dec. 1994.

cal analysis of animal models failed to show retinal toxicity of Sm and one analogue, even at repeated toxic doses [17, 18]. Moreover, the synthesis of suitable analogues might overcome the putative ocular toxicity. Therefore, we have extensively studied the antitumour activity and retinotoxicity of EdSm. In this report the results are summarised and discussed.

MATERIALS AND METHODS

Drugs

EdSm was synthesised at the department of Organic Chemistry of the University of Nijmegen, The Netherlands [17], and was acquired in a lyophilised form. The drug was dissolved in phosphate buffered saline (pH 7.4) and stored at 4°C in dark flasks. Solutions with the required drug concentration were prepared just before administration by dilution with isotonic NaCl.

Maximal tolerable dose

The acute toxicity of EdSm has been determined in normal CD2F1 mice. The 50% and 10% lethal doses (LD_{50} and LD_{10}, respectively) were determined after administration on various routes and schedules. For bolus injections we used five dose levels and six mice per dose level. For continuous administration of EdSm to normal and tumour-bearing mice, we used Alzet osmotic minipumps 1003 D and 1007 D (Alza, Palo Alto, California, U.S.A.). The 1003 D minipump has a volume of 100 μl and a flow capacity of 1.06 ± 0.05 μl/h continuously over 3 days. The 1007 D minipump also has a volume of 100 μl, but a flow capacity of 0.5 μl/h continuously over 7 days. These minipumps were filled with an EdSm solution in isotonic NaCl. Pumps were implanted intraperitoneally (i.p.) or subcutaneously (s.c.) under ether anaesthesia. Three mice were used per dose level in osmotic minipumps.

In vitro *xenografts*

Experiments with human tumour xenografts were performed by Dr H.H. Fiebig, Department of Internal Medicine, Division of Haematology–Oncology, University of Freiburg, D-7800 Freiburg, Germany. Human tumours, established in serial passage in nude mice, as described by Fiebig and colleagues [19], were used. A single-cell suspension of the solid human tumours was obtained by mechanical disaggregation with scissors and subsequent incubation with enzymes, as described by Fiebig and colleagues [20]. The two-layer soft-agar culture system introduced by Hamburger and Salmon [21] was modified as described by Fiebig and colleagues [19]. In the colony assay, EdSm was applied by continuous exposure until the end of the experiment. Three dose levels were studied in triplicate and in each experiment six untreated cultures were used as controls. 5-Fluouracil (100 μg/ml, continuous exposure) was added as a positive reference drug. A compound was considered active if it reduced colony formation to 30% or less of the control value.

Antitumour activity

Three human tumours were used to study the antitumour activity of EdSm *in vivo*: a non-small cell lung carcinoma (LXFG 529), a gastric carcinoma (GXF 251) (both studies were tested by Dr H.H. Fiebig), and an ovary carcinoma (FMa), (tested by Dr E. Boven, Free University Hospital, Amsterdam, The Netherlands). The murine leukaemia tumours P388 and L1210 and the solid tumours B16 melanoma and RC renal cell carcinoma were obtained from G. Atassi, Brussels. They were passaged and maintained in animals as recommended by the NCI. The experiments with the osteosarcomas C22LR and LL, the Lewis Lung carcinoma and M5076 sarcoma were performed at the Radiobiological Institute TNO in Rijswijk as reported by Zylicz and associates [18]. The osteosarcoma CP368 was tested at CIVO-TNO, Zeist, The Netherlands, by Dr P. Lelieveld. In the treatment of subcutaneously growing human and murine tumours EdSm was administered to tumour-bearing animals, with tumour sizes between 25 and 50 mm^3, at a dose level of 5 and 10 mg/kg on days 1, 5 and 9. Animals were randomised before treatment.

Studies on retinotoxic effects

CD2F1 mice were obtained from Charles River Breeding Laboratories, Inc. Male mice weighing between 18 and 22 g were inoculated intraperitoneally with 10^5 L1210 cells. Acceptable median control survival times range from 8–11 days for L1210 i.p. tumour. EdSm and cisplatin were administered i.p. at various doses on days 1, 5 and 9 after tumour implantation. Eye toxicity was investigated in tumour-bearing animals which were recorded as cures after drug treatment [22].

