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The Localisation of $TPPS_4$ in Some Organs and Its Possible Nephrotoxicity in Rats

T. ZIMA[1], M. JIRSA[2], M. JIRSA JR.[2], V. BRADOVÁ[2], J. STEJSKAL[3], J. ŽABKA[2], C. POVÝŠIL[4], M. JANEBOVÁ[1]

[1]First Institute of Medical Chemistry and Biochemistry, [2]First Department of Medicine, First Faculty of Medicine, [3]Department of Pathology, Second Faculty of Medicine, [4]Second Department of Pathology, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

Photodynamic therapy (PDT) is now being used more frequently in carefully selected cases of malignancies. The drugs used for PDT are mostly derivatives of haematoporphyrine (HPD) and its active component photofrine II. Another compound prepared by total synthesis is meso-tetra-(4-sulfonatophenyl)-porphine ($TPPS_4$) but its application in human medicine was rejected because of its neurotoxicity. Our $TPPS_4$ was prepared by the method of Busby *et al.* in the modification of Jirsa and Kakač (1987). This product is purer and without neurotoxic effects. In this study, we concentrated our attention on the effect of $TPPS_4$ on nephrotoxicity and its accumulation in some organs. As the parameters of toxic kidney damage we used urine levels of N-acetyl-beta-D-glucosaminidase (NAG), serum creatinine levels, glomerular filtration rate (GFR) and proteinuria. $TPPS_4$ was administered i.v. in a dose of 25 mg/kg b.w. The animals were observed for 21 days after drug application. Urine and blood samples were collected over 24-hour periods on days 0, 5 and 21. The serum creatinine level was significantly higher only on day 5 (65.0 ± 1.46 μmol/l vs 56.5 ± 2.69 μmol/l on day 0, $p<0.05$). There were no significant changes in GFR, proteinuria or NAG activity in the urine during the experiment. AST serum activity was increased. We determined the concentration of $TPPS_4$ (pmol/mg w.w.) in rat organs on the 21st day after the injection. The concentration of $TPPS_4$ was high in kidneys (30.8 ± 5.5), liver (13.5 ± 2.0), lungs (11.7 ± 4.6) and spleen (9.7 ± 1.5), while the concentration in heart and brain was low. We conclude that $TPPS_4$ has the highest concentration in the kidney 21 days after its administration and does not exert any nephrotoxic effects during this period.

Key words

Nephrotoxicity – NAG – Organ localisation – Meso-tetra-(4-sulfonatophenyl)-porphine ($TPPS_4$) – Renal function – Photodynamic therapy

Introduction

The phenomenon of apparent selective accumulation of parenterally administered porphyrins in tumours has formed the basis for its anticipated diagnostic and therapeutic applications for many decades (Schwartz *et al.* 1995). Several porphyrine derivatives such as meso-tetraphenylporphyrines sulfonated to a different degree are currently being investigated for their possible use as photosensitisers in the treatment of cancers (Moan *et al.* 1987).

Photodynamic therapy (PDT) has recently been more frequently used in properly selected cases of malignancies (e.g. skin and breast cancers). PDT is one of the alternative methods of therapy besides the administration of cytostatics, and radiation or surgical therapy. The drugs used for PDT are mostly derivatives of haematoporphyrine (HPD) and its most active polymer photofrine II.

Synthetic compounds have an exact chemical composition, stability at room temperature and good solubility in water. Meso-tetra-(4-sulfonatophenyl)-porphine ($TPPS_4$) is one of these compounds which was first synthesized by Winkelman (1962), but its application in human medicine was rejected because of its neurotoxicity. Our $TPPS_4$ was prepared by the method of Busby *et al.* (1975) in a modification of Jirsa and Kakač (1987). This product is purer and without neurotoxic effects (Střelečková *et al.* 1995).

The majority of biological photosensitisers acts *via* the singlet oxygen pathway (Jori and Spikes 1984).

As indicators of toxic kidney damage, we used urine levels of N-acetyl-beta-D-glucosaminidase (E.C. 3.2.1.30.) (NAG). This tubular enzyme is considered to be the most sensitive marker. It is a lysosomal enzyme belonging to the hydrolase group and its activity is present in most organs and tissues and also in body fluids.

Damage of various parts of the nephron is combined with a specific elevation of the activity of various enzymes in the urine. It is apparent that NAG and beta-galactosidase are the most frequently used enzymes for the determination of glomerular lesions, damage of the proximal tubules and of renal papilla (Tucker *et al.* 1980, Bourbouze *et al.* 1984).

In this study, we concentrated on the effect of $TPPS_4$ on nephrotoxicity and we measured its accumulation in organs and distribution in the body.

Material and Methods

Experimental arrangement

Female Wistar rats weighing approximately 180-220 g were housed in individual metabolic cages with free access to distilled water and standard rat chow. $TPPS_4$ was dissolved in saline solution immediately before the administration and 25 mg/kg b.w. were injected into the tail vein.

