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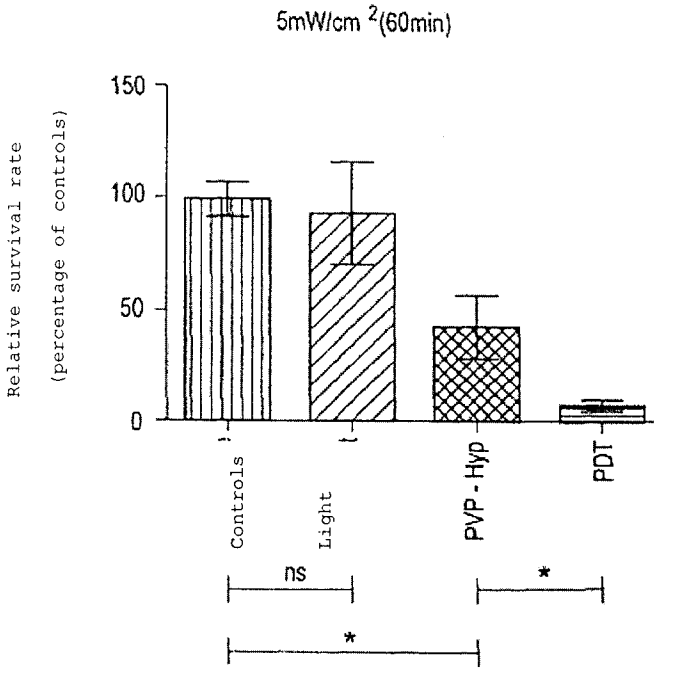

(57) Abrégé/Abstract:
A formulation which can be used as a photosensitizer in the therapy of cancer, for example bladder cancer, contains polyvinylpyrrolidone-bound or polyvinylpyrrolidone-complexed hypericin sodium salt.

ABSTRACT

A formulation which can be used as a photosensitizer in the therapy of cancer, for example bladder cancer, contains polyvinylpyrrolidone-bound or polyvinylpyrrolidone-complexed hypericin sodium salt.

FORMULATION OF HYPERICIN FOR PHOTODYNAMIC THERAPY

FIELD

The invention relates to a novel formulation of hypericin, which can be used in photodynamic therapy.

BACKGROUND

Photodynamic therapy (PDT) is a method suitable for treatment of tumors and premalignant changes in the skin and mucous membranes of various hollow organs (Juarranz et al., 2008; Agostinis et al., 2011).

PDT is based on the interaction of three components: photosensitizer, light in the visible range and oxygen.

After systemic or topical application of a photosensitizer, the photosensitizer accumulates in the malignant tissue. The photosensitizer can be excited by using light of a suitable wavelength. In the excited state, energy is transferred to a reactant, for example, molecular oxygen. In doing so, reactive oxygen molecules are created, and these in turn damage cellular structures of the tumor tissue, thereby initiating cellular processes, such as apoptosis and necrosis (Agostinis et al., 2011; Allison and Sibata, 2010).

An ideal photosensitizer for PDT exhibits selective accumulation in tumor cells, minimal or no systemic toxicity and is photochemically efficient.

Hypericin 1,3,4,6,8,13-hexahydroxy-10,11-dimethylphenantro (1,10,9,8-opqra)perylene-7,14-dione has already been described in the literature as a potential photosensitizer (Agostinis et al., 2002).

In vitro studies have demonstrated the efficacy of hypericin in PDT in a number of cell lines (Karioti and Bilia, 2010).

In addition, *in vivo* animal studies have confirmed the potential of hypericin for use in PDT (Bhuvaneswari et al., 2010; Chen et al., 2003; Liu et al., 2000; Sanovic et al., 2011).

Hypericin is hydrophobic and insoluble in water. For this reason, hypericin has in the past been dissolved with the help of the organic solvent dimethyl sulfoxide (DMSO) or a water-soluble polymer, polyethylene glycol (PEG).

Animal experiments in a rat model have shown encouraging results with regard to PDT of bladder carcinoma. In these experiments, hypericin was introduced into the tumor cells with the help of polyethylene glycol. Up to 98% of the tumor cells could be killed with a hypericin dose of 30 µM and irradiation with light (595 nm) at an intensity of 25 to 50 mW/cm^2 (Kamuhabwa et al. 2003).

For clinical use, however, a water-soluble formulation of hypericin that has tumor selectivity and can be energized with light in the visible range is required.

The document WO 01/89576 A2 describes how the solubility of hypericin can be increased by the additive polyvinylpyrrolidone (povidone, PVP).

Use of PVP hypericin in PDT is also described in WO 2014/079972 A1. WO 2014/079972 A1 relates in particular to a device that can be used in PDT of hollow organs, such as the human bladder.

