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RESEARCH PAPER

Formulation of a Zinc(II)-Phthalocyanine-Containing Topical Gel for Photodynamic Therapy of Hyperproliferative Skin Conditions

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ABSTRACT

Zinc(II)-phthalocyanine is a lipophilic photosensitizer that shows promise as an agent for photodynamic therapy of hyperproliferative conditions. In order to address the treatment of skin conditions, we have developed a topical formulation of this molecule. Only a limited number of solvents were able to solubilize the dye to any extent and of these, the most suitable for formulation purposes was N-methyl-2-pyrrolidone. We, therefore, incorporated the photosensitizer in a nonaqueous gel formulation using this solvent. Under standard storage conditions, there was little or no degradation or aggregation of the dye and this formulation will be used to explore the potential of photodynamic therapy in various skin diseases.
Key Words: *N-methyl-2-pyrrolidone; Photodynamic therapy; Photosensitizer; Topical gel; Zinc(II)-phthalocyanine.*

INTRODUCTION

Photodynamic therapy (PDT) is a treatment modality that takes advantage of the fact that certain photosensitive tetrapyrolic molecules, such as porphyrins, phthalocyanines, and bacteriochlorins, are able to accumulate in and be selectively retained by abnormal or hyperproliferative cells and tissues. Subsequent photoactivation of these molecules in the presence of oxygen may lead to destruction of the target tissue, while sparing surrounding normal tissue, as a result of singlet oxygen mediated peroxidative damage (1,2).

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Although at present, PDT is considered predominantly a modality for the ablation of solid tumors, there is growing interest in its potential for the treatment of a variety of oncological, cardiovascular, dermatological, and ophthalmic indications (3).

Zinc(II)-phthalocyanine (Zn-Pc) is a promising second-generation photosensitizer with advantageous chemical, photophysical, and pharmacological properties, and has been extensively investigated in preclinical animal models as a phototherapeutic agent for the treatment of solid tumors (4). Because the molecule is extremely hydrophobic and almost completely water insoluble, it has been formulated in liposomes for parenteral administration (4,5). Incorporation of Zn-Pc molecules into the bilayer of small unilamellar vesicles also provided an environment in which the photosensitizer was maintained predominantly in the monomeric state, an essential prerequisite for effective photodynamic activity (5). In addition, liposomal incorporation facilitated the delivery of Zn-Pc to the tumor target in experimental animal models (4,6,7).

PDT has been used successfully to treat patients with superficial skin tumors (8,9), and recently studies have been initiated to determine its potential for the resolution of lesions in plaque psoriasis (10,11), a disease characterized by epidermal hyperproliferation and abnormal keratinocyte differentiation (12). In order to explore the efficacy of photoactivated Zn-Pc in dermatoses such as psoriasis, we have assumed a requirement for a stable topical formulation that maintains the photosensitizer in the monomeric state, contains excipients likely to be acceptable to the regulatory authorities, and delivers the dye to the target keratinocyte cell population in the epidermis. This communication describes the development of such a formulation.

MATERIALS AND METHODS

Materials

Zn-Pc (MW 577.92) was supplied by Dr. H.-G. Capraro, CIBA-Geigy Ltd., Basel, Switzerland, as a chemically pure compound that is stable in solid form up to 250°C; the chemical structure of this achiral compound and its light absorption profile are shown in Fig. 1.

N-Methyl-2-pyrrolidone (NMP) was obtained as Pharmasolve™ from ISP Europe, Guildford, U.K. Labrafil M1944 CS (apricot kernel oil PEG-6 esters), Labrafil M2130 CS (hydrogenated palm/palm kernel oil PEG-6 esters), Labrasol (saturated polyglycolized glyceride), and Transcutol (diethylene glycol monoethylether) were supplied by Gattefossé sa, Saint-Priest, France, and polyethylene glycol 400 (PEG 400), was supplied by Cremophor EL and Cremophor RH40 by BASF, Cheadle, U.K. Hydroxypropyl cellulose type M (HPC-M) was from Nippon Soda Co. Ltd., Tokyo, Japan. All other chemicals were obtained from BDH Biochemicals, Poole, U.K., and were of the highest purity available.

