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Effect of Chloroquine Wash-out Period of Photosensitizers in the Skin and Selected Organs in Rats

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Summary

In the last decade, photodynamic therapy has become an alternative method for the diagnosis and therapy of tumors. In human medicine hematoporphyrin derivatives, sulfonated hydrophilic meso-tetraphenylporphyrin ($TPPS_4$) and an oligomer of hematoporphyrin (Photosan 3), are widely used. Chloroquine is used for the treatment of porphyria cutanea tarda for its power to release porphyrins from the liver tissue. The kinetics of two porphyrin photosensitizers $TPPS_4$ and Photosan 3 in the skin and some organs as well as the effect of chloroquine on the porphyrin excretion and their accumulation in skin and organs of Wistar rats were studied. $TPPS_4$ exhibited maximum fluorescence in skin 48 h after application with decreasing to basal level from the 8th to the 14th day. Maximum fluorescence was reached at 72 hours after Photosan 3 application and it decreased to basal level during 96 hours after application. $TPPS_4$ caused significantly higher fluorescence compared to Photosan 3. Chloroquine after oral administration did not change the fluorescence of skin, but it significantly decreased the $TPPS_4$ concentration in rat organs if chloroquine treatment started 3 days or 2 weeks after $TPPS_4$ application. Chloroquine significantly decreased the serum $TPPS_4$ concentration during the period of 28 days. Fluorescence of skin was significantly higher and lasted longer after application of $TPPS_4$ compared to Photosan 3. Chloroquine after oral administration did not influence the fluorescence of the skin, but it significantly decreased the $TPPS_4$ concentration in rat organs. This effect could be useful in photodynamic therapy for mobilizing exogenous porphyrins from tissues after parenteral photodynamic therapy.

Key words

Photosensitizers • Photodynamic therapy • Meso-tetra-(4-sulfonatophenyl)-porphine-$TPPS_4$ • Photosan 3 • Chloroquine

Introduction

Photodynamic therapy and fluorescence endoscopy is becoming an alternative method for therapy and diagnosis of tumors. This method is based on the discovery that some porphyrins accumulate selectively in the tumor tissue after intravenous application. The selective accumulation of parenterally administered porphyrins in tumors has been the basis for anticipated diagnostic and therapeutic applications in human medicine (Winkelman *et al.* 1967, Moan *et al.* 1987, Schwartz *et al.* 1995).

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The photosensitizing property of porphyrins is caused by singlet oxygen formation after irradiation (Jori and Spikes 1984, Parker and Stanbro 1984). In human medicine, hematoporphyrin derivatives (Photosan 3, Photofrin), sulfonated hydrophilic meso-tetraphenyl-porphines ($TPPS_4$) are mainly used porphyrin-photosensitizers. Meso-tetra-(4-sulfonato-phenyl)-porphine ($TPPS_4$) first used by Winkelman (1962), is a stable drug soluble in water at room temperature which gives a greater yield of singlet oxygen but its application in human medicine was rejected for its neurotoxicity (Rodgers 1985). Our $TPPS_4$ was prepared by the method of Busby *et al.* (1975) with modified purification described by Jirsa and Kakač (1987). This product is free of neurotoxic effects (Střelečková *et al.* 1995).

Chloroquine is used for the treatment of porphyria cutanea tarda (Wallace 1996). The main effect of this drug is depletion of porphyrins accumulated in the liver cell. We supposed that after application of chloroquine, the same mechanism could shorten the presence of $TPPS_4$ in the skin and organs. Sensitive and reliable early detection of fluorescence indicating the presence of malignancy is important because it enables early diagnosis and effective therapy. Several detection systems have been designed and developed (Baumgartner *et al.* 1987, Straigh *et al.* 1991). Our reflectance fluorometer allowed detection of very weak fluorescence signals. Such a detection system has not been used in photodynamic diagnostics yet (Stádník *et al.* 1997).

In this study, we directed our interest to the kinetics of the two mostly used porphyrin-photosensitizers ($TPPS_4$ and Photosan 3) in the skin and some organs as well as to the effect of chloroquine on $TPPS_4$ accumulation in the skin and organs.