PVP hypericin manifests a selective accumulation in tumor cells *in vitro* and *in vivo* (Kubin et al., 2008; Vandepitte et al., 2011).

SUMMARY

The invention is based on the object of making available a sterile and stable formulation of hypericin that can be used for clinical administration in PDT.

This object is achieved with a formulation of hypericin having the features described herein.

Preferred and advantageous embodiments of the formulation of hypericin according to the invention are also described.

DETAILED DESCRIPTION

It has surprisingly been found that the formulation of hypericin according to the invention is stable and can be used under clinical conditions only if hypericin is present in the form of a salt.

An evaluation of the formulation of hypericin according to the invention in animal experiments has surprisingly shown that, at a dose of 30 µM hypericin in a stable formulation with PVP according to example 1, a required light intensity of 5 or 25 mW/cm^2 at a wavelength of 595 nm and 120 minutes treatment time in the bladder (instillation time) is sufficient to kill 98% of the tumor cells. The same result of 98% killed tumor cells is also obtained with the same light intensity and 40 µM hypericin after 15 minutes or 30 minutes of exposure time and treatment with light at a wavelength of 610 nm. Likewise an instillation time of 1 hour with 20 µM hypericin and the same light intensity and 570 nm light frequency yields a kill rate of 97%, and an instillation time of 120 minutes with 9 µM hypericin, the same light intensity and treatment at 600 nm yields a kill rate of 95%. Thus, with a light intensity of 5 to 25 mW/cm^2 and a light frequency of 570 to 610 nm, hypericin concentrations of 9 to 40 µM and treatment times between 15 and 120 minutes, a kill rate of 95% to 98% of the tumor cells is achieved (application examples 1, 2, 3 and 4).

The efficacy of PDT depends to a significant extent on the total amount of light. At the same time, the probability of local adverse effects is also increased by increasing the light intensity.

With the help of the formulation according to the present invention, an improved accumulation in malignant tissue is achieved, so that a greatly reduced light intensity of only 5 mW/cm^2 to max. 25 mW/cm^2 is sufficient to kill tumor cells.

Selective enrichment of the formulation of hypericin according to the invention and the surprisingly low light intensity needed for PDT in the animal model when using the formulation of hypericin according to the invention allows its use in treatment of lesions in various body cavities that can be reached with the required dose of light.

Examples of the formulation of hypericin according to the invention (hypericin-PVP complex) are given below.

General procedures for preparing a formulation with sodium hypericinate as an active ingredient.

The goal is to prepare a formulation containing hypericin for use as a photosensitizer in the field of photodynamic therapy.

The formulation according to the invention is prepared from a salt of hypericin, in particular sodium hypericinate.

To define the hypericin content of the starting material, mainly the water content is needed in addition to the determination of the concentration, and in the case of sodium hypericinate, the sodium content must be determined. The chemico-physical

properties may have an influence on the formulation of the pharmaceutical substance.

For clinical use, stability of the formulation according to the invention is required. This stability is ensured by the composition of the finished product and, at the same time, also relates to the preparation process. A sufficient stability of the bulk solution can be achieved by means of the buffer systems used even during preparation up to the stage of lyophilization of the finished product.

Various additives may be used as the buffer systems, preferably yielding a physiologically tolerable pH for the bulk solution as well as the reconstituted solution and achieving an osmotic pressure of 290 mOsmol/kg after reconstitution with 50 mL water for injection. Phosphate or citrate buffer systems may be used primarily.

After completion of the bulk solution from the ingredients mentioned above, the corresponding amount of the bulk solution is bottled in injection vials and lyophilized.

Example 1:

A solution with a target initial weight of 90.0 mg hypericin is prepared from sodium hypericinate.

To 1875 mg PVP k25 is added 5.0 g hypericin solution and dissolved completely.

This solution is quantitatively topped off to 250.0 g with a phosphate buffer solution. The final concentration of this solution is 0.0225 mg hypericin/g solution.

For lyophilization, a defined amount of the resulting bulk solution is bottled in vials, and the finished lyophilizate is prepared with a corresponding lyo program.

Example 2:

The procedure described in Example 1 is followed, but instead of PVP k25, PVP k17 is used for complexing sodium hypericinate.

Example 3:

The procedure described in Example 1 is followed, but instead of PVP k25, PVP k30 is used for complexing sodium hypericinate.

Example 4:

The procedure described in Examples 1, 2 or 3 is followed, but instead of the phosphate buffer solution, a citric acid buffer solution is used.

The bulk solutions prepared as described in Examples 1 through 4 can be produced with different hypericin contents.