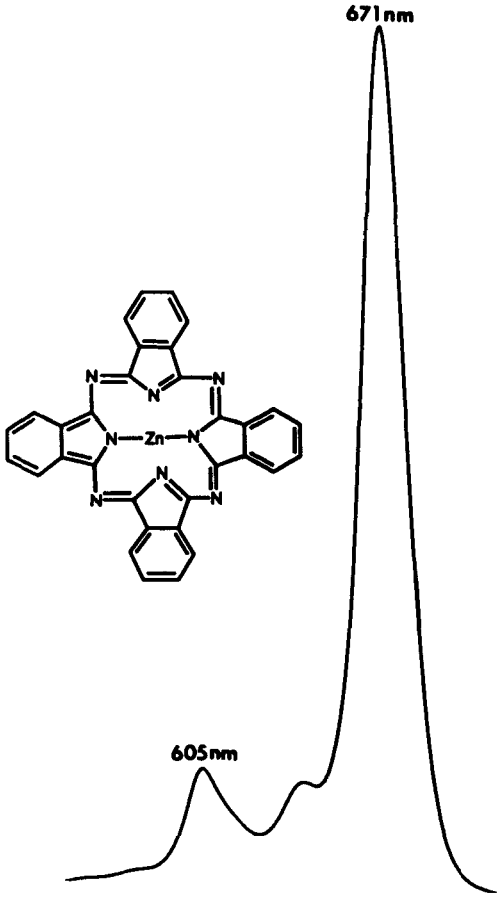

Figure 1. Structure and action spectrum of Zn-Pc.

Physical Measurements

The aggregation state of Zn-Pc in various solvents was determined as the monomeric index (MI). Solutions (100 μg/ml) were scanned in a Perkin-Elmer LS-5 Luminescence spectrophotometer, and the ratio of the absorption peaks at 671 nm and 606 nm was used to determine the MI as described by Valduga and co-workers (13). The concentration of Zn-Pc was determined spectrophotometrically (671 nm). The viscosity of gel

formulations was determined using a Carrimed rheometer equiped with a 4-cm, 2° stainless steel cone, a 52-μm gap setting, a sample volume of 0.6 ml, and a torque setting of 40,000 dynes/cm^2 at 25°C.

RESULTS AND DISCUSSION

Zn-Pc is lipophilic (log $P > 8$), insoluble in water and poorly soluble in common organic solvents (14). We, therefore, examined the solubility of Zn-Pc in a range of solvents, some of which were known from previous studies to solubilize other photosensitizers or had a demonstrated skin penetration enhancing capacity (15). The results of these studies are presented in Table 1; it is apparent that relatively few of these solvents are able to solubilize Zn-Pc at concentrations greater than 1 mg/ml. It is clear from studies of parenterally administered liposomal Zn-Pc that, in combination with appropriate total light doses, significant photodynamic effects can be achieved with 100-300 ng Zn-Pc/g tissue (4). Therefore, assuming about 1-2% dye penetration into the epidermis after topical application (unpublished observation) and application of 0.1-0.2 ml formulation/cm^2, we calculated the need to incorporate 50-150 μg Zn-Pc/ml formulation in order to achieve therapeutic levels of dye in the target tissue. In order to limit the concentration of solvent in the formulation, to circumvent the potential for stability problems due to incorporation of the the dye at or near the saturation point and to reduce the likelihood of solvent incompatibility with viscosity modifying agents or other components of the formulation, it was decided to utilize NMP (Table 1) as solvent for Zn-Pc.

To exclude water from the formulation, NMP containing sufficient Zn-Pc to achieve final dye concentrations of 12.5, 62.5, and 125 μg/ml was diluted with PEG-400. All 3 formulations contained 5% v/v NMP and the viscosity was enhanced by the inclusion of 1.5% w/v of HPC-M (Table 2). Batches were routinely prepared by dissolving the appropriate concentration of Zn-Pc in NMP, diluting with PEG-400 to give 5% v/v NMP, adding HPC-M, and heating the mixture to 80°C

Table 1

Solubility of Zn-Pc in Various Solvents

Solvent	Zn-Pc Solubility (mg/ml)	Skin Penetration Enhancer	Acceptable Toxicology
Water	<0.001	N	Y
Dimethyl sulfoxide	<1	Y	N
Piperidine	<1	N	N
Pyridine	<1	N	N
Tetrahydrofuran	<5	N	N
N-Methyl pyrrolidone	<5	Y	Y[a]
Oleic acid	<0.01	Y	Y
Propylene glycol	<0.01	N	Y
Cremophor RH40/EL	<0.01	N	Y
PEG 400	<0.01	N	Y
Transcutol	<0.1	Y	Y
Ethanol	<0.01	Y	Y
Ethomeen C25	<0.01	N	Y
Isopropyl myristate	<0.01	N	Y
Isopropyl palmitate	<0.01	N	Y
Labrafil	<0.01	N	Y
Labrasol	<0.01	N	Y
Liquid paraffin	<0.01	N	Y
Sesame, peanut, olive oils	<0.01	N	Y