Rhodopsin assay. Animals were dark-adapted for 24 h to ensure maximal regeneration of rod visual pigment (opsin to rhodopsin). All procedures were carried out in dim red light (> 610 nm). After pentobarbital anaesthesia the eyes were extirpated, placed in light-tight containers and directly frozen at −80°C. The animals were sacrificed afterwards. Extraction and assay of rhodopsin have been described previously [23]. Rhodopsin was measured by difference spectroscopy and the rhodopsin concentration was calculated from the difference in absorbance at 500 nm before and after illumination, using a molar absorbance coefficient of 403 000.

Opsin assay. For quantitative determination of opsin, the earlier described enzyme-linked immunosorbent assay (ELISA) was used [24, 25].

Histopathology. Eyes were fixed in Bouin solution and embedded in paraffin according to standard procedures [26]. Sections of 5 μm were stained with haematoxylin and eosin, and subsequently analysed microscopically.

RESULTS

The cytostatic activity of EdSm was studied in 11 human tumour xenografts *in vitro* in doses ranging from 0.01 to 1.0 μg/ml (0.0278–2.78 μM) given by continuous exposure (Table 1). A clear dose–response relationship was established. At the dose of 0.01 μg/ml 2 out of 11 human tumours (18%) were sensitive, at 0.1 μg/ml this was 45% and at 1.0 μg/ml all tumours responded, suggesting that if an EdSm concentration of about 2.8 μM can be maintained at the cellular tumour level over a longer period *in vivo*, this tumour will respond positively to EdSm treatment.

Before determining the antitumour activity of EdSm in different animal tumour models, we investigated the acute toxicity in normal CD2F1 mice. The 50% and 10% lethal doses (LD_{50} and LD_{10}, respectively) were determined after i.p. EdSm administration on various schedules; the results are summarised in Table 2. For single dose treatment the intravenous (i.v.) route was more tolerable than the i.p. route; the LD_{10} values are 48.8 and 24.9 mg/kg, respectively. This difference disappeared upon daily administration. Additionally, these data indicate that EdSm is better tolerated when it is given more frequently at lower doses. Daily i.p. administration for 9 days results in a LD_{10}

Table 1. In vitro *effect of EdSm on* 11 *human tumour cells derived from xenografts in nude mice expressed as the number of responses/the number tested*

Tumour	Passage No.	Drug concentration (μg/ml) 0.01	0.1	1
CXF 609	8	—	—	++
GXF 251	15	++	+++	+++
GXF 97	23	—	+	+++
LXFA 526	15	—	++	+++
LXFG 529	6	+	++	+++
LXFK 605	10	—	—	+++
LXFK 650	12	++	+++	+++
LXFL 1029	4	—	—	+++
MAXF 401	12	—	++	+++
TXF 423	11	—	—	+++
XF 299	15	—	+	+++
Sensitive*		2/11	5/11	11/11

Tumours were considered to be responsive if the colony count was ≤ 30% of the control at continuous exposure.
CXF, colon cancer xenograft; GXF, gastrix; LXF, lung; A, adeno, G, large cell, K, small cell; MAXF, mammary; TXF, testicular; XF, miscellaneous cancer xenograft.
* Sensitive = ++ and +++; T/C > 50% = —; 30% < T/C ≤ 50% = +; 10% ≤ T/C ≤ 30% = ++; T/C < 10% = +++.

Table 2. *Tolerable doses of EdSm in mice after administration on various schedules*

Schedule	Route	LD_{10}	LD_{50}	*m*	*R*
SD	i.p.	24.9	30.0	11.8	0.959
D1, 5, 9	i.p.	37.2	44.1	13.0	0.993
D1–9*	i.p.	54.0	72.0		
CI	i.p.	2.96	4.48	5.3	0.998
SD	i.v.	48.8	57.7	13.1	0.995
D1–7*	i.v.	48.3			

SD, single dose; D1, 5, 9, treatment on day 1, 5 and 9; D1–7 and D1–9, daily treatment for 7 or 9 days, respectively; CI, continuous infusion during 3 days, using osmotic minipumps (1003D); i.p., intraperitoneal; i.v., intravenous; LD_{10}, cumulative doses in mg/kg that results in 10% lethality; LD_{50}, cumulative doses in mg/kg that results in 50% lethality; *m*, the coefficient signifying the shape of the dose–effect curve for the agent used; *R*, the linear correlation coefficient of the dose–effect curves.
* Results presented by Zylicz and associates [18].

of 54 mg/kg. However, EdSm is less tolerable when given as a continuous infusion, resulting in a LD_{10} dose of 2.96 mg/kg over 3 days.