The efficacy of the formulation of hypericin according to the invention was tested in a preclinical study using the formulation described in Example 1.

Application Examples:

The formulation of hypericin according to the invention for PDT was tested on rats in a preclinical orthotopic bladder tumor model. In all examples, the tumors were treated with the formulation of hypericin according to the invention in various concentrations from 9 to 40 µM, at different light intensities

of 5 or 25 mW/cm^2, at different light frequencies of 570 to 610 nm and different instillation times.

Example 1. After a 2-hour instillation with 30 µM of the formulation of hypericin according to the invention and different light intensities (5 or 25 mW/cm^2) with light of a wavelength of 595 nm, up to 98% of the tumor cells could be killed.

Example 2. After a 1-hour instillation with 20 µM of the formulation of hypericin according to the invention and different light intensities (5 or 25 mW/cm^2) with light of the wavelength 570 nm, up to 97% of the tumor cells could be killed.

Example 3. After a 15- or 30-minute instillation with 40 µM of the formulation of hypericin according to the invention and different light intensities (5 or 25 mW/cm^2) with light of the wavelength 610 nm, up to 98% of the tumor cells could be killed.

Example 4. After a 2-hour instillation with 9 µM of the formulation of hypericin according to the invention and different light intensities (5 or 25 mW/cm^2) with light of the wavelength 600 nm, up to 95% of the tumor cells could be killed.

The results of these studies on the rat model are shown in Figs. 1 and 2. In the diagrams, "ns" stands for "not significant" and "*" [asterisk] stands for "significant." The diagrams in Figs. 1 and 2 illustrate the survival of tumor cells after treatment with light and the formulation of hypericin according to the invention. The bladder tissue was dissociated 24 hours after the treatment, and the surviving cells were determined with the help of a clonogenic assay in comparison with the controls (without PVP hypericin and light).

The relative survival of the cells under PDT conditions (PVP hypericin according to Example 1 and treatment with light) amounts to (represented as the mean value + SD): 7.4% (±6.4%) when using 5 mW/cm^2 and 2.4% (±4.0%) at 25 mW/cm^2 and a light treatment time of 60 minutes. This is illustrated in two diagrams (Figs. 1 and 2).

REFERENCES

Agostinis, P.; Berg, K.; Cengel, K. A.; Foster, T. H.; Girotti, A. W.; Gollnick, S. O.; Hahn, S. M.; Hamblin, M. R.; Juzeniene, A.; Kessel, D.; Korbelik, M.; Moan, J.; Mroz, P.; Nowis, D.; Piette, J.; Wilson, B. C.; Golab, J. Photodynamic Therapy of Cancer: An Update. CA Cancer J Clin. 2011 July-August; 61(4): 250-281.

Agostinis P.; Vantieghem, A.; Merlevede, W.; de Witte, P. A. Hypericin in Cancer Treatment: More Light on the Way. Int J Biochem Cell Biol. 2002 March; 34(3): 221-241.

Allison, R. R.; Sibata, C. H. Oncologic Photodynamic Therapy Photosensitizers: A Clinical Review. Photodiagnosis Photodyn Ther. 2010 June; 7(2): 61-75.

Bhuvaneswari, R.; Thong, P. S.; Gan, Y. Y.; Soo, K. C.; Olivo, M. Evaluation of Hypericin-Mediated Photodynamic Therapy in Combination with Angiogenesis Inhibitor Bevacizumab Using In Vivo Fluorescence Confocal Endomicroscopy. J Biomed Opt. 2010 January-February; 15(1): 011114. Erratum in: J Biomed Opt. 2010.

Chen, B.; Ahmed, B.; Landuyt, W.; Ni, Y.; Gaspar, R.; Roskams, T; de Witte, P. A. Potentiation of Photodynamic Therapy with Hypericin by Mitomycin C in the Radiation-Induced Fibrosarcoma-1 Mouse Tumor Model. Photochem Photobiol. 2003 September; 78(3): 278-282.

Juarranz, A.; Jaen, P.; Sanz-Rodriguez, F.; Cuevas, J.; Gonzalez, S. Photodynamic Therapy of Cancer. Basic Principles and Applications. Clin Transl Oncol. 2008 March; 10(3): 148-154.

Karioti, A.; Bilia, A. R. Hypericins as Potential Leads for New Therapeutics. Int J Mol Sci. 2010 Feb. 4; 11(2): 562-594.

Kubin, A.; Meissner, P.; Wierrani, F.; Burner, U.; Bodenteich, A.; Pytel, A.; Schmeller, N. Fluorescence Diagnosis of Bladder Cancer with New Water Soluble Hypericin Bound to Polyvinylpyrrolidone: PVP-Hypericin. Photochem Photobiol. 2008; 84(6): 1560-1563.

