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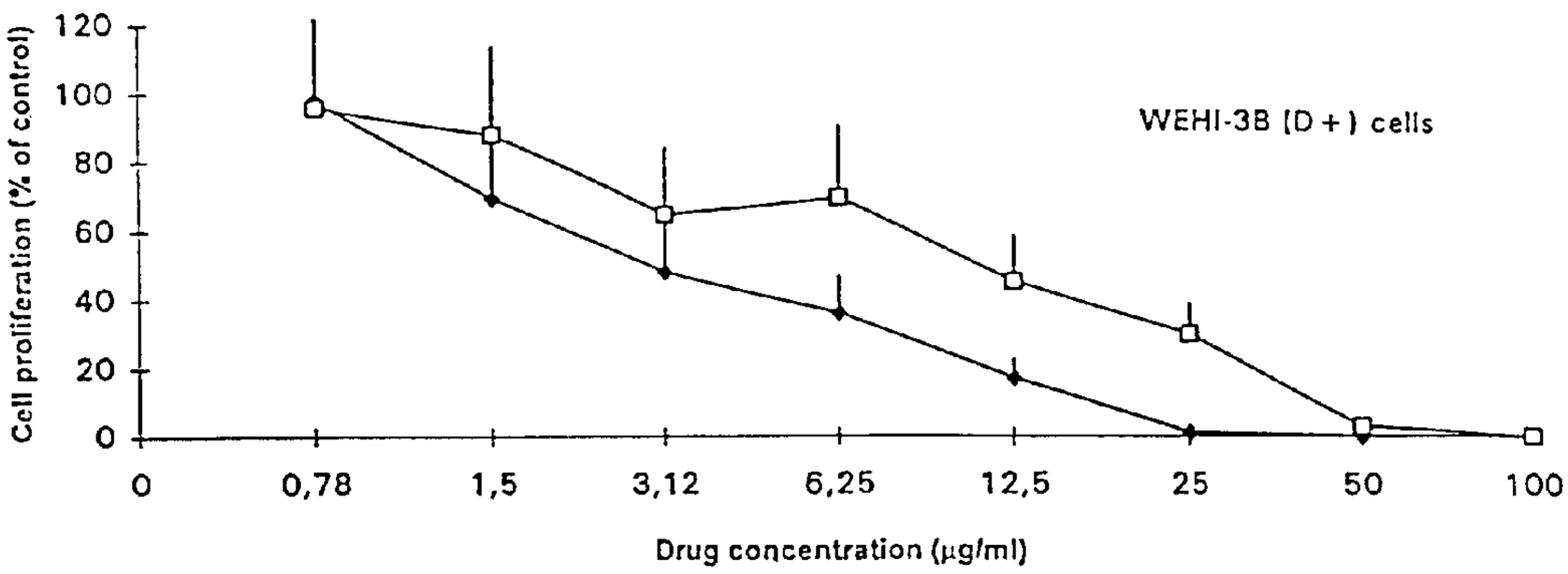
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(54) **METHODE ANTITUMORALE PAR ADMINISTRATION DE DERIVES DE PARTRICINE**

(54) **ANTITUMORAL METHOD BY ADMINISTRATION OF PARTRICIN DERIVATIVES**



- Dose-response curve of the cytotoxic effect on *in vitro* cell proliferation (MTT test).

  (◆) SPA-S-843 (□) Amphotericin B

  Each point is the mean ± s.e. of 3 experiments conducted in triplicate.

(57) The use of some semisynthetic derivatives of the antifungal polyene partricin to selectively hinder the growth of tumor cells is here described. Their use can be associated with the use of other known antineoplastic agents, in particular in the cases where a resistance to these agents is present or has developed. The invention can be applied to systems of cell cultures as well as to the therapeutic practice.

ABSTRACT

The use of some semisynthetic derivatives of the antifungal polyene partricin to selectively hinder the growth of tumor cells is here described.

Their use can be associated with the use of other known antineoplastic agents, in particular in the cases where a resistance to these agents is present or has developed.

The invention can be applied to systems of cell cultures as well as to the therapeutic practice.

ANTITUMORAL METHOD BY ADMINISTRATION OF PARTRICIN DERIVATIVES.

Partricin, a mixture of partricin A and partricin B (The Merck Index XII Ed., no. 7181), is a polyene substance obtained through fermentation of a special *S. aureofaciens* strain, and exhibiting very high antifungal activity.

Since the substance as such is rather toxic to be used in therapy, several structural modifications were brought to it by semisynthetic route, using partricin itself or its individual constituents as the raw material, specially partricin A. Examples of the afore-said derivatives are given, among others, in European Patent Applications EP-A-0434943 and EP-A-0489308 as well as in U.S. Patent 3780173 (see also the Merck Index 12th Ed., 5891) and US 3961047.

Several of these derivatives were found more active as antifungal agents, and much less toxic, than the starting substances and than the present reference standard in therapeutic practice, that is, amphotericin B.

In the course of experimental investigations, wherein partricin derivatives were tested on cell

cultures with a view to preventing their contamination by fungi - which was suggested by the high antifungal activity of these derivatives, coupled with their excellent tolerance by eukaryote cells at the concentrations active against fungi and moulds - we have surprisingly found - and it is the subject of the present patent application - that some tumor cell lines were extraordinarily sensitive to such substances; also at very reduced concentrations (but, at any rate generally higher than those carrying out antifungal activity in the respective cell culture), and for a limited exposure time, a total blockage of cell growth was observed, with final complete mortality of the cells.

The classes of partricin derivatives suitable for the use and methods of this invention comprise esters and amides on the carboxyl group, optionally substituted on the primary amino group of the mycosamine radical with acyls.

