ANTITUMORAL AGENTS WITH A BENZOPHENANTHRIDINE STRUCTURE AND FORMULATIONS CONTAINING THEM

The present invention relates to the use of benzophenanthridine alkaloids and the salts thereof for the preparation of medicaments for the treatment of tumors.

The invention also relates to the novel salts of said alkaloids and compositions containing them.

State of the art

The first aim of oncology is complete eradication of the tumor by any means, even when this entails serious side effects; the motto *primum non nocere* ("first, do no harm") is not used as a guideline in the treatment of tumors, but preferably replaced with *primum succerere ("first, hasten to help")*.

Oncological treatment usually involves radical surgery, targeted radiotherapy including photodynamic therapy, high doses of chemotherapy drugs, radiotherapy, and treatment with cytokines (IL2) and monoclonal antibodies. Despite the large, constantly increasing number of available treatments, the success rate in terms of cures is still unsatisfactory, especially in the case of the chemotherapy, due to the onset of drug resistance.

In solid tumors, chemotherapy reduces the tumor mass rapidly, but not completely; a few particularly resistant cells can keep the tumor active and it then develops, often with fatal results. These cells were recently classified as tumor stem cells, which possess strong proliferation potential and adaptability. Said cells constitute approx. 5% of the tumor mass, and are resistant to all known chemotherapy agents in current use. The existence of tumor stem cells was established for the first time in myeloid leukaemia (Bonnet D, Dick JE. *Nature Medicine, 3, 730-737 (1997)*), and subsequently demonstrated in breast

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and brain tumors (Al-Hajj M, et al., *Natl Acad Sci, USA, 100.* 3983-3988, 2003, Sing SK, et al., *432, 396-401*, Nature 2004).

More recently, stem cells have been isolated from melanomas, colon tumors and pulmonary microtomes (Ricci-Vitali, Nature *445, 111-115* (2007); Fang D., Cancer Res., *65, 9328-9337* 2005, Eramo, *Cell Death and Differentiation, advance online publication Nov 30. 2007 Doi: 10.1038/sj.cdd.4402283).*

These cells, as stated above, are not controlled by the standard treatment, and regenerate the tumor. There is consequently a clear need to develop compounds able to inhibit tumor stem cells, possibly by inducing apoptotic processes.

Description of the invention

It has now been found that benzophenanthridine alkaloids, especially sanguinarine, chelerythrine, chelidonine and the salts thereof with luteic, phosphatidic or hyaluronic acids have an antiproliferative effect on multiresistant tumor stem cells.

In particular, the salts with luteic acid give the molecules a potent cytotoxic action towards cancer cells and an equally potent antiangiogenic, anti-inflammatory and analgesic action.

In a first aspect, the invention therefore relates to the use of benzophenanthridine alkaloids or the salts thereof for the preparation of medicaments for the treatment of tumors.

In a second aspect, the invention provides novel salts of benzophenanthridine alkaloids with phosphatidic or hyaluronic acids. Said salts are particularly useful for targeted topical or systemic administration.

In a further aspect, the invention provides complexes of benzophenanthridine or isoquinoline alkaloids with human albumin in nanoparticulate form and suspensions containing said complexes which can be

administered intravenously or orally. The nanoparticles selectively reach the tumor, where they perform a cytotoxic and anti-angiogenic tumor mass reducing action. This formulation is particularly useful for the treatment of solid tumors and the more common forms of leukaemia.

Phosphatidic acids contain residues of fatty acids, which can be the same or different, with saturated or unsaturated straight chains comprising 12 to 22 carbon atoms.

Dipalmitoyl- and distearoyl-phosphatidic acids which considerably increase oral and topical bioavailability are preferred.

The salt of sanguinarine with a phosphatidic acid is particularly preferred.

The salts according to the invention are able to induce apoptosis of tumor stem cells at sub-micromolar concentrations.

The salt of sanguinarine with luteic acid has proved *in vitro* a potent inhibiting activity towards melanoma and colon stem cells at a concentration of 200 ng/ml. Its behaviour towards other oncological stem cells is identical. These alkaloids can consequently be considered new-generation antitumoral agents, which are able to treat human tumors effectively. For tumors of the colon, liver, pancreas and cervix the most appropriate form is the salt with luteic acid dispersed in a suitable vehicle, whereas for the treatment of skin tumors such as melanoma, the salt with a phosphatidic acid can advantageously be administered topically directly to the area affected by the tumor.

Complexes with albumin are suitable to be administered by loco-regional injection at a dose much lower than the toxic dose, and for very long periods; selectivity towards tumor stem cells compared with normal cells and the potent anti-angiogenic effect produce a rapid reduction and eventual eradication of the tumor mass.

The product most suitable for oral and topical administration is the salt of sanguinarine with luteic acid. Said salt has proved particularly effective in

the treatment of oropharynx, head and neck tumors, melanoma and cervical tumors, to a greater extent than the unsalified alkaloid, and it also interacts with any viruses associated with the tumor.

Salts with hyaluronic acid which increase cell uptake of the alkaloid and allow eradication of the tumor are also advantageous.