Kamuhabwa, A. A.; Roskams, T.; D'Hallewin, M. A.; Baert, L.; Van Poppel, H.; de Witte, P. A. Whole Bladder Wall Photodynamic Therapy of Transitional Cell Carcinoma Rat Bladder Tumors Using Intravesically Administered Hypericin. Int J Cancer. 2003 Nov. 10; 107(3): 460-467.

Liu, C. D.; Kwan, D.; Saxton, R. E.; McFadden, D. W. Hypericin and Photodynamic Therapy Decreases Human Pancreatic Cancer In Vitro and in Vivo. J Surg Res. 2000 September; 93(1): 137-143.

Sanovic, R.; Verwanger, T.; Hartl, A.; Krammer, B. Low Dose Hypericin-PDT Induces Complete Tumor Regression in BALB/c Mice Bearing CT26 Colon Carcinoma. Photodiagnosis Photodyn Ther. 2011 December; 8(4): 291-296.

Vandepitte, J.; Van Cleynenbreugel, B.; Hettinger, K.; Van Poppel, H.; de Witte, P. A. Biodistribution of PVP-Hypericin and Hexaminolevulinate-Induced PpIX in Normal and Orthotopic Tumor-Bearing Rat Urinary Bladder. Cancer Chemother Pharmacol. Cancer Chemother Pharmacol. 2011 April; 67(4): 775-781.

Vandepitte, J.; Roelants, M.; Van Cleynenbreugel, B.; Hettinger, K.; Lerut, E.; Van Poppel, H.; de Witte, P. A. Biodistribution and Photodynamic Effects of Polyvinylpyrrolidone-Hypericin Using Multicellular Spheroids Composed of Normal Human Urothelial and T24 Transitional Cell Carcinoma Cells. J. Biomed Opt. 2011 January-February; 16(1): 018001.

CLAIMS:

1. Use, for photosensitizing a tumor in a photodynamic therapy of tumors, of a photosensitizer reconstituted from a lyophilizate obtained from a stable complex or a stable compound of an alkali salt of hypericin and a polymeric complexing agent in a buffer system of a phosphate buffer or a citric acid buffer, comprising about 0.0225 mg/g hypericin in said buffer system;

wherein the photosensitizer has a concentration of 9 to 40 µM of hypericin; and

wherein the polymeric complexing agent is selected from the group consisting of polyethylene glycol and poly-N-vinylamide.

2. Use, for preparation of a medicament for photosensitizing a tumor in a photodynamic therapy of tumors, of a photosensitizer that is reconstituted from a lyophilizate obtained from a stable complex or a stable compound of an alkali salt of hypericin and a polymeric complexing agent in a buffer system of a phosphate buffer or a citric acid buffer, comprising about 0.0225 mg/g hypericin in said buffer system;

wherein the photosensitizer has a concentration of 9 to 40 µM of hypericin; and

wherein the polymeric complexing agent is selected from the group consisting of polyethylene glycol and poly-N-vinylamide.

3. The use of claim 1 or 2, wherein the alkali salt of hypericin is a sodium salt or a potassium salt.

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4. The use of claim 1 or 2, wherein the photosensitizer has a concentration of 30 µM of hypericin.

5. The use of claim 1 or 2, wherein the light to be employed during the photodynamic therapy has an intensity of 5 to 25 mW/cm^2.

6. The use of claim 5, wherein the light is at a wavelength of from 570 nm to 610 nm.

7. The use of claim 1 or 2, wherein the illumination during the photodynamic therapy will occur for a period of 15 to 120 minutes.

8. The use according to claim 1 or 2, wherein the complexing agent is a poly-N-vinylamide.

9. The use according to claim 8, wherein the poly-N-vinylamide is polyvinylpyrrolidone (PVP) of various degrees of polymerization and crosslinking.

10. The use according to claim 9, wherein the polyvinylpyrrolidone is PVP k17, PVP k25 or PVP k30.

11. Use, for photosensitizing in a photodynamic therapy of cancer, of a photosensitizer that is reconstituted from a lyophilizate obtained from a stable complex or a stable compound of an alkali salt of hypericin and a polymeric complexing agent in a buffer system of a phosphate buffer or a citric acid buffer, comprising about 0.0225 mg/g hypericin in said buffer system;

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wherein the photosensitizer has a concentration of 9 to 40 µM of hypericin; and

wherein the polymeric complexing agent is selected from the group consisting of polyethylene glycol and poly-N-vinylamide.