Methods

Experimental design

Female Wistar rats weighing approximately 200-220 g were housed in standard cages with free access to destiled water and standard rat chow. Four groups ($TPPS_4$, $TPPS_4$ + chloroquine, Photosan 3, chloroquine) each consisting of 6 animals were examined. The photosensitizers were injected into the inquinal vein in a single dose 5 mg/kg b.w.. Chloroquine administration started 14 days after $TPPS_4$ application, it was applied orally 8 mg/kg b.w. twice a week (for two weeks). Two groups ($TPPS_4$, $TPPS_4$ + chloroquine) each consisting of 24 animals were examined. The photosensitizers were injected into inquinal vein in a single dose 5 mg/kg b.w.. Chloroquine was administered on day 3, 7, 10, 14, 17, 21, 24, and 27 after $TPPS_4$ administration. It was applied orally in a dose 8 mg/kg b.w.

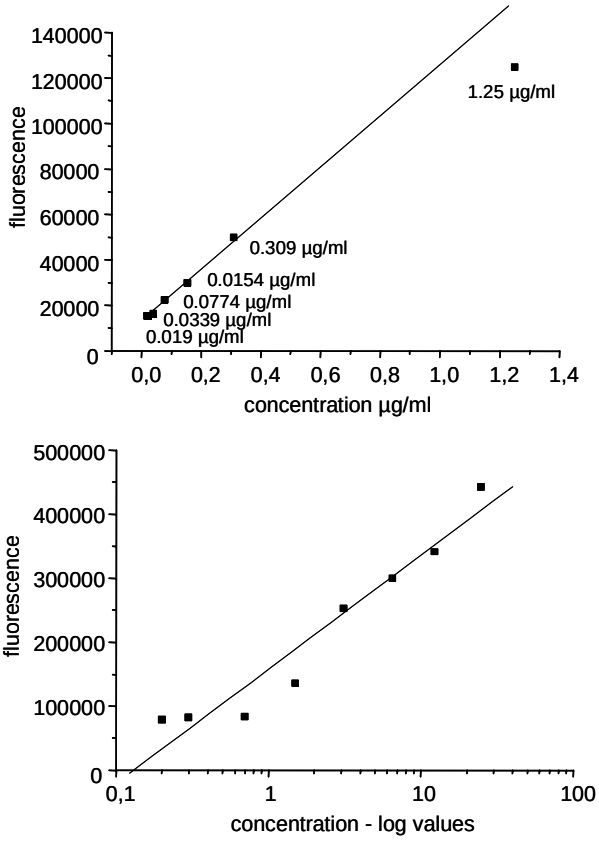

Fig. 1. Calibration curve of the reflectance fluorometer using 10 mm glass cells (upper panel) or gel circles (lower panel).

Chemicals and analytical process

$TPPS_4$ was prepared and purified according to the method of Jirsa and Kakač (1987), Photosan 3 was provided by prof. H. Mueller von der Haegen (Seehof-Laboratorium and Entwicklungsgesellschaft BRD) and chloroquine was from Alkaloida (Hungary).

The concentration of $TPPS_4$ and Photosan 3 in rat organs and the serum were measured by spectrofluorometric assay (emission 550-700 nm, excitation 416 nm) after extraction with 0.1 M NaOH.

Red skin fluorescence was measured by reflectance fluorometry (600-700 nm). Our reflectance fluorometric apparatus allowed detection of very weak fluorescence signals. Its operation is based on the synchronous lock-in amplifier detection technique. The detection system is fully automated. Its basic operation parameters are: the dynamic range 125 dB (from 10 dBm to –115 dBm for the Si detector), and the resolution

0.001 dB in the wavelength range 250-2000 nm (Stádník *et al.* 1997).

The calibration *in vitro* of our reflectance fluorometer and its comparison with a standard laboratory fluorometer are shown in Figure 1. The results for $TPPS_4$ concentration range 0.1936-12.39 ng/ml.

Statistical analysis

We used parametric ANOVA test with Bonferroni correction, non-parametric Kruskal-Wallis ANOVA test, Dunn´s test, and non-parametric unpaired Mann-Whitney test.