The derivatives of partricin A and/or B which we have used, are the same as described in European Patent applications EP-A-0434943, EP-A-0489308, US 3780173 and US 3961047 are, in practice, esters and amides on the carboxyl group at C-18 of the macrolide ring,

optionally substituted on the amino group of the mycosamine radical with an acyl group and their salts with organic and inorganic acids acceptable in the pharmacological and pharmaceutical practice.

In particular, the amide derivatives of partricin A and/or B described in European Patent application EP-A-0434943 (corresponding to US patent 5.296.597) are secondary and tertiary amides of the carboxyl group at C-18 of the macrolide ring. In partricin A and/or B derivatives described in patent application EP-A-0489308 (corresponding to US patent 5.298.495), the carboxyl group at C-18 of the macrolide ring may be unsubstituted or may form a C_1-C_6 alkyl ester or an amide, neutral or containing an additional basic nitrogen containing function, and the primary amino group of the mycosamine portion forms an amide bond with the carboxyl group of an aliphatic acid of 2-4 carbon atoms containing an additional basic nitrogen containing group.

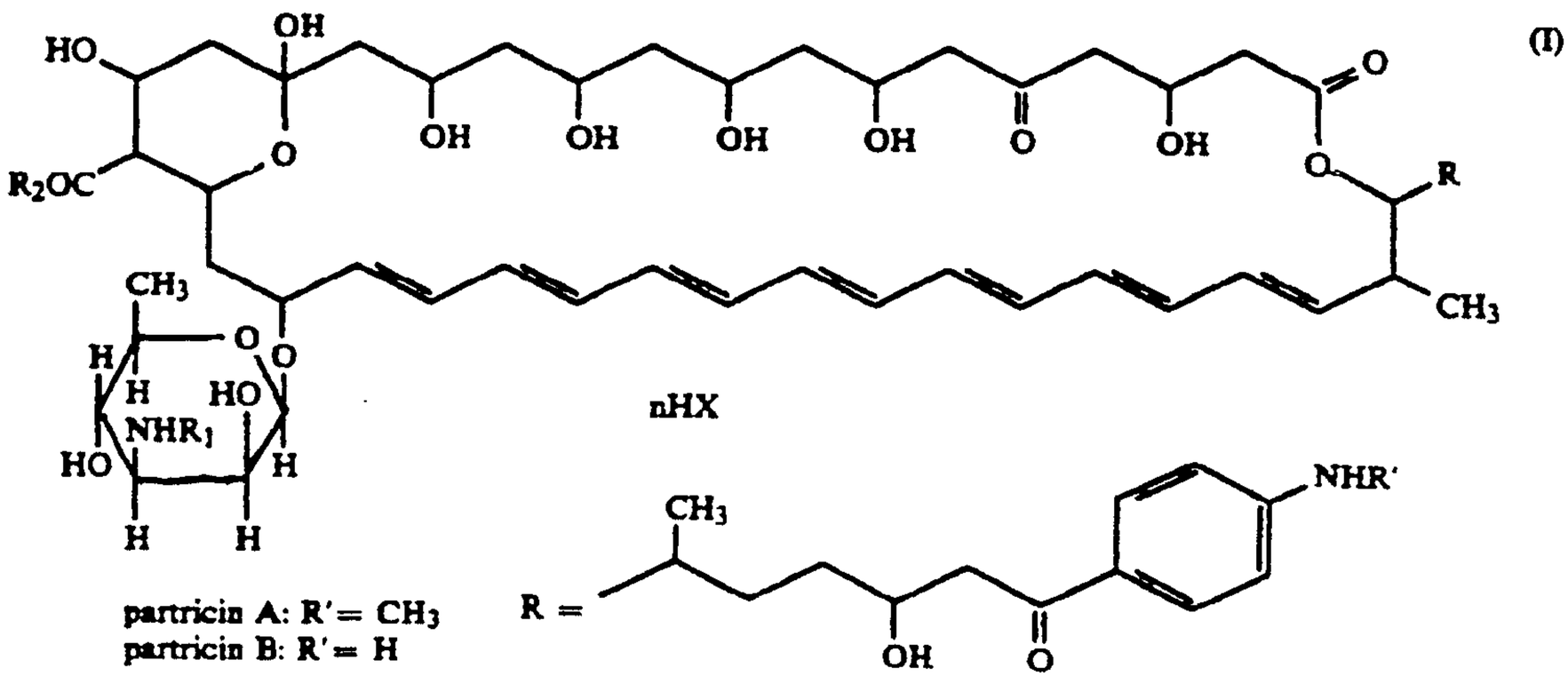
Other derivatives of partricin suitable for the use and methods of this invention are the alkyl esters of partricin, e.g. C_1-C_4 alkyl esters, in particular, the methyl ester of partricin complex (mepartricin)

and of partricin A and/or B, described in US 3780173 and US 3961047.

The description and examples of the afore-said European patents, their corresponding US 5296597 and US 5298495 counterparts, of US 3780173 and US 3961047 relative to the preparation of partricin A and/or B derivatives mentioned above, are incorporated by reference in the present patent application.

The scope of this invention includes the use of those derivatives of partricin A and/or B having the usual spatial configuration of partricin as well as those derivatives of partricin A and/or B having ''all trans'' configuration which can be obtained, for instance, by UV or sun light exposure (Bruzzese T. et al, Il Farmaco Ed. Sci. 4, 331-334, 1974).