[a]Regulatory status under review; not currently present in a pharmaceutical product licensed for human use.
Note: Information on skin penetration enhancement was obtained from the literature (15-17). If a current pharmaceutical product contained the solvent, it was considered acceptable; data were obtained from a standard source (18).

Table 2

Composition of Zn-Pc Topical Gel Formulations

Zn-Pc Concentration	Zn-Pc (mg/100 g)	NMP (g/100 g)	PEG-400 (g/100 g)	HPC-M (g/100 g)
12.5	1.09	4.47	94.22	1.31
62.5	5.44	4.47	94.22	1.31
125	10.9	4.47	94.22	1.31

with continuous stirring. After maintaining this temperature for 10 min, the mixture was cooled to room temperature and a clear gel obtained. These NMP-containing gels were assigned the descriptor CGH 840.

There has been increased interest in recent years in the use of liposomes as carriers for topical delivery (19–21) and, because Zn-Pc has been successfully incorporated into liposomes for parenteral administration (4–7), we initially investigated the feasibility of formulating liposomal Zn-Pc in various gel systems. Although methods were developed to obtain liposomal gel formulations, in all cases the photosensitizer aggregated in the liposomal bilayer upon storage over a range of temperatures (data not shown). In contrast, Zn-Pc incorporated into the CGH 840 gels described in this communication were considerably less prone to aggregation. Formulations containing the 3 Zn-Pc concentrations of 12.5, 62.5, and 125 μg/ml were stored under dark conditions at 4°C, 22°C, and 37°C for up to 17 months and stability monitored by measuring two parameters: the aggregation state of the Zn-Pc (MI) and the maximum absorbance at 671 nm. Since Zn-Pc is susceptible to photobleaching, the gels were stored in the dark. Data obtained with gels containing the highest Zn-Pc concentration is shown in Table 3. It can be seen that, with gels containing 125 μg/ml, there was no evidence of aggregation at any of the storage temperatures. There was, however, a small drop in absorbance at 671 nm between days 200 and 485 at the highest temperature only. With gels containing 62.5 μg/ml, there was evidence of aggregation at 37°C between days 308 and 485 with concomitant reduction in absorbance at λ_{max}; at 4°C and 22°C, the MI values obtained at 485 or 515 days were, respectively, 99% and 97% with little or no reduction in absorbance at 671 nm, indicating no aggregation and no degradation of the dye. In contrast, CGH 840 containing the lowest amount of Zn-Pc (12.5 μg/ml) showed marked reduction in 671 nm absorbance of Zn-Pc at all 3 storage temperatures over the 485- to 515-day period and complete aggregation (0% MI) after 200 days at 37°C. Thus, the formulations least prone to Zn-Pc degradation and aggregation were those containing the highest concentrations of photosenstizer studied and stored at the lower temperatures. The gels were found to possess good preservative properties, reducing viable counts of *Candida albicans, Aspergillus niger, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Escherichia coli* strains sufficiently over a 28-day test

Table 3

Effect of Storage at 4°C, 22°C, and 37°C on the MI and 671 nm Absorbance of Zn-Pc in CGH 840 Preparations Containing 125 μg/ml of Photosensitizer

Time (days)	Monomeric Index (%)	Absorbance at 671 nm
	Stored at 4°C	
0	93	0.657
24	100	N.D.
100	96	0.690
200	97	0.711
308	100	0.736
515	99	0.791
	Stored at 22°C	
0	93	0.657
24	98	0.702
100	98	0.685
200	95	0.655
308	97	0.659
485	96	0.671
	Stored at 37°C	
0	93	0.657
24	98	0.665
100	93	0.620
200	96	0.653
308	96	0.607
485	95	0.548

period to satisfy BP 1988 antimicrobial preservative test criteria. It has been established that NMP has antimicrobial activity (22). The viscosity of the gels fell within the range 5000–6000 mPa.