12. Use, for preparation of a medicament for photosensitizing in a photodynamic therapy of cancer, of a photosensitizer that is reconstituted from a lyophilizate obtained from a stable complex or a stable compound of an alkali salt of hypericin and a polymeric complexing agent in a buffer system of a phosphate buffer or a citric acid buffer, comprising about 0.0225 mg/g hypericin in said buffer system;

wherein the photosensitizer has a concentration of 9 to 40 µM of hypericin; and

wherein the polymeric complexing agent is selected from the group consisting of polyethylene glycol and poly-N-vinylamide.

13. The use of claim 11 or 12, wherein the cancer is bladder cancer.

14. The use of claim 11 or 12, wherein the alkali salt of hypericin is a sodium salt or a potassium salt.

15. The use of claim 11 or 12, wherein the photosensitizer has a concentration of 30 µM of hypericin.

16. The use of claim 11 or 12, wherein the light to be employed during the photodynamic therapy has an intensity of 5 to 25 mW/cm^2.

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17. The use of claim 16, wherein the light is at a wavelength of from 570 nm to 610 nm.

18. The use of claim 11 or 12, wherein the complexing agent is a poly-N-vinylamide.

19. The use of claim 18, wherein the poly-N-vinylamide is polyvinylpyrrolidone (PVP) of various degrees of polymerization and crosslinking.

20. The use of claim 19, wherein the polyvinylpyrrolidone is PVP k17, PVP k25 or PVP k30.

21. Use, for photosensitizing in a photodynamic therapy of tumors, of a photosensitizer that is a stable complex or a stable compound of a sodium or potassium salt of hypericin and a polymeric complexing agent selected from the group consisting of polyethylene glycol and poly-N-vinylamide, wherein the photosensitizer is reconstituted from a lyophilizate obtained from a solution of the sodium or potassium salt of hypericin, containing about 0.0225 mg of hypericin/g of solution; the polymeric complexing agent and a buffer system comprising a phosphate buffer or a citric acid buffer,

wherein the lyophilizate is for reconstitution to obtain the photosensitizer having a concentration of 9 to 40 µM of hypericin.

22. Use, for preparation of a medicament for photosensitizing in a photodynamic therapy of tumors, of a photosensitizer that

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is a stable complex or a stable compound of a sodium or potassium salt of hypericin and a polymeric complexing agent selected from the group consisting of polyethylene glycol and poly-N-vinylamide, wherein the photosensitizer is reconstituted from a lyophilizate obtained from a solution of the sodium or potassium salt of hypericin, containing about 0.0225 mg of hypericin/g of solution; the polymeric complexing agent and a buffer system comprising a phosphate buffer or a citric acid buffer,

wherein the lyophilizate is for reconstitution to obtain the photosensitizer having a concentration of 9 to 40 µM of hypericin.

23. Use, for photosensitizing in a photodynamic therapy of cancer, of a photosensitizer that is a stable complex or a stable compound of a sodium or potassium salt of hypericin and a polymeric complexing agent selected from the group consisting of polyethylene glycol and poly-N-vinylamide, wherein the photosensitizer is reconstituted from a lyophilizate obtained from a solution of the sodium or potassium salt of hypericin, containing about 0.0225 mg of hypericin/g of solution, the polymeric complexing agent and a buffer system comprising a phosphate buffer or a citric acid buffer,

wherein the lyophilizate is for reconstitution to obtain the photosensitizer having a concentration of 9 to 40 µM of hypericin.

24. Use, for preparation of a medicament for photosensitizing in a photodynamic therapy of cancer, of a photosensitizer that is a stable complex or a stable compound of a sodium or

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potassium salt of hypericin and a polymeric complexing agent selected from the group consisting of polyethylene glycol and poly-N-vinylamide, wherein the photosensitizer is reconstituted from a lyophilizate obtained from a solution of the sodium or potassium salt of hypericin, containing about 0.0225 mg of hypericin/g of solution, the polymeric complexing agent and a buffer system comprising a phosphate buffer or a citric acid buffer,

wherein the lyophilizate is for reconstitution to obtain the photosensitizer having a concentration of 9 to 40 µM of hypericin.

25. The use of claim 23 or 24, wherein the photodynamic therapy of cancer is photodynamic therapy of bladder cancer.

26. The use of any one of claims 21 to 25, wherein the photosensitizer has a concentration of 30 µM of hypericin.

27. The use of any one of claims 21 to 25, wherein the light to be employed during the photodynamic therapy has an intensity of 5 to 25 mW/cm^2.

28. The use of claim 27, wherein the light is at a wavelength of from 570 nm to 610 nm.

29. The use of any one of claims 21 to 25, wherein the illumination during the photodynamic therapy will occur for a period of 15 to 120 minutes.