The animals were observed for 21 days after the drug application. Urine and blood samples were collected over 24 hour periods on days 0, 5 and 21. Urine and serum were frozen and examined at the end of the whole experiment.

Analytical methods

Urine was filtered prior to the analytical enzyme assay. The biochemical parameters in the urine and serum were determined using diagnostic kits (Hoffman-la-Roche, Switzerland) on a Cobas Mira analyser (Hoffman-la-Roche, Switzerland).

Fluorometric methods were used for determination of NAG activity in the urine (Haragsim and Zima 1992).

The concentration of $TPPS_4$ in tissues was measured by spectrofluorometric assay (emission 550–700 nm, excitation 416 nm) after extraction with 0.1 M NaOH.

Histology

Rat kidneys, liver, heart, muscles, brain, lungs and spleen were examined by light microscopy after haematoxyline-eosine staining and by fluorescence microscopy (Fluoval, filters BG 12 and G247).

Statistical analysis

We used ANOVA one-way analysis and the Bonferroni p value.

Results

Biochemical results

We examined serum creatinine, total protein, urea, AST, ALT, GFR, the enzyme waste of NAG in urine and proteinuria. Their relative amount correlated with the creatinine level in millimoles to eliminate the influence of various urine volumes (Table 1.). The serum activity of gama-glutamyl-transferase and the concentration of bilirubin were at the detection level of the kit. There were no significant deviations from the normal value during the measurements.

The concentrations of $TPPS_4$ (pmol/mg w.w.) in several organs of rats on the 21st day after the intravenous administration are presented in Table 2.

The kidney had the highest amount of $TPPS_4$, compared to spleen, lung and liver where the concentration was approximately one half of the values found in the kidney. Other organs contained only small quantities of $TPPS_4$. Activity in the brain were on the detection level of the method.

Histological results and localisation of $TPPS_4$ in various organs

No pathological changes in the kidney, spleen, heart, muscle, bone marrow, brain and lung were found in the light microscope. Moderate enlargement of the portal connective tissue with an increasing number of inflammatory cells was found in the liver.

We determined localisation of $TTPS_4$ in rat organs by fluorescence microscopy 24 hours and 21 days after the administration.

In all the examined organs, massive red fluorescence in was found in arteries and arterioles one and 21 days after $TPPS_4$ injection.

Weak fluorescence in the cortical tubuli with no fluorescence in the glomeruli was observed in the kidney. After 3 weeks, the fluorescence in tubuli decreased (Figs 1 and 2).

In the liver, weak fluorescence was found in the portal connective tissue. Hepatocytes displayed only very low green autofluorescence. After 3 weeks, there was a small decrease of fluorescence in the enlarged portal (periportal) connective tissue. Besides the portal region, only sporadic fluorescence of the enlarged connective tissue was present. Kupffer cells contained lipopigment inclusions only (Figs 3 and 4).

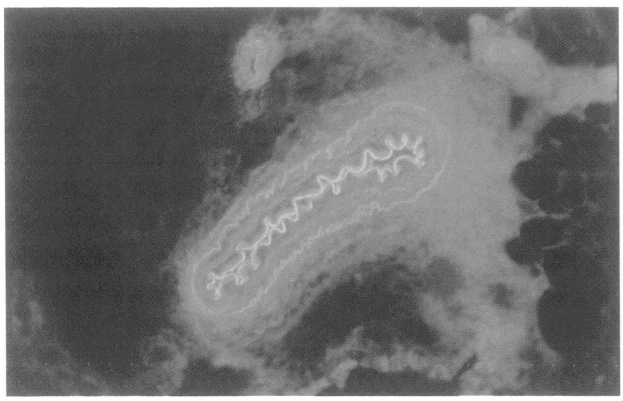

Fig. 1. *Kidney (24 h after i.v. administration of TPPS$_4$) – red fluorescence in arteries and arterioles, yellow autofluorescence of elastic membranes. No fluorescence in glomeruli and small red fluorescence in cortex tubuli.*

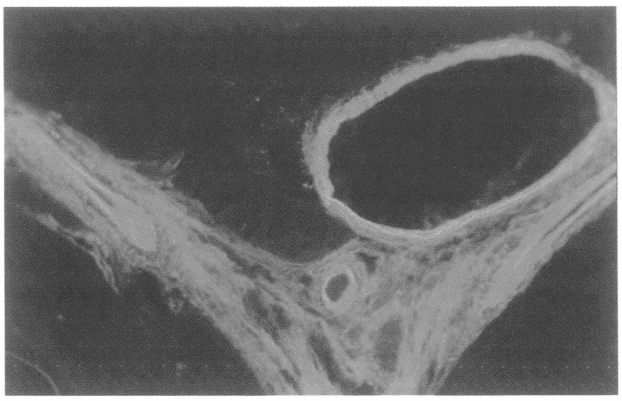

Fig. 3. *Liver (24 h after i.v. administration of TPPS$_4$) – red fluorescence in arteries and arterioles, weak fluorescence in portal connective tissue, hepatocytes show only weak green autofluorescence.*