Zn–Pc shows great promise as a second-generation photosensitizer. It can be produced in high yields by a one-step synthetic procedure to give a chemically pure compound with a high degree of chemical stability (4). It has an absorbance maximum of 671 nm, and light of this wavelength shows good penetration into tissues and a low degree of overlap with the sunlight emission spectrum, ensuring minimal skin phototoxicity as a result of fortuitous exposure to sunlight (23). Furthermore, Zn–Pc has a high extinction coefficient, which allows low therapeutic drug doses to be used. It also has a singlet oxygen quantum yield sufficiently high to facilitate efficient target tissue destruction at suitable drug:light doses. When appropriately formulated, it displays excellent selectivity for implanted tumors and participates in effective tumor destruction when activated by light (4,24,25). Like other lipophilic photosensitizers, it localizes in the membrane systems of target cell populations and effects direct cell killing from this locus (7,26).

Although the physicochemical properties of Zn–Pc are advantageous for tissue photosensitization, they give rise to substantial problems with respect to formulation. In particular, the extreme lipophilicity, propensity for aggregation, and lack of solubility in most commonly used pharmaceutical excipients combined to make Zn–Pc difficult to formulate for topical administration. Molecules of Zn–Pc readily aggregate in an aqueous environment and we, therefore, attempted to develop a water-free formulation. Of the potential excipients tested, NMP, a relatively innocuous solvent (27–29), proved satisfactory with respect to stable incorporation of drug at suitable concentrations (Table 1) and the NMP/PEG-400 system did not promote aggregation of the photosensitizer (Table 3). Skin has a relatively high lipid content and the intercellular lipids form broad multilamellar sheets that contribute to the regulation of the barrier function of the stratum corneum (30,31). It is possible that the application to the skin surface of CGH 840 will enable photosensitizer molecules to gain access to the epidermis and, particularly, the basal cell layer at the dermal–epidermal junction in nonaggregated form via these lipid channels. We plan to investigate the mode of penetration of Zn–Pc into ex vivo human skin in order to optimize the formulation with respect to photodynamic efficacy.

REFERENCES

1. H. I. Pass, Photodynamic therapy in oncology: mechanisms and clinical use, J. Natl. Cancer Inst., 85, 443–456 (1993).
2. D. Dolphin, Photomedicine and photodynamic therapy, Can. J. Chem., 72, 1005–1013 (1994).
3. J. G. Levy, Photodynamic therapy, TIB Tech., 13, 14–18 (1995).
4. K. Schieweck, H.-G. Capraro, U. Isele, P. van Hoogevest, M. Ochsner, T. Maurer, and E. Batt, CGP 55 847, liposome-delivered zinc(II)-phthalocyanine as a phototherapeutic agent for tumours, SPIE Proc., 2078, 107–118 (1994).
5. U. Isele, P. van Hoogevest, R. Hilfiker, H.-G. Capraro, K. Schieweck, and H. Leuenberger, Large-scale production of liposomes containing monomeric zinc phthalocyanine by controlled dilution of organic solvents, J. Pharm. Sci., 83, 1608–1616 (1994).
6. U. Isele, K. Schieweck, R. Kessler, P. van Hoogevest, and H.-G. Capraro, Pharmacokinetics and body distribution of liposomal zinc phthalocyanine in tumor-bearing mice: influence of aggregation state, particle size, and composition, J. Pharm. Sci., 84, 166–173 (1995).
7. W. G. Love, S. Duk, R. Biolo, G. Jori, and P. W. Taylor, Liposome-mediated delivery of photosensitizers: localization of zinc (II)-phthalocyanine within implanted tumors after intravenous administration, Photochem. Photobiol., 63, 656–661 (1996).
8. T. Warloe, Q. Peng, H. Heyerdahl, J. Moan, H. B. Steen and K.-E. Giercksky, Photodynamic therapy with 5-aminolevulinic acid induced porphyrins and DMSO/EDTA for basal cell carcinoma. SPIE Proc., 2371, 226–235 (1995).
9. H. Heinritz, W. Benzel, R. Sroka, and H. Iro, Photodynamic therapy of superficial skin tumors following local application of delta-aminolevulinic acid, Adv. Otorhinolaryngol., 49, 48–52 (1995).
10. W.-H. Boehnecke, K. König, R. Kaufmann, W. Scheffold, O. Prümmer, and W. Sterry, Photodynamic therapy in psoriasis: suppression of cytokine production in vitro and recording of fluorescence modification during treatment in vivo, Arch. Dermatol. Res., 286, 300–303 (1994).
11. J. G. Levy, C. A. Jones, and L. A. Pilson, The preclinical and clinical development of Photofrin® and benzoporphyrin derivative: a reflection on opportunities and challenges, J. Photochem. Photobiol. B: Biol., 30, 79–82 (1995).
12. C. Camisa, Psoriasis, Blackwell Scientific Publications, Boston, 1994.
13. G. Valduga, E. Reddi, and G. Jori, Spectroscopic studies on Zn(II)-phthalocyanine in homogeneous and