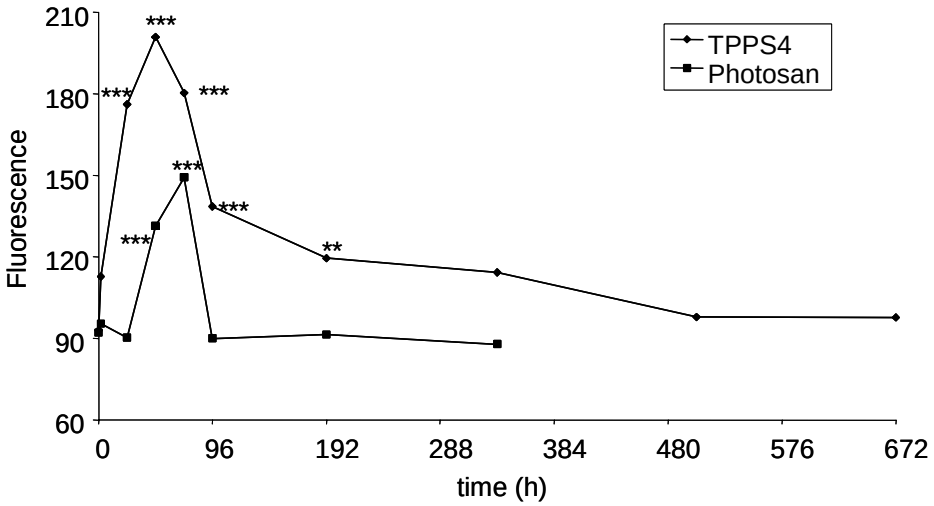

Fig. 2. Fluorescence of the rat skin after $TPPS_4$ and Photosan 3 application. **p<0.01, ***p<0.001 compared to time 0.

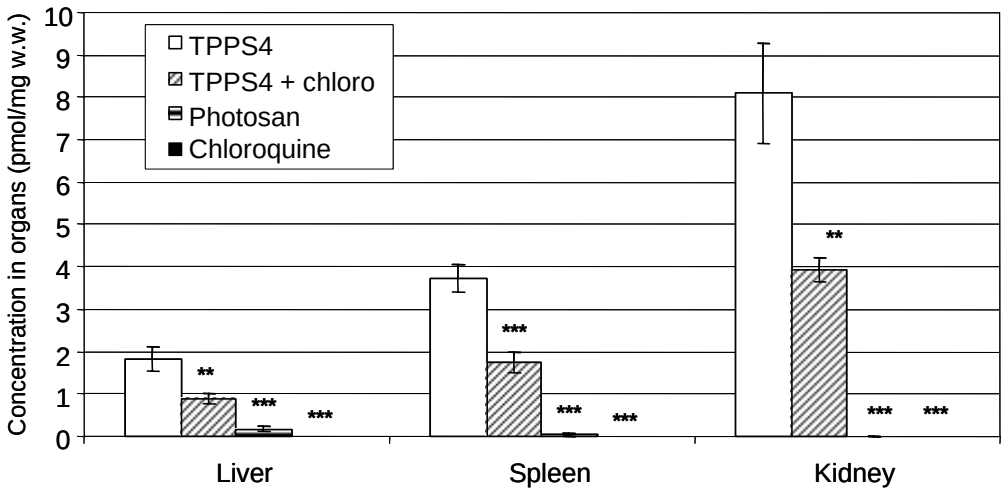

Fig. 3. Concentration of photosensitizers in the rat organs 28 days after application and the effect of chloroquine (N=6). Data are means ± S. E. M. **p<0.01, ***p<0.001 compared to $TPPS_4$ group evaluated by parametric, unpaired ANOVA test with Bonferoni p value.

Results

Red skin fluorescence was measured by reflectance fluorometry (600-700 nm) in the course of 28 days. After $TPPS_4$ administration, maximum fluorescence in skin was found 48 h after application, decreasing to basal levels in the course of 8 to 14 days. After Photosan 3 application, maximum fluorescence was reached 72 h after the injection with a decrease to basal levels within 96 h. The skin fluorescence after $TPPS_4$ application thus significantly exceeded that of Photosan 3 between 2-336 h ($p<0.001$) (Fig. 2).

Chloroquine after oral administration did not change the fluorescence of the skin, but it significantly

decreased the $TPPS_4$ concentration in the liver, spleen and kidney if chloroquine treatment started 2 weeks after $TPPS_4$ application (Fig. 3).

Chloroquine administered on day 3, 7, 10, and 14 after $TPPS_4$ application significantly decreased the serum $TPPS_4$ concentration on day 8 and 15 (Table 1).