Partricin A and /or B derivatives which are particularly useful for the use accoding to this invention are represented by the following formula (I) :



(I)

wherein

R' is a hydrogen atom or a methyl group;

R_1 is an aminoacyl radical $-CO(CH_2)_mNR_3R_4$, wherein m= 1,2 or 3, R_3 and R_4, which can be the same or different, are a hydrogen atom or a C_1-C_3 alkyl group or form with the nitrogen atom to which they are bound, a five- or six-membered heterocyclic ring optionally containing another hetero atom selected from the group consisting of O,S and N, wherein said N may be substituted by a methyl or 2-hydroxyethyl group;

R_2 is a hydroxy, C_1-C_6 alkoxy, NR_3R_4 amino or $-NH-(CH_2)_m-NR_1R_4$ aminoalkylamino group, wherein m, R_3 and R_4 are as defined above, or a substituted $-NH-(CH_2)_m-R_5$ alkylamino group, wherein m is as defined above, and R_5 is a five- or six-membered heterocyclic ring, wherein the heteroatom is nitrogen which may be substituted with a methyl or ethyl group;

X is the anion of a pharmaceutically acceptable organic or inorganic acid, preferably selected from the group consisting of aspartic, glutamic, glycolic, glucuronic, gluconic and ascorbic acid; and

n is 0,1 or 2.

One of the compounds found most effective in the course of our study is a partricin A derivative, that is, N-dimethylamino-acetyl-partricin A 2-dimethylaminoethylamide. Following transformation to the corresponding salts, in particular, to diascorbate (laboratory code SPA-S-843), the compound becomes water soluble, unlike many natural polyenes, and can easily be employed in *in vitro* cultures, as well as easily used in pharmaceutical formulations for the clinical application by parenteral route (injectable ampuls, infusions etc.).

The above reported finding relative to the surprisingly marked toxicity against some tumor cell lines, leads us to foresee the use of these compounds in the clinical practice as antineoplastic drugs, after having tested *in vitro* cell sensitivity of each tumor line (for example, leukemia, lymphoma, myeloma , melanoma, carcinoma, sarcoma and other tumors).

Their utilisation is also foreseeable in combination with other drugs of better known antineoplastic activity (platinum derivatives, doxorubicin, vincristine, methotrexate, 5-fluorouracil etc.)

considering, at any rate, that their interaction (common with the other polyenes) with the membrane sterols, is damaging to cell membrane which favours intracellular penetration and accumulation of the other antineoplastic drugs, leading to synergic effects.

A representative example of SPA-S-843 cell toxicity on murine WEHI-3B (D+) myelomonocytic leukemia cells (Pessina et al., ''Susceptibility of leukemia cell lines to quinolones and induction of resistance to ciprofloxacin in WEHI-3B (D+) leukemia cells ''Cancer J., *6*, 291-7, 1993) is here reported with no limiting intent of the invention.

Substances: SPA-S-843 and amphotericin B were dissolved in sterile bidistilled water to a concentration of 2000 µg/ml. The working solutions were prepared in McCoy medium supplemented with penicillin+streptomycin (100 U/ml + 100 µg/ml) at the concentrations of from 200 µg/ml to 1.56 µg/ml by subsequent serial 1:2 dilutions.

The murine WEHI-3B (D+) myelomonocytic leukemia cells were maintained through subcultures in 25-cm^2 plastic bottles in McCoy medium (Seromed, Germany) containing 5% FCS (Gibco, USA).

Cell sensitivity to the toxic action of SPA-S-843 in comparison with that of amphotericin B, taken as reference substance, was determined by two different methods:

A) Microtiter test (MTT) (Pessina et al. ''In vitro short-term and long-term cytotoxicity of fluoroquinones on murine cell line'', Ind. J. Exp. Biol., *32*, 113-8, 1994).

B) Clonogenic test in agar (Pessina et al. ''Inhibition of murine leukemia cell - WEHI-3B and L1210 - Proliferation by cholera toxin B subunity'', Biochem. Biophys. Acta, 1013, 206-11, 1989).

Description of tests

Cytotoxicity by MTT: it is a microtiter test using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT - Sigma, USA) according to Mossman T. (J. Immunol. Methods, *65*, 55-63, 1983) modified according to Kriegler A.B. et al. (J. Immunol. Methods *103*, 93-102, 1987).

In brief, 50µl of a 1:2 serial dilution from 2µg/ml to 0.015 µg/ml of each of the two drugs were placed in 96 wells of flat-bottom Microtiter plates then each well was added with a 50µl cell suspension (6 x 10^3

cells/ml). The cultures were incubated for 7 days at 37°C in air+5%CO_2 and finally 20µl of MTT at a conc. of 5 mg/ml in PBS were added to each well. After 3-h incubation at 37°C the cultures were destroyed with 100µl of a lytic buffer prepared with sodium dodecylsulfate/dimethylforma-mide/acetic acid/HCl. After further overnight incubation, the optical density (OD) at 620 nm was determined with a microplate LP200 detector. The O.D. of the wells with no cells was considered as 0% proliferation. The O.D. of the wells with growing cells in drug-free medium was considered as 100% proliferation. The drug cytotoxicity was calculated as the minimum concentration leading to 50% inhibition of cell growth *in vitro* (IC_{50}).

Cytotoxicity by clonogenic test in agar

The clonogenic capacity of WEHI-3B(D+) cells was tested by culturing 300 cells in 0.3 ml of complete enriched medium [McCoy + 10% FCS, 10 mM sodium pyruvate, 4% NEAA (10x), 4% Mem Vit (100x)] + 0.3% agar in 35 mm Petri plates.