Based on these LD_{10} values the antitumour activity of EdSm was determined in animal tumour models. As presented in Table 3, all i.p. inoculated murine tumours (L1210, P388, B16 and RC) responded positively to daily i.p. EdSm administration (%T/C > 135%). The L1210 model was used to investigate the effect of different treatment schedules. When EdSm was given in three doses (D1, 5, 9), the antitumour activity against L1210 was still comparable to that observed upon daily administration (158% and 172%, respectively). On the other hand, continuous infusion i.p. for 3 or 7 days lowered the activity to 130 and 120%, respectively, and no antitumour response was seen when these minimpumps were placed s.c., despite the increased EdSm dose

Table 3. *Summary of the antitumour activity of EdSm in* 8 *murine tumours and* 3 *human tumour xenografts* in vivo

Tumour	Site	Schedule	Route	Dose*	Response†	Ref.
L1210	IP	D1–9	i.p.	55	172	[18]
L1210	IP	D1, 5, 9	i.p.	15	158	[16]
L1210	IP	CI D1–3	i.p.	1	130	
L1210	IP	CI D1–3	s.c.	2	100	
L1210	IP	CI D1–7	i.p.	2	120	
L1210	IP	CI D1–7	s.c.	2	100	
L1210	SC	D1–7	i.v.	48	139	[18]
P388	IP	D1–9	i.p.	55	150	[18]
B16	IP	D1–7	i.p.	35	156	[38]
RC	IP	D1–9	i.p.	37	197	[18]
RC	SC	D1–7	i.v.	42	138	[18]
M5076	SC	D1–9	i.p.	55	100	[18]
C22LR	SC	D2–10	i.p.	55	100	[18]
CP369	SC	D1, 5, 9	i.p.	15	100	
LL	SC	D1, 5, 9	i.p.	22.5	100	[18]
LXFK 529	SC	D1, 5	i.p.	20	100	
GXF 251	SC	D1, 5, 9	i.p.	30	100	
FMa	SC	D1, 5, 9	i.p.	30	100	

For explanations see also Table 2. i.p., intraperitoneally; i.v., intravenously; s.c., subcutaneously.
* Cumulative dose in mg/kg; † Median survival time in % of treated over control (% T/C).

of 2 mg/kg. From the s.c. implanted tumours appreciable T/C values (> 135%) were only obtained with L1210 and RC. No significant antitumour response was measured in the M5076, C22LR, CP369 and LL tumour models. The two human tumour xenografts most sensitive to EdSm *in vitro*; i.e. the large cell lung cancer LXFG 529 and the gastric carcinoma GXF 251, as well as the ovary carcinoma FMa were tested *in vivo*. EdSm at a dose of 5 or 10 mg/kg, given i.p. on days 1, 5 (LXFG 529) or 1, 5, 9 (GXF 251 and FMa), did not exert any antineoplastic effect in any of these tumours.

Retinopathy

Toxic effects of EdSm on the protein biosynthetic capacity of the retina were assessed by assaying the rhodopsin and opsin levels in tumour-bearing mice, cured after treatment with doses of EdSm which were toxic for normal standards (LD_{50}). The photoreceptor cell–RPE (retinal pigment epithelium) complex accomplishes a high turnover rate of visual pigment (ca. 10% per day) and in order to maintain a constant level the photoreceptor cell has a high biosynthetic capacity for the protein opsin (5–10 pmol/10^6 cells per day) [27]. Perturbations like inhibition of this process will, therefore, rapidly lead to a reduction in ocular opsin content, and may eventually effect an almost complete loss in visual pigment protein. For conversion of the apoprotein opsin into the photoactive pigment rhodopsin, the photoreceptor cell relies on the production of the required chromophore, 11-*cis* retinal, by the enzymatic machinery of the retinal pigment epithelium [28]. Selective inhibition of this process could result in impaired ocular opsin levels and reduced rhodopsin levels. In order to incorporate possible additive effects due to tumour- and other disease-related pathogenicity, the toxicity of EdSm was investigated in tumour-bearing mice which survived on relatively high doses of EdSm or Sm, in combination with cisplatin, which at high doses can also cause retinotoxicity [29]. In none of these cases could a significant reduction in either

opsin or rhodopsin levels be measured (Table 4). None of the eyes of mice treated with cisplatin and EdSm showed histological changes.