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30. The use according to any one of claims 21 to 25, wherein the poly-N-vinylamide is polyvinylpyrrolidone (PVP) of various degrees of polymerization and crosslinking.

31. The use according to claim 30, wherein the polyvinylpyrrolidone is PVP k17, PVP k25 or PVP k30.

32. A formulation for use in photosensitizing a tumor in a photodynamic therapy of tumors, comprising a photosensitizer that is reconstituted from a lyophilizate obtained from a stable complex or a stable compound of an alkali salt of hypericin and a polymeric complexing agent in a buffer system of a phosphate buffer or a citric acid buffer, comprising about 0.0225 mg/g hypericin in said buffer system;

wherein the photosensitizer has a concentration of 9 to 40 µM of hypericin; and

wherein the polymeric complexing agent is selected from the group consisting of polyethylene glycol and poly-N-vinylamide.

33. A formulation for use in photosensitizing in a photodynamic therapy of cancer, comprising a photosensitizer that is reconstituted from a lyophilizate obtained from a stable complex or a stable compound of an alkali salt of hypericin and a polymeric complexing agent in a buffer system of a phosphate buffer or a citric acid buffer, comprising about 0.0225 mg/g hypericin in said buffer system;

wherein the photosensitizer has a concentration of 9 to 40 µM of hypericin; and

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wherein the polymeric complexing agent is selected from the group consisting of polyethylene glycol and poly-N-vinylamide.

34. The formulation for use according to claim 33, wherein the cancer is bladder cancer.

35. A formulation for use in photosensitizing in a photodynamic therapy of tumors, comprising a photosensitizer that is a stable complex or a stable compound of a sodium or potassium salt of hypericin and a polymeric complexing agent selected from the group consisting of polyethylene glycol and poly-N-vinylamide, wherein the photosensitizer is reconstituted from a lyophilizate obtained from a solution of the sodium or potassium salt of hypericin, containing about 0.0225 mg of hypericin/g of solution; the polymeric complexing agent and a buffer system comprising a phosphate buffer or a citric acid buffer,

wherein the formulation has a concentration of 9 to 40 µM of hypericin.

36. A formulation for use in photosensitizing in a photodynamic therapy of cancer, comprising a photosensitizer that is a stable complex or a stable compound of a sodium or potassium salt of hypericin and a polymeric complexing agent selected from the group consisting of polyethylene glycol and poly-N-vinylamide, wherein the photosensitizer is reconstituted from a lyophilizate obtained from a solution of the sodium or potassium salt of hypericin, containing up to about 0.0225 mg of hypericin/g of solution, the polymeric

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complexing agent and a buffer system comprising a phosphate buffer or a citric acid buffer,

wherein the lyophilizate is for reconstitution to obtain the photosensitizer having a concentration of 9 to 40 µM of hypericin.

37. The formulation for use according to claim 36, wherein the photodynamic therapy of cancer is photodynamic therapy of bladder cancer.

38. The formulation of any one of claims 32 to 37, wherein the salt of hypericin is sodium salt.

39. The formulation of any one of claims 32 to 37, wherein the salt of hypericin is potassium salt.

40. The formulation of any one of claims 32 to 37, wherein the photosensitizer has a concentration of 30 µM of hypericin.

41. The formulation of any one of claims 32 to 37, wherein the light to be employed during the photodynamic therapy has an intensity of 5 to 25 mW/cm^2.

42. The formulation of claim 41, wherein the light is at a wavelength of from 570 nm to 610 nm.

43. The formulation of any one of claims 32 to 37, wherein the illumination during the photodynamic therapy will occur for a period of 15 to 120 minutes.

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44. The formulation of any one of claims 32 to 37, wherein the complexing agent is polyethylene glycol.

45. The formulation of any one of claims 32 to 37, wherein the complexing agent is poly-N-vinylamide.

46. The formulation of claim 45, wherein the poly-N-vinylamide is polyvinylpyrrolidone (PVP) of various degrees of polymerization and crosslinking.

47. The formulation according to claim 46, wherein the polyvinylpyrrolidone is PVP k17, PVP k25 or PVP k30.

48. The formulation according to any one of claims 32 to 47, wherein the formulation is prepared for intravenous, intracavity, inhalative, oral, intraperitoneal and topical administration.

49. The formulation according to any one of claims 32 to 47, wherein the formulation is prepared in a hydrophilic vehicle.

50. The formulation according to any one of claims 32 to 47, wherein the formulation is prepared in a hydrophobic vehicle.

51. The formulation according to any one of claims 32 to 47, wherein the formulation is prepared in the form of a solution, a cream, a gel, an aerosol, emulsions or as a patch.