SPA-S-843 and amphotericin B (100 µl) had previously been added to the Petri plates at serial concentrations of from 25 to 0.19 µg/ml. The control plates contained 100 µl of culture medium. The colonies were counted

after 7 days of incubation at 37°C in an atmosphere of air + 5% CO_2.

Results

The cytotoxicity of the two drugs on cell proliferation, evaluated with the MTT test, is expressed as % of the O.D. values measured in the controls (untreated cells, O.D. 1133.5 ± 48).

The diagrams in Fig. 1 report the dose/response curves. As confirmed by linear regression analysis, the toxic effect of SPA-S-843 and of amphotericin B shows a dose-related kinetics, however, SPA-S-843 cytotoxicity on WEHI-3B(D+) cells appears substantially higher. The histograms in Fig. 2 show the IC10, the IC50 and the IC90 of the two drugs on the same cell line. The study of the differences between IC values confirms that SPA-S-843 IC10, IC50 and IC90 are significantly lower, which demonstrates that its cytotoxicity is substantially higher than that of amphotericin B.

The results of the cytotoxicity study of the two drugs on cell clonogenicity, evaluated through the agar test, were expressed as percent of the number of colonies counted in the controls (untreated cells; number of WEHI-3B (D+) colonies = 212 ± 62).

The diagrams in Fig. 3 show the dose-response curves confirming SPA-S-843 substantially higher cytotoxicity. The histograms in Fig. 4 report the IC10, IC50 and IC90 values. In this case too these values indicate that, in the WEHI-3B(D+) cells, all the IC values regarding amphotericin B are significantly higher than those regarding SPA-S-843, which substantially confirms the latter drug higher cytotoxicity on the cells under examination even in a typical clonogenicity test.

All in all, the foregoing results show that in both tests of cytotoxicity of the two drugs on murine myelomonocytic leukemia cells, these cells are more sensitive to SPA-S-843 than to amphotericin B, which latter drug is well known for its cytotoxic action against all eukaryote cells.

This finding is quite surprising in that SPA-S-843, as several other partricin derivatives, were reported in literature to be generally better tolerated by animal cells. US patent 5.298.495, for instance, reports that the lytic activity on erythrocytes of partricin A derivative SPA-S-843, and of other derivatives of it, appears at very high concentrations, of the order of 18 µg/ml or more (SPA-S-843 free base: total hemolytic conc. - THC > 18 µg/ml). This scarce cytotoxicity

towards RBC and other mammalian cells, results in a scarce systemic toxicity in the animals; in fact, as reported in the a/m US patent, the dose, lethal to 50% of the animals (LD_{50}) by i.v. route in mice is 58 mg/kg. A comparison can be drawn with the corresponding values of amphotericin B whose THC is 2 µg/ml and LD_{50} is 4.77 mg/kg i.v. in mice (T. Bruzzese et al., Eur.J.Med.Chem., *31* , 965-72, 1996). As suggested by the foregoing comments and by Fig. 1-4 herewith attached, SPA-S-843 toxicity occurs at sensibly lower concentrations in the case of some tumor cells such as, for instance, the WHEI-3B(D+).

The observation of this unexpected highly selective cell toxicity of some partricin derivatives on some tumor cell lines, will allow these drugs to be given, following, possibly, a suitable screening of *in vitro* cytotoxicity, a selective indication for the treatment of some tumors due to cells sensitive to the said derivatives. Selectivity of the antineoplastic activity will be assessed, for instance, by preliminarily performing an *in vitro* cytotoxicity test on tumor cells and healthy cells obtained from the same organ or tissue affected by the tumor, using a derivative of partricin A and/or B selected for the treatment of the

tumor. This comparison could also be drawn using other healthy cells obtained from the same organism affected by the tumor, (for instance, an animal, preferably a mammal, most preferably, a human) which may potentially be considered sensitive to the cytotoxic effect of the partricin derivative selected for treatment.

As already mentioned, the partricin derivatives could also be used as adjuvants of other antineoplastic drugs (e.g., platinum derivatives, doxorubicin, vincristine, methotrexate, 5-fluorouracil etc.).

It is know, for instance, that some tumor cell lines, or some cells in particular, are, or may become, resistant to the best known antineoplastic drugs, which often leads to recurrence of the disease, and serious prejudice to the successful outcome of chemiotherapy. It has also been suggested that some drugs, active on cell membrane through complexation with the sterols of cell wall, such as amphotericin B (for instance, Kuwano et al., ''Techniques to reverse or circumvent drug resistance in vitro'', Prog. Clin. Biol. Res. 223, 163-71, 1986; Morikage et al., ''Reversal of cisplatin resistance with amphotericin B in a non-small cell lung tumor cell line'', Japan J. Cancer Res., 82 , 747-51, 1991) can interact with the cells resistant to the

antineoplastic drug, thus modifying their membrane permeability and accumulating the chemotherapeutic agent into the cell, thereafter restoring cell sensitivity to the drug. We have experimentally tested cisplatin (CP) chemotherapeutic activity on thyroid medullary carcinoma (TMC) cells and have confirmed, through a cytotoxicity test (MTT), that their resistance to cisplatin was substantially counteracted by pre-treatment with SPA-S-843 (2 μg/ml).

Notwithstanding this relatively low concentration, SPA-S-843 was able to potentiate cisplatin cytotoxicity against resistant cells up to the level of naturally sensitive cells; in conclusion, incubation with SPA-S-843 was able to potentiate cisplatin antineoplastic activity to such an extent as to make it cytotoxic for all TMC cells.As an example of the application of the partricin derivatives as adjuvant of usual antineoplastic agent in patients that have become irresponsive to the treatment with said antineoplastic agents a case is hereby reported of a 71 year old female patient having undergone, in June 1993, left hemicolectomy with preaortocaval lymphoadenectomy, splenectomy and left adnexectomy because of G2 adenoma at Stage C2. The patient was successively treated with

adjuvant chemotherapy based on 5-fluorouracil and folinic acid.