DISCUSSION

The toxicity of Sm analogues in mice is related to their lipophilicity; it decreases with increasing lipophilic character [17]. Therefore, EdSm could be administered at much higher doses than Sm, e.g. LD_{50} of 8 mg/kg for EdSm compared with 0.26 mg/kg for Sm for the i.p. schedule D1–9. Osmotic minipumps have been very useful in the study of the effects of continuous drug treatment [30–34]. Chronic administration of EdSm using 100 μl osmotic minipumps over 3 days turned out to be less tolerable (LD_{10} = 2.96 mg/kg) and less therapeutic than repeated bolus injections. Thus, the therapeutic window of EdSm treatment when given chronically seems to be low.

Previous studies have demonstrated that exposure of Chinese hamster fibroblast cells to Sm for 1 h inhibits over 99% of their protein synthetic capacity, but is lethal for only 25% of these cells, due to the fact that after removal of the drug the cells recovered their protein synthesising ability. This reversibility disappeared after longer periods of incubation [35]. Similarly, chronic EdSm treatment might cause an irreversible protein imbalance due to permanent inhibition of protein synthesis, which would enhance its toxicity to normal tissues. When lower doses are given at frequent intervals the cumulative tolerable dose for EdSm increases and the antitumour response is improved. Under those conditions, appreciable antitumour activity for EdSm (> 135%) is obtained when the tumour site as well as the drug treatment is i.p., and in two s.c. implanted tumours (L1210 and RC) when EdSm was given i.v. (139% and 138%, T/C respectively). Worth mentioning is that no antitumour activity of EdSm was noticed when the tumour was implanted s.c. and drug treatment was i.p. It is not clear yet whether this is due to the treatment schedule (D1, 5, 9 instead of D1–9) or mainly due to i.p. instead of i.v. administration. The strongest antitumour responses were obtained upon daily EdSm administration, i.p. when the tumour was implanted i.p. and i.v. when the tumour was implanted s.c. From this we conclude that the application route of choice for EdSm in animal tumour models should implement i.p. administration for i.p. tumours and i.v. administration for s.c. tumours.

Table 4. Rhodopsin and opsin concentrations in eyes of CD2F1 mice, 6–8 months of age, which were alive and tumour-free 100 days after combined treatment with cisplatin and EdSm in the L1210 tumour model

Treatment	% Survivors	*Rhodopsin/eye	†Opsin/eye
Group I	33	0.64 ± 0.05	0.65 ± 0.06
Group II	67	0.66 ± 0.03	0.65 ± 0.03
Group III	60	0.67 ± 0.04	0.68 ± 0.02
Group IV	67	0.66 ± 0.02	0.68 ± 0.06
Group V	67	0.63 ± 0.05	0.61 ± 0.07
Control		0.71 ± 0.07	

Mice from all these groups were histologically checked for degeneration of the neural retina and retina pigment epithelium.

Group I, cisplatin 4 mg/kg; group II, cisplatin 4 mg/kg and EdSm 10 mg/kg; group III, cisplatin 3 mg/kg and EdSm 5 mg/kg; group IV, cisplatin 3 mg/kg and EdSm 10 mg/kg; group V, cisplatin 3 mg/kg and Sm 1.5 mg/kg [14, 22].

* Spectroscopy, $n = 4 \pm$ S.D.; † ELISA, $n = 4 \pm$ S.D.

Under the auspices of the National Cancer Institute (Bethesda, Maryland, U.S.A.) additional toxicological studies in rats and monkeys have been performed [36]. Retinotoxicity was assessed by electroretinography (ERG) and eye histology. Abnormal electroretinograms were only observed in moribund rats, probably due to their poor general condition. No ERG changes were seen in monkeys. Histopathological changes were not detected in any animal. The plasma membrane of reticulocytes is rather impermeable for Sm [37]. On the basis of these studies, the Sm-related retinopathy is suggestive of a poor general condition, and possibly of inappropriate drug schedules. Since the first reports on retinal toxicity of Sm preparations concern late-stage patients, to a large degree in cachectic condition [4, 5], we extended these analyses to tumour-bearing mice and included the more lipophilic Sm derivative EdSm, which will more easily cross the blood–retina barrier. No evidence was found for any retinotoxic action of Sm or EdSm, either on a supracellular level (retinal morphology) or on a subcellular level (protein biosynthesis, intracellular and intercellular transport). These novel observations are fully in line with our previous results. We have to conclude that Sm-derivatives are not retinotoxic.