The formulations will be prepared by well-known processes such as those described in "Remington's Pharmaceutical Handbook", Mack Publishing Co., N.Y., USA, together with suitable excipients.

The examples set out below illustrate the invention in detail.

Example I - Preparation of luteic acid sanguinarine salt

3.68 g of sanguinarine chloride are dissolved in 100 ml of ethanol and added under stirring with 3.6 g of potassium luteate and the mixture is reacted for 3 hours. The formed potassium chloride is filtered off and the solution is concentrated to small volume. 5.6 g of salt are obtained.

Example II - Preparation of 1, 2-dipalmitoyl-sn-glycero-3-phospho-rac - (1- glycerol) sanguinarine salt

3.7 g of sanguinarine chloride are dissolved in 50 ml of methanol; this solution is added under stirring with 7.5 g of 1,2-dipalmitoyl - sn - glycero-3-phospho - rac- (1-glycerol) 1,2-dipalmitoyl-sn-glycero-3-phospho - rac-(1- glycerol) sodium salt and the mixture is left under stirring for 2 h. The resulting solution is diluted with an equal volume of methylene chloride and the mixture is left under stirring for 0.5h. The precipitated sodium chloride is filtered off and the filtrate is concentrated to dryness under vacuum at a temperature not higher than 35°C. 10 g of a reddish solid having melting point of 70°C are obtained.

Example III - Preparation of nanoparticles of albumin with sanguinarine

1 g of sanguinarine are dissolved in 15 ml of dioxane at room

temperature and added to a solution of 5 g of human albumin in 300 ml of saline. The mixture is left under stirring in sterile environment for 4 hours. The resulting turbid solution is subjected to ultrasounds for 1 minute. The physical appearance and the color of the suspension change. The solution is freeze-dried without adding excipients.

Example IV

Following the procedures of Example III, the sanguinarine solution is prepared and the solute is freeze-dried under sterile conditions in containers which will contain 10 mg of sanguinarine after completion of the process.

The lyophilisate is used in the loco-regional treatment of the oropharynx tumor.

Example V - 1 Gram chewable tablets for the treatment of head and neck tumor

Sanguinarine luteate	10.00 mg
Soy lecithin	30.00 mg
Anhydrous citric acid	10.00 mg
Lactose	240.00 mg
Mannitol	550.00 mg
Methyl cellulose	40.00 mg
Palmitoyl stearate	50.00 mg
Berry flavour	40.00 mg
Ammonium Glycyrrhizinate	0.5 mg
Talc	10.00 mg

Example VI - Soft-gelatin capsules for the treatment of cervical tumor

Sanguinarine luteate	10.00 mg
Soy lecithin	50.00 mg
Beeswax	50.00 mg

Ammonium Glycyrrhizinate		10.00 mg
Vegetable oil	q.s. to	800.00 mg

Example VII - Soft-gelatin capsules for the treatment of cervical tumor

Sanguinarine luteate		10.00 mg
Soy lecithin		50.00 mg
Beeswax		50.00 mg
Sodium succinyl-glycyrrhetate		10.00 mg
Vegetable oil	q.s. to	800.00 mg

Example VIII - Oil-in-water emulsion of phosphatidic acid sanguinarine salt for the topical treatment of melanoma

Di-palmitoylphosphatidic acid sanguinarine salt	0.40 g
Propylene glycol	10.00 g
Isopropyl myristate	5.00 g
Cetyl alcohol	5.00 g
Polysorbate 80	3.00 g
Carbomer	0.40 g
Methyl parahydroxy benzoate	0.10 g
Propyl parahydroxy benzoate	0.05 g
Purified water q.s. to	100 g

CLAIMS

1. The use of benzophenanthridine alkaloids or the salts thereof for the preparation of medicaments for the treatment of tumors.

2. The use as claimed in claim 1 wherein the benzophenanthridine alkaloids are selected from sanguinarine, chelerythrine, chelidonine.

3. The use as claimed in claim 2 wherein the alkaloid is sanguinarine.

4. The use as claimed in any one of claims 1 to 3 wherein the alkaloids are in the form of salt with luteic acid or with a phosphatidic acid.

5. The use as claimed in claim 4 of sanguinarine luteate.

6. The use as claimed in claim 4 of a sanguinarine salt with a phosphatidic acid.

7. The use as claimed in any one of claims 1 to 3 wherein the alkaloids are present in the form of nanoparticles consisting of complexes of said alkaloids with albumin.

8. The use as claimed in any one of claims 1-7 wherein tumors derive from hyperproliferation of multiresistant tumor stem cells.

9. The use as claimed in any one of claims 1 - 8 wherein tumors are oropharynx, head and neck, or cervical tumors, or melanoma.

10. Salt of benzophenanthridine alkaloids with phosphatidic acid or hyaluronic acid.

11. The salt as claimed in claim 10 with phosphatidic acid.

12. The salt as claimed in claim 11 wherein the phosphatidic acid is di-palmitoyl- or di-stearoyl-phosphatidic acid.

13. The sanguinarine salt as claimed in claim 11 or 12.

14. The salt of claim 11, 12 o 13 as an anti-melanoma agent.

15. Pharmaceutical compositions comprising a salt of claims 10 - 14 as the active ingredient.