Following chemotherapy, the dosage of carcino-embryonic antigen (CEA) showed that CEA levels had come down from 262 ng/ml to almost normal levels (5 ng/ml). In February 1997 the disease recurred at the left adrenal gland site, with development of solid afinalistic retroperitoneal tissue.

The patient was submitted to laparotomy with total resection, followed at a later time by chemotherapy by slow infusion of 5-fluorouracil (500 mg) and folinic acid (250 mg) doses. The treatment was first according to the usual scheme (phase A),and 3 weeks later combined with the semisynthetic polyene SPA-S-843 (50 mg i.v., Phase B).

The assay for CEA, carried out with a CEA-RIA (RIA = Radio-Immuno Assay) monoclonal one-step kit (Boehringer Mannheim, Germany) (Disaia P.J. et al., Am.J.Obster.Gynecol., 121, 159-163,1993) after phase A gave a value of 272ng/ml, while after phase B was 65ng/ml.

The patient is presently receiving radiotherapy for antalgic purposes.

Worth mentioning is the onset of resistance to the usual chemotherapeutic agents, administered alone, while their combination with the polyene re-established the sensitivity to them, possibly due to interaction of the polyene with sterols of the cell membrane and increase of cell permeability.

The above mentioned ester and amide derivatives of partricin A and/or B can be used in a method of treatment of animals, preferably mammals,most preferably humans, in which tumoral cells are present and/or tend to develop and which are in need of antitumoral treatment.

According to said method the dosage of partricin A and/or B derivative to be administered to said animals preferably mammals, most preferably humans, depends on the weight and age of the animal, preferably mammals, most preferably humans, the severity of the desease (i.e. the amount and/or the stage of development of the tumoral cells present in the animal) and may be adjusted according to the response of the tumor affected animal, preferably mammal, most preferably human to the specific partricin A and/or B derivative and the judgement of the person who controls the administration of the said partricin A and/or B

derivative to said animal, preferably mammal, most preferably human.

In general, the effective dosage ranges from about 0.025 to about 3 mg a day per kilogram of body weight, preferably from about 0.15 to about 1.5 mg a day per kilogram of body weight. Said dosage may be administered as a single dose or subdivided in two or more fractions. Said dosage can be given every day or at intervals (e.g. every 2 to 7 days) depending on the pathological conditions and the severity of the disease. The usual administration route is the parenteral route, with the intravenous route being preferred, in particular in the form of drip infusion to be administered in a time period of 0.5 to 8 hours.

SPA-S-843 pharmaceutical formulations, intended for use as antineoplastic agents, contain 1 to 100 mg of active ingredient, preferably 5 to 50 mg, and are prepared following the usual techniques. These formulations preferably contain ascorbic acid/sodium ascorbate as an antioxidant and stabiliser and are more often preserved in the solid form, f.e., following freeze-drying. An example of a typical, but not limiting, formulation for the intravenous use is as follows:

SPA-S-843	25	mg

Ascorbic acid	4.5 mg
Lactose	250 mg

Sterile bidistilled water q.s. to 5 ml

The solution is generally lyophilised, to be dissolved in 5% glucose solution (50-500 ml) before use to be administered as a slow infusion.

When the derivatives of partricin A and/or B are used as adjuvants of the antineoplastic activity of other antineoplastic drugs,they can be administered concomitatly with the usual by antineoplastic agent or sequentially. Accordingly, the pharmaceutical formulations can contain both active ingredients in a single dosage form, or the same active ingredients can be contained in separate dosage forms permitting their composition as a single dosage form (e.g., a solution, or a suspension for administration other than intravenous administration) at the time of use. Alternatively, the two active ingredients can be enclosed in separate dosage forms allowing their sequential administration during antineoplastic therapy.

Therefore, according to the above description one of the main aims of this invention is the use of a partricin A and/or partricin B derivative as defined

above for the manufacture of a medecine for use in a method of treatment of a neoplastic disease, said treatment being performed with the partricin A and or B derivative alone or in combination with an usual antineoplastic drug.

CLAIMS

1. A method for the treatment of animals, preferably mammal, most preferably humans, in which tumoral cells are present and/or tend to develop and which are in need of antitumoral treatment, comprising administering to said animals, preferably mammals, most preferably humans an amount of a derivative of partricin A and/or B which is selectively toxic to the tumoral cells.

2. A method according to claim 1, wherein the said derivatives are esters or amides on the carboxyl group at C-18 of the macrolide ring, optionally substituted on the amino group of the mycosamine radical with an acyl group and their salts with organic and inorganic acids acceptable in the pharmacological and pharmaceutical practice.

3. A method according to claim 2, wherein the said amide derivatives of partricin A and/or B are secondary or tertiary amides of the carboxyl group at C-18 of the macrolide ring.

4. A method according to claim 2, wherein the the said ester derivatives of partricin A and/or B are alkyl ester, preferably C_1-C_6 alkyl esters, most preferably methyl esters.

5. A method according to claim 2, wherein the said ester or amide derivatives of partricin A and/or B are derivatives in which the carboxyl at C-18 of the macrolide ring forms a C_1-C_6 alkyl ester or an amide, neutral or containing an additional basic nitrogen containing function, and/or the primary amino group of the mycosamine portion forms an amide bond with the carboxyl group of an aliphatic acid of 2-4 carbon atoms containing an additional basic nitrogen containing group.