1. Owen SP, Dietz A, Camiener GW. Sparsomycin, a new antitumour antibiotic. *Antimicrob Agents Chemother* 1962, **3**, 772–779.
2. Argoudelis AD, Herr RR. Sparsomycin, a new antitumour antibiotic. II. Isolation and characterization. *Antimicrob Agents Chemother* 1962, **3**, 780–786.
3. National Institute of Health. Developmental Therapeutic Program, Division of Cancer Treatment. Cancer Chemotherapy National Service Centre, Bethesda, MD, USA. Screening data sparsomycin (NSC-59729) 1964, 1–28.
4. Close HP, McFarlane JR. Ocular toxicity with sparsomycin (NSC 59729) in a phase I study: a preliminary report. *Cancer Chemother Rep* 1964, **43**, 29–31.
5. McFarlane JR, Yanoff M, Scheie HG. Toxic retinopathy following sparsomycin therapy. *Arch Ophthal* 1966, **76**, 532–540.
6. National Cancer Institute. Bethesda, MD. Repeated-dose intravenous toxicity of NSC-59729 (sparsomycin) in rats and monkeys, including electroretinograph studies. Research report No. PH-43-65-61 1967, 1–44.
7. Zylicz Z, Grip WJ de, Wagener DJTh, *et al.* Studies on retinotoxic potential or a novel antitumour antibiotic—sparsomycin—in rats. *Anticancer Res* 1989, **9**, 929–928.
8. Contreras A, Carrasco L. Selective inhibition of protein synthesis in virus-infected mammalian cells. *J Virol* 1979, **29**, 114–122.
9. Goldberg IH, Mitsugi K. Sparsomycin, an inhibitor of aminoacyl transfer to polypeptide. *Biochem Biophys Res Commun* 1966, **23**, 453–459.
10. Goldberg IH, Mitsugi K. Sparsomycin inhibition of polypeptide synthesis promoted by synthetic and natural polynucleotides. *Biochemistry* 1967, **6**, 372–382.
11. Goldberg IH, Mitsugi K. Inhibition by sparsomycin and other antibiotics of the puromycin-induced release of polypeptide from ribosomes. *Biochemistry* 1967, **6**, 383–391.
12. Tada K, Trakatellis AC. Mechanism of action of sparsomycin on protein synthesis. *Antimicrob Agents Chemother* 1979, **3**, 227–230.
13. Theocharis DA, Coutsogeorgopoulos C. Mechanism of action of sparsomycin in protein synthesis. *Biochemistry* 1992, **31**, 5861–5868.
14. Zylicz Z, Wagener DJTh, Rennes H van, *et al.* *In vivo* potentiation of cis-diamminedichloroplatinum (II) antitumour activity by pretreatment with sparsomycin. *Cancer Lett* 1986, **32**, 53–59.
15. Zylicz Z, Hofs HP, Wagener DJTh. Potentiation of cisplatin antitumour activity on L1210 leukaemia s.c. by sparsomycin and three of its analogues. *Cancer Lett* 1989, **46**, 153–157.
16. Hofs HP, Wagener DJTh, De Valk-Bakker V, *et al.* Potentiation of cisplatin antitumour activity by ethyldeshydroxysparsomycin in L1210 leukemia. *Anticancer Res* 1992, **12**, 167–170.
17. Broek LAGM van den, Lazaro E, Zylicz Z, *et al.* Lipophilic analogues of sparsomycin as strong inhibitors of protein synthesis and tumour growth: a structure–activity relationship study. *J Med Chem* 1989, **32**, 2002–2015.