52. A method for the production of a photosensitizing formulation for photodynamic therapy, comprising:

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complexing a sodium or potassium salt of hypericin to a polymeric complexing agent selected from the group consisting of a polyethylene glycol, a poly-N-vinylamide, and polyvinylpyrrolidone (PVP) to form a stable complex or a stable compound, wherein:

the photosensitizing formulation comprises the stable complex or stable compound of the sodium or potassium salt of hypericin, wherein the photosensitizing formulation is reconstituted from a lyophilizate obtained from a solution of the sodium or potassium salt of hypericin, containing about 0.0225 mg hypericin/g solution, the polymeric complexing agent and a buffer system comprising a phosphate buffer or a citric acid buffer, and

the lyophilizate is reconstituted to obtain the photosensitizing formulation, having a concentration of 9 to 40 µM of hypericin.

53. A method for the production of a formulation for photodynamic therapy, comprising:

complexing a sodium or potassium salt of hypericin to a polymeric complexing agent selected from the group consisting of a polyethylene glycol, a poly-N-vinylamide, and polyvinylpyrrolidone (PVP) to form a stable complex or a stable compound, wherein:

the formulation is a stable complex or a stable compound of a sodium or potassium salt of hypericin, reconstituted from a lyophilizate obtained from a solution of the sodium or potassium salt of hypericin, containing about 0.0225 mg hypericin/g solution, the polymeric complexing agent and a buffer system comprising a phosphate buffer or a citric acid buffer, and

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the lyophilizate is reconstituted to yield the formulation, having a concentration of 9 to 40 µM of hypericin,

wherein the complexing is performed in aqueous solution.

54. The method according to claim 52, wherein the formulation is prepared for intravenous, intracavity, inhalative, oral, intraperitoneal and topical administration.

55. The method according to claim 52, wherein the formulation is prepared in a hydrophilic vehicle.

56. The method according to claim 52, wherein the formulation is prepared in a hydrophobic vehicle.

57. The method according to claim 52, wherein the formulation is prepared in the form of a solution, a cream, a gel, an aerosol, emulsions or as a patch.

58. The method according to claim 53, wherein the aqueous solution is decanted into injection flasks and freeze-dried.

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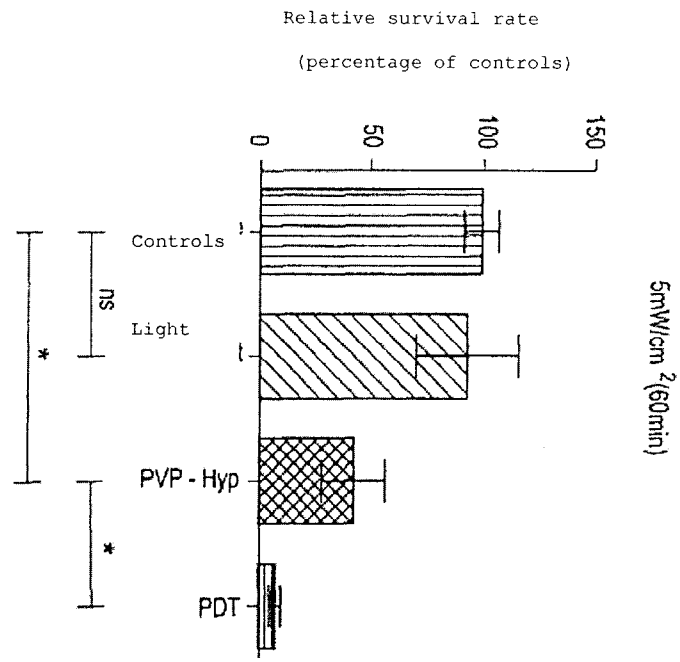

Relative survival rate
(percentage of controls)
0
50
100
150
Controls
Light
PVP - Hyp
PDT
ns
*
*
5mW/cm 2 (60min)