6. A method according to claims 1 to 5, wherein said derivatives are made water-soluble through salification with pharmaceutically acceptable, non toxic acids.

7. A method according to claims 1 to 6, wherein the said derivatives are N-dimethylaminoacetyl-partricin A 2-dimethylaminoethylamide and its diascorbate.

8. A method according to claims 1 to 7, wherein the derivative of partricin A and/or B has the ''all trans'' spatial configuration.

9. A method according to claims 1 to 8, wherein the tumors are represented by solid or non-solid tumors.

10. A method according to claims 1 to 9, wherein the tumors comprise leukemia, lymphoma, myeloma, melanoma, carcinoma and sarcoma.

11. A method according to claims 1 to 10, wherein the antitumoral treatment is rendered selective by preliminary *in vitro* sensitivity tests of both tumor and healthy cells from the organism affected by the tumor, towards the derivative of partricin A and/or B selected to treat the tumor.

12. A method according to claims 1 to 11, wherein the partricin derivatives are used in combination with other known antineoplastic agents.

13. A method according to claim 12, wherein the afore-said antineoplastic agents are represented by platinum derivatives, doxorubicin, vincristine, methotrexate and 5-fluorouracil.

14. A method according to claim 13, wherein the platinum derivatives are represented by cisplatin or 5-fluorouracil.

15. A method according to claims 1 to 14, wherein the said derivatives are administered by parenteral route and the dosage ranges from about 0.025 to about 3 mg a day, preferably from about 0.15 to about 1.5 mg a day, per kilogram of body weight.

16. A pharmaceutical formulation for use in a method according to any of claims 1 to 15, containing from 1 to 100 mg of a derivative of partricin A and/or B, preferably 5 to 50 mg.

17. Formulations for the use according to claims 1 to 16, wherein ascorbic acid/sodium ascorbate is present as an antioxidant and stabiliser.

18. Formulation according to claims 16 to 17, characterised by the fact that the partricin derivative and the known antineoplastic agent are enclosed in the same dosage form or in separate dosage forms permitting their composition as a single dosage form at the time of use.

19. Formulation according to claims 16 to 17, characterised by the fact that the partricin derivative and the antineoplastic agent are enclosed in separate dosage forms allowing their sequential administration during treatment of the tumor.

20. A method to selectively inhibit in vitro or ex vivo the growth of tumor cells, consisting in providing said cells with a derivative of partricin A and/or B, of claims 1 to 8, in such amount as to prove to be cytotoxic for the said tumor cells and much less

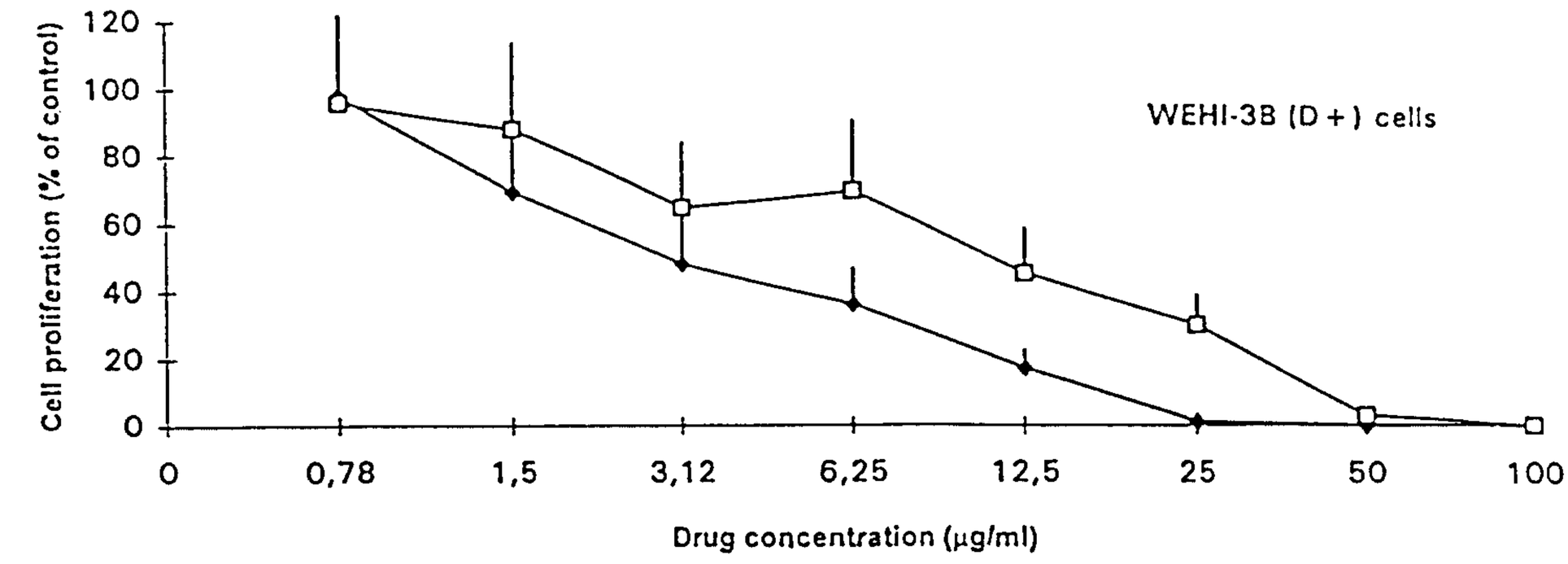
cytotoxic for the healthy cells of the same organism affected by the tumor.

21. A method according to claim 20, wherein the cells are taken from an organism affected by the tumor which is an animal, preferably a mammal, most preferably human.