The fluorescence of skin was significantly higher and lasted longer after the application of $TPPS_4$ compared to Photosan 3. Chloroquine after oral administration did not influence the fluorescence of skin, but it significantly decreased the $TPPS_4$ concentration in rat organs.

Table 1. Serum concentration of $TPPS_4$ (nmol/ml) and the effect of chloroquine.

Time	N	$TPPS_4$	$TPPS_4$ + chloroquine
3 days after $TPPS_4$ before chloroquine	12	0.346±0.017	0.326±0.009
8 days after $TPPS_4$ after 2 doses of chloroquine	12	0.118±0.006	0.082±0.003***
15 days after $TPPS_4$ after 4 doses of chloroquine	6	0.063±0.004	0.041±0.001**

Data are means ± S.E.M., ** p<0.01, *** p<0,001 difference between groups at studied periods of time evaluated by non-parametric Mann-Whitney test

Discussion

The results of our experiments have demonstrated that $TPPS_4$ fluorescence after the same parenteral dose as Photosan 3 reaches higher values and remains in the skin for a longer period of time. This is a disadvantage compared with hematoporphyrin derivatives. This negative effect may perhaps be compensated by the greater efficacy of $TPPS_4$ which permits to use much smaller therapeutical doses (Parker and Stanbro 1984, Rodgers 1985). Although the animals were kept in illuminated cages and shaved on some parts of the body, no local manifestation of skin photosensitivity was observed. It is a question whether there exists a direct dependence between porphyrin concentration in the skin and manifestation of skin sensitivity.

The concentration of $TPPS_4$ in some organs is comparable to the data published by Winkelman (1962), and our previously published data (Zima *et al.* 1997). The $TPPS_4$ concentration in organs continues to decrease after 28 days of application, while the concentration of Photosan 3 is significantly lower in rat tissues. It was suggested that several porphyrins are selectively accumulated in tumors and some organs (Winkelman *et al.* 1967).

Chloroquine is used as a therapeutic drug in human porphyria cutanea tarda for decreasing the concentration of uroporphyrine in the liver and other organs. Chloroquine in a single compartment model with increasing concentrations inhibits the total production of porphyrins, reduces intracellular concentration of porphyrins and increases transversal permeation of porphyrins through the cellular membrane (Kotal *et al.* 1984). Chloroquine influenced directly but nonspecifically the membrane permeability, apparently mainly the vacuolar membrane (Kotal *et al.* 1988). It was also suggested that chloroquine stimulates the exocytosis of porphyrins (Kotal and Kotyk 1988). Chloroquine forms complexes with porphyrins (Egger *et al.* 1996) and these compounds are easily eliminated from the tissues into the urine.

Egger *et al.* (1996) sugested that chloroquine is unlikely to be useful after photodynamic therapy for mobilizing exogenous porphyrins from tissues such as the liver, spleen and kidney if the hematoporphyrin (50 mg/kg b.w. i.p.) and chloroquine (100 mg/kg b.w. s.c.) were used. We found an analogy between $TPPS_4$, as a polar porphyrine compound, and uroporphyrine excretion.

Peroral chloroquine administration significantly decreased the $TPPS_4$ concentration in rat organs (liver, kidney, speen) if chloroquine treatment started 2 weeks after $TPPS_4$ administration during long periods of treatment. In shorter periods (8 days), we found only a small decrease of $TPPS_4$ concentration without an effect on day 28. Chloroquine significantly decreased $TPPS_4$ concentration in the serum. We did not register changes of red skin fluorescence after chloroquine administration using reflectance fluorometer.

We suppose that chloroquine administration may be useful after $TPPS_4$ i.v. injection. This effect could be useful in photodynamic therapy for mobilizing exogenous porphyrins from tissues and also could decrease the side effect of photodynamic therapy – skin sensibilization.

We can conclude that the $TPPS_4$ caused higher skin fluorescence compared to Photosan 3 after application of the same dose. Chloroquine did not affect skin fluorescence, but it significantly decreased the $TPPS_4$ concentration in the serum. During a shorter period (8 days), we found only a small decrease of $TPPS_4$ concentration. The chloroquine effect may begin by decreasing the serum concentration and during longer period the decrease could also be observed in organs.

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