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 (TPPS₄) 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 TPPS₄ 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. TPPS₄ 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. TPPS₄ caused significantly higher fluorescence compared to Photosan 3. Chloroquine after oral administration did not change the fluorescence of skin, but it significantly decreased the TPPS₄ concentration in rat organs if chloroquine treatment started 3 days or 2 weeks after TPPS₄ application. Chloroquine significantly decreased the serum TPPS₄ concentration during the period of 28 days. Fluorescence of skin was significantly higher and lasted longer after application of TPPS₄ compared to Photosan 3. Chloroquine after oral administration did not influence the fluorescence of the skin, but it significantly decreased the TPPS₄ 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-TPPS₄ • 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).
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-tetraphenylporphines (TPPS₄) are mainly used porphyrin-photosensitizers. Meso-tetra-(4-sulfonato-phenyl)-porphine (TPPS₄) 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 TPPS₄ 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 TPPS₄ 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 (TPPS₄ and Photosan 3) in the skin and some organs as well as to the effect of chloroquine on TPPS₄ 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 destilled water and standard rat chow. Four groups (TPPS₄, TPPS₄ + 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 TPPS₄ application, it was applied orally 8 mg/kg b.w. twice a week (for two weeks). Two groups (TPPS₄, TPPS₄ + 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 TPPS₄ administration. It was applied orally in a dose 8 mg/kg b.w.

Chemicals and analytical process

TPPS₄ 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 TPPS₄ 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 TPPS₄ 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.

![Figure 2](image-url)

**Fig. 2.** Fluorescence of the rat skin after TPPS₄ and Photosan 3 application. **p<0.01, ***p<0.001 compared to time 0.

![Figure 3](image-url)

**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 TPPS₄ group evaluated by parametric, unpaired ANOVA test with Bonferroni p value.

**Results**

Red skin fluorescence was measured by reflectance fluorometry (600-700 nm) in the course of 28 days. After TPPS₄ 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 TPPS₄ 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.

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>TPPS$_4$</th>
<th>TPPS$_4$ + chloroquine</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days after TPPS$_4$ before chloroquine</td>
<td>12</td>
<td>0.346±0.017</td>
<td>0.326±0.009</td>
</tr>
<tr>
<td>8 days after TPPS$_4$ after 2 doses of chloroquine</td>
<td>12</td>
<td>0.118±0.006</td>
<td>0.082±0.003***</td>
</tr>
<tr>
<td>15 days after TPPS$_4$ after 4 doses of chloroquine</td>
<td>6</td>
<td>0.063±0.004</td>
<td>0.041±0.001**</td>
</tr>
</tbody>
</table>

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, spleen) 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 TPPS4 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 sensitization.

We can conclude that the TPPS4 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 TPPS4 concentration in the serum. During a shorter period (8 days), we found only a small decrease of TPPS4 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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References


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