22. A method according to claims 1 to 8 and 20 to 21, wherein administration of partricin A and/or B derivative is combined with administration of a known antineoplastic agent, preferably selected among the derivatives of platinum, doxorubicin, vincristine, methotrexate and 5-fluorouracil.

23. Use of a partricin A and/or B derivative for the manufacture of a drug to be used according to any of the methods according to any of claims 1 to 15 and 20 to 22.

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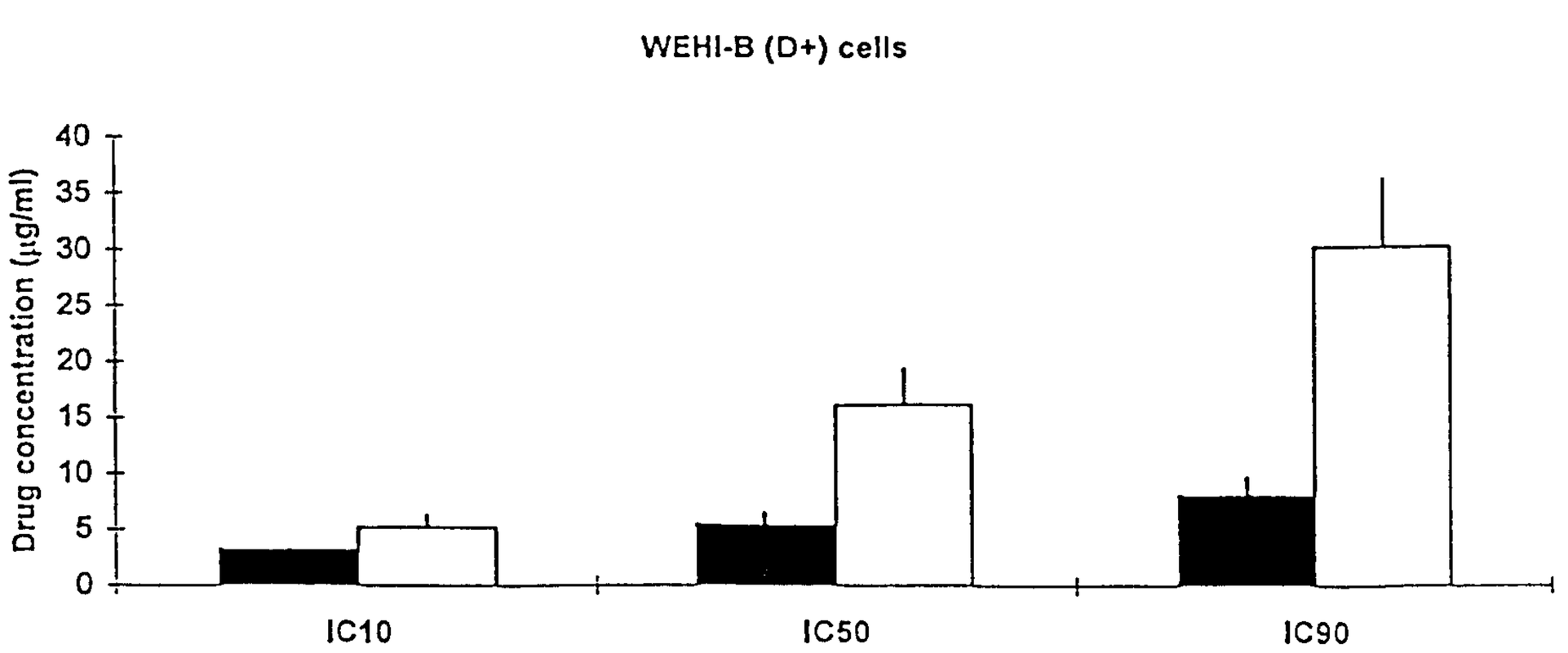


- Dose-response curve of the cytotoxic effect on *in vitro* cell proliferation (MTT test).

  (◆) SPA-S-843 (□) Amphotericin B

  Each point is the mean ± s.e. of 3 experiments conducted in triplicate.