18. Zylicz Z, Wagener DJTh, Rennes H van, *et al. In vivo* antitumour activity of new sparsomycin and its analogues in eight murine tumour models. *Invest New Drugs* 1988, **6**, 285–292.
19. Fiebig HH, Berger DP, Köpping K, Ottenheijm HCJ, Zylicz Z. *In vitro* and *in vivo* anticancer activity of mitozolomide and sparsomycin in human tumour xenografts, murine tumours and human bone marrow. *J Cancer Res Clin Oncol* 1990, **116**, 550–556.
20. Fiebig HH, Schmid JR, Bieser W, Henss H, Lohr GW. Colony assay with human tumour xenografts, murine tumour and human bone marrow. Potential for anticancer drug development. *Eur J Cancer Clin Oncol* 1987, **23**, 937–948.
21. Hamburger AW, Salmon SE. Primary bioassay of human tumour stem cells. *Science* 1977, **197**, 461–463.
22. Hofs HP, Wagener DJTh, De Valk-Bakker V, *et al.* Correlation of the *in vitro* cytotoxicity of ethyldeshydroxysparsomycin and cisplatin with the *in vivo* antitumour activity in murine L1210 leukaemia and two resistant L1210 subclones. *Cancer Chemother Pharmac* 1993, **31**, 289–294.
23. De Grip WJ, Daemen FJM, Bonting SL. Isolation and purification of bovine rhodopsin. *Meth Enzym* 1980, **65**, 301–302.
24. Schalken JJ, Janssen JJM, Sanyal S, Hawkins RK, Grip WJ de. Development and degeneration of retina in rds mutant mice: immunoassay of the rod visual pigment rhodopsin. *Biochim Biophys Acta* 1990, **1033**, 103–109.
25. Schalken JJ, Grip WJ de. Enzyme-linked immunosorbent assay for quantitative determination of the visual pigment rhodopsin in total eye-extracts. *Exp Eye Res* 1986, **43**, 431–439.
26. Broekhuyse RM, Kuhlmann ED, Winkens HJ. Experimental autoimmune posterior uveitis accompanied by epitheloid cell accumulations (EAPU). A new type of experimental ocular disease induced by immunization with PEP-65, a pigment epithelial polypeptide preparation. *Exp Eye Res* 1992, **55**, 819–829.
27. Young RW, Dioz B. The renewal of protein in retinal rods and cones. *J Cell Biol* 1968, **39**, 169–184.
28. Bernstein PS, Law CC, Rando RR. Biochemical characterization of the retinoid isomerase system of the eye. *J Biol Chem* 1987, **262**, 16848–16857.
29. Wilding G, Caruso R, Lawrence TS, *et al.* Retinal toxicity after high-dose cisplatin therapy. *J Clin Oncol* 1985, **3**, 1683–1689.
30. Thompson JS, Saxena SK, Greaton C, Schultz G, Sharp JG. The effect of the route of delivery of urogastrone on intestinal regeneration. *Surgery* 1989, **106**, 45–51.
31. Wood JM, Jobber RA, Baum HP, Gasparo M de, Nussberger J. Biochemical effects of prolonged renin inhibition in marmosets. *J Hypertension* 1989, **7**, 615–618.
32. Hernandez TD, Heninger C, Wilson MA, Gallager DW. Relationship of agonist efficacy to changes in GABA sensitivity and anticonvulsant tolerance following chronic benzodiazepine ligand exposure. *Eur J Pharmac* 1989, **170**, 145–155.
33. Sanjar S, Aoki S, Boubekeur K, *et al.* Inhibition of PAF-induced eosinophil accumulation in pulmonary airways of guinea pigs by anti-asthma drugs. *Jpn J Pharmac* 1989, **51**, 167–172.
34. Szemeredi K, Zukowska-Grojec Z, Bagdy G, Fekete MIK, Kopin IJ. Opposite effects of chronic cortisol treatment on pre- and postsynaptic actions of clonidine in pithed rats. *J Auton Pharmac* 1989, **9**, 35–43.
35. Bhuyan BK, Scheidt LG, Fraser TJ. Cell cycle phase specificity of antitumour agents. *Cancer Res* 1972, **32**, 398–407.
36. National Cancer Institute, Bethesda, MD. Repeated-dose intravenous toxicity of NSC-59729 (sparsomycin) in rats and monkeys, including electroretinograph studies. Research report No. PH-43-65-61 1967, 1–43.
37. Colombo B, Felicetti L, Baglioni C. Inhibition of protein synthesis in reticulocytes by antibiotics. *Biochim Biophys Acta* 1966, **119**, 109–119.
38. Hofs HP, Wagener DJTh, Valk-Bakker V de, *et al.* Preclinical antitumour activity of ethyldeshydroxysparsomycin in combination with cisplatin. *Invest New Drugs* 1995 (in press).

Acknowledgements—We are very grateful to Dr Koopmans and his coworkers at the Central Animal Laboratory for excellent animal care and assistance during these experiments. This work was financially supported by the Dutch Cancer Society, by Pharmachemie B.V., The Netherlands and by the Maurice and Anna de Kock Foundation, The Netherlands.