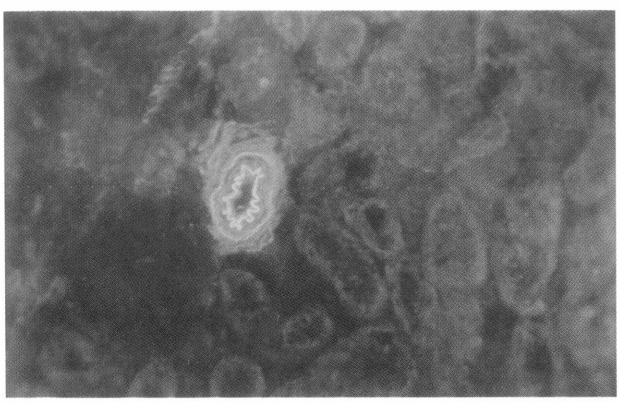

Fig. 2. *Kidney (21 days after i.v. administration of TPPS$_4$) – red fluorescence in arteries and arterioles, weak green autofluorescence in tubuli.*

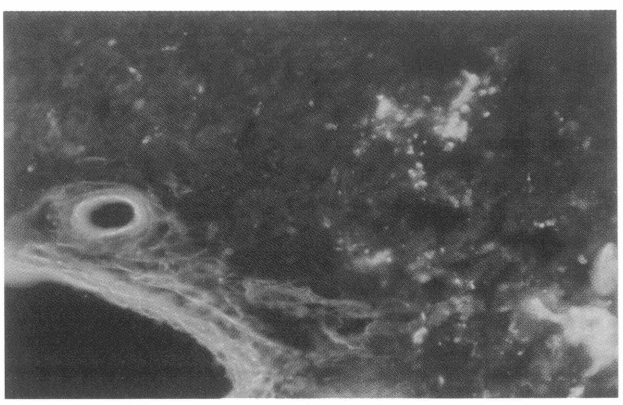

Fig. 4. *Liver (21 days after i.v. administration of TPPS$_4$) – red fluorescence in arteries and arterioles, weaker fluorescence in enlarged portal connective tissue, weak yellow autofluorescence in lipid particles and lipopigment in Kupffer cells. Enlarged connective tissue shows red fluorescence.*

Strong red fluorescence of the capsule and trabeculae was found in the spleen, while the lungs exhibited diffuse fluorescence of the interstitium. In the heart, red fluorescence was present in the vessels, weak fluorescence in the endocardium and none in the myocytes. In the brain, red fluorescence was only found in small vessels of the meninges.

Table 1. Biochemical data in the serum (means ± S.E.M.).

Parameter	N	Time (day) 0	5	21	ANOVA
Total protein (g/l)	8	62.1±1.4	62.7±1.2	65.5±1.2	n.s.
ALT (μkat/l)	8	0.52±0.10	0.33±0.01	0.34±0.01	n.s.
AST (μkat/l)	8	1.26±0.04	$1.55\pm0.05^{+}$	$2.16\pm0.11^{xxx***}$	0.0001
Urea (mmol/l)	8	6.75±0.19	7.28±0.53	7.97±0.40	n.s.
Creatinine (μmol/l)	8	56.5±2.7	$65.0\pm1.5^{+x}$	56.8±2.1	0.05
Proteinuria (g/mmol Cr)	8	1.1±0.4	1.4±0.4	0.9±0.2	n.s.
NAG (nkat/mmol Cr)	8	0.98±0.38	1.61±0.62	0.68±0.27	n.s.
GFR (ml/min)	8	0.63±0.14	0.54±0.19	0.61±0.17	n.s.

Data are means ± S.E.M., $^{+}p<0.05$ difference between day 0 and 5, $^{***}p<0.001$ difference between day 0 and 21, $^{x}p<0.05$, $^{xxx}p<0.001$ difference between day 5 and 21.

Table 2. Concentration of $TPPS_4$ in various organs 21 days after injection

Organ	Concentration (pmol/mg w.w.)
Brain	0.063±0.006***
Heart	4.2±0.8***
Spleen	9.7±1.5**
Lung	11.7±4.6*
Liver	13.5±2.0*
Kidney	30.8±5.5

*N = 8, means±S.E.M., * $p<0.05$, ** $p<0.01$, *** $p<0.0001$ compared to the kidney.*

Discussion

The concentration of $TPPS_4$ in various organs is comparable to the data published by Winkelman (1962). However, we studied this for a longer period after the administration. According to the literature, it was suggested that several porphyrins were selectively localised in tumours and in some organs (Winkelman *et al.* 1967).

$TPPS_4$ in amounts five times higher than the highest therapeutic dose