Fig. 1

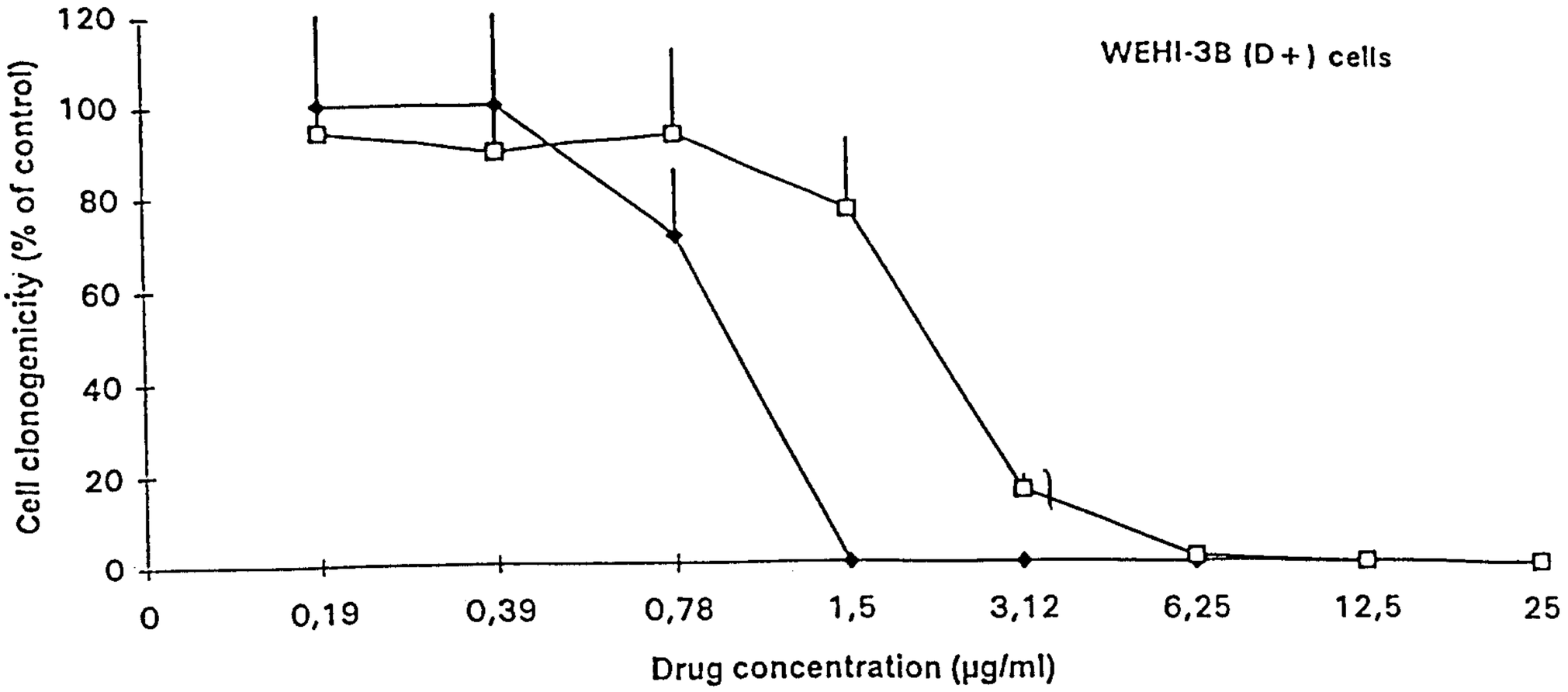


- IC values of SPA-S-843 and amphotericin B assessed in *in vitro* cell proliferation test .

  (■) SPA-S-843 (□) Amphotericin B

  Each value is the mean of 3 experiments conducted in triplicate.

Fig. 2

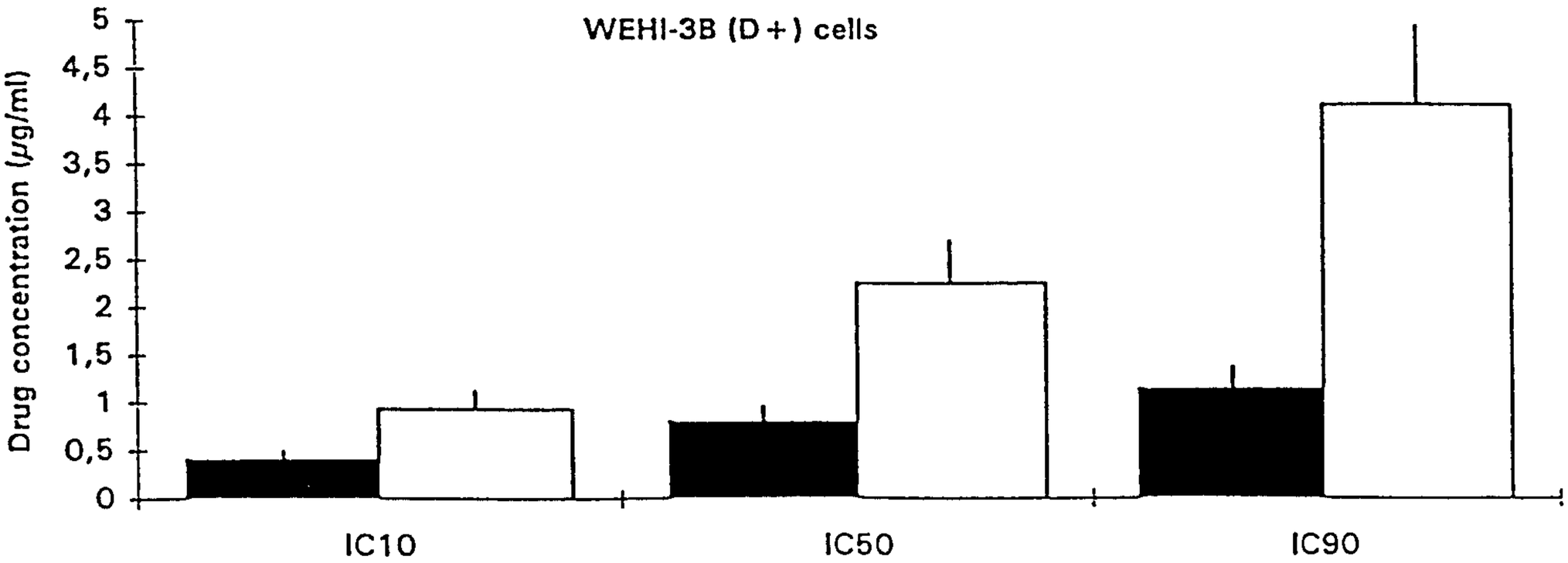


- Dose-response curve of the cytotoxic effect on cell clonogenicity (agar test).

(◆) SPA-S-843 (□) Amphotericin B

Each point is the mean ± s.e. of 2 experiments conducted in duplicate.

Fig. 3



- IC values of SPA-S-843 and amphotericin B assessed in *in vitro* cell clonogenicity test .

(■) SPA-S-843 (□) Amphotericin B

Each value is the mean of 2 experiments conducted in duplicate.

Fig. 4