Fig. 1

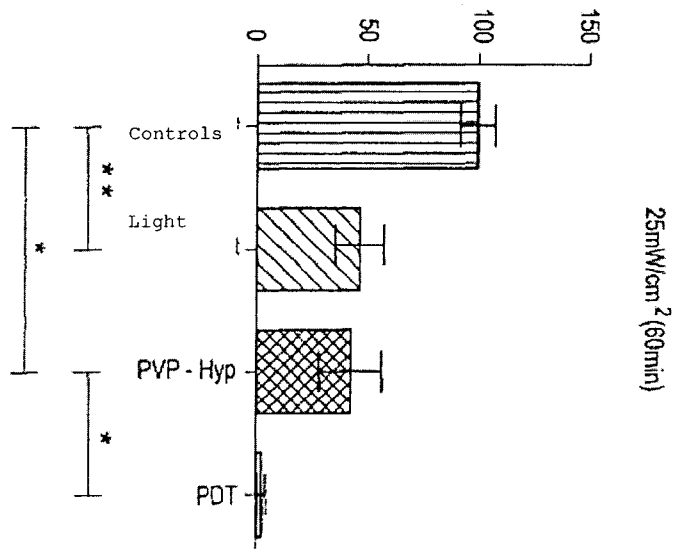
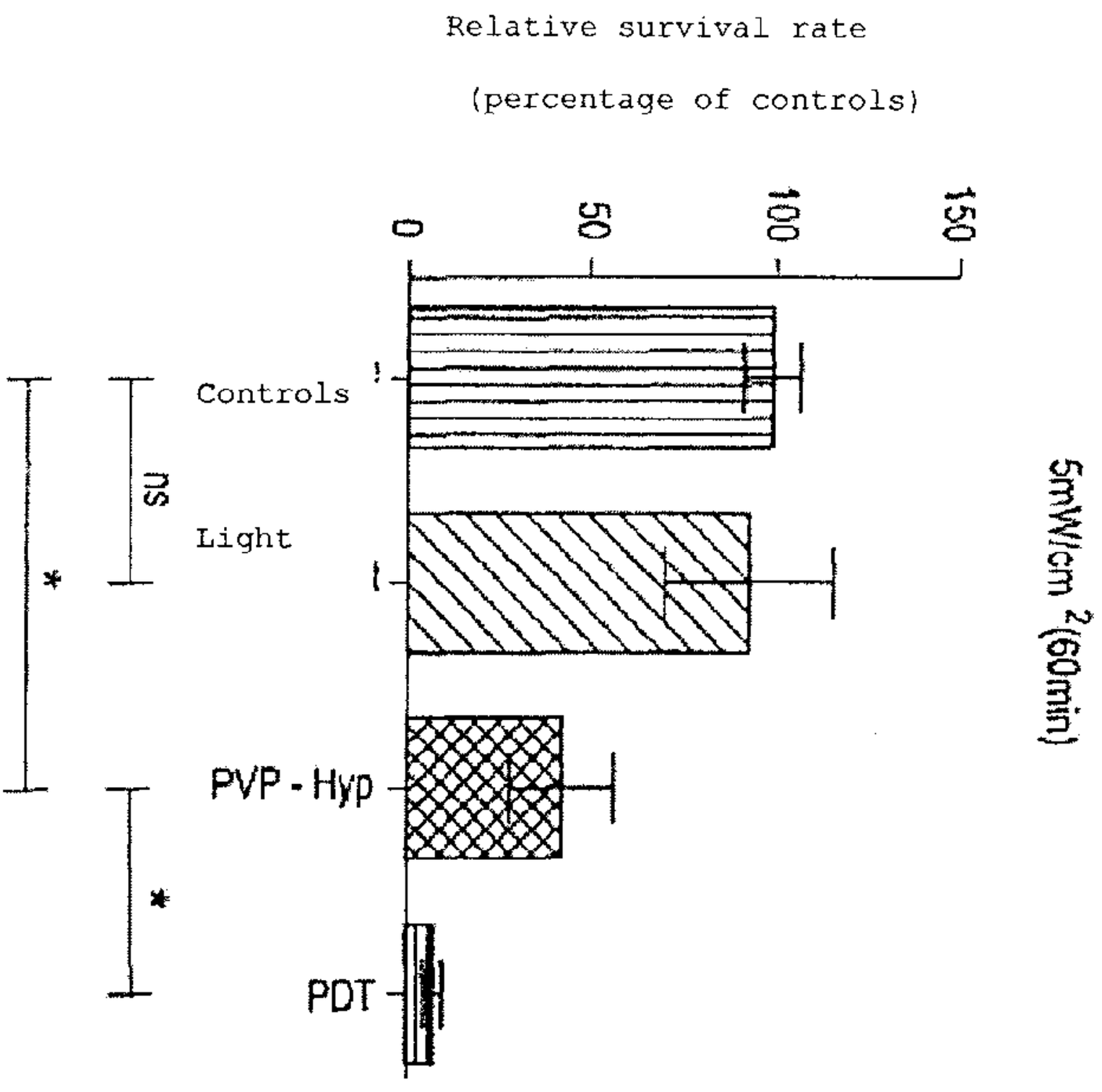

Relative survival rate
(percentage of controls)
0
50
100
150
Controls
Light
PVP - Hyp
PDT
**
*
*
25mW/cm 2 (60min)

Fig. 2


Relative survival rate
(percentage of controls)
0
50
100
150
Controls
Light
PVP - Hyp
PDT
ns
*
*
5mW/cm 2(60min)