microheterogeneous systems, J. Inorg. Biochem., 29, 59–65 (1987).
14. J. D. Spikes, Phthalocyanines as photosensitizers in biological systems and for the photodynamic therapy of tumors, Photochem. Photobiol., 43, 691–699 (1986).
15. B. W. Barry, Mode of action of penetration enhancers in human skin, J. Controlled Release, 6, 85–97 (1987).
16. T. M. Turunen and A. Urtti, Penetration enhancers in transdermal drug delivery, Acta Pharm. Fenn., 101, 3–10 (1992).
17. G. C. Santus and R. W. Baker, Transdermal enhancer patent literature, J. Controlled Release, 25, 1–20 (1993).
18. A. Wade and P. J. Weller, eds., *Handbook of Pharmaceutical Excipients*, 2nd ed., The Pharmaceutical Press, London, 1994.
19. J. Lasch and W. Wohlrab, Liposome-bound cortisol: a new approach to cutaneous therapy, Biomed. Biochim. Acta., 45, 1295–1299 (1986).
20. J. du Plessis, K. Egbaria, and N. Weiner, Influence of formulation factors on the deposition of liposomal components into the different strata of the skin, J. Soc. Cosmet. Chem., 43, 93–100 (1992).
21. C. Nastruzzi, E. Esposito, E. Menegatti, and P. Walde, Use and stability of liposomes in dermatological preparations, J. Appl. Cosmetol., 11, 77–91 (1993).
22. Eur. Patent Appl. 38416 A2. Desinfektionsmittelformulierungen mit spezifischem Wirkungsmechanismus, Oct. 28, 1981 (to Intermedicat).
23. M. Ochsner, Light scattering of human skin: a comparison between zinc(ii)-phthalocyanine and Photofrin II®, J. Photochem. Photobiol. B: Biol., 32, 3–9 (1996).
24. E. Reddi, C. Zhou, R. Biolo, E. Menegaldo, and G. Jori, Liposome- or LDL-administered Zn(II)-phthalocyanine as a photodynamic agent for tumours. I. Pharmacokinetic properties and phototherapeutic efficiency, Br. J. Cancer, 61, 407–411 (1990).
25. C. Milanesi, C. Zhou, R. Biolo, and G. Jori, Zn(II)-phthalocyanine as a photodynamic agent for tumours. II. Studies on the mechanism of photosenstised tumour necrosis. Br. J. Cancer, 61, 846–850 (1990).
26. W. G. Love, E. C. Havenaar, P. J. Lowe, and P. W. Taylor, Uptake of zinc(II)-phthalocyanine by HepG2 cells expressing the LDL receptor: studies with the liposomal formulation CGP 55 847, SPIE Proc., 2078, 381–388 (1994).
27. W. Bartsch, G. Sponer, K. Dietmann, and G. Fuchs, Acute toxicity of various solvents in the mouse and rat, Artzneim. Forsch., 26, 1581–1583 (1976).
28. P. J. Becci, L. A. Gephart, F. J. Koschier, W. D. Johnson and L. W. Burnette, Subchronic feeding study in beagle dogs of *N*-methylpyrrolidone, J. Appl. Toxicol., 3, 83–86 (1983).
29. J. M. Ansell and J. A. Fowler, The acute oral toxicity and primary ocular and dermal irritation of selected *N*-alkyl-2-pyrrolidones, Fd. Chem. Toxic., 26, 475–479 (1988).
30. P. M. Elias, Epidermal lipids, barrier function, and desquamation, J. Invest. Dermatol., 80, 44s–49s (1983).
31. G. Imokawa, S, Akasaki, M. Hattori, and N. Yoshizuka, Selective recovery of deranged water-holding properties by stratum corneum lipids, J. Invest. Dermatol. 87, 758–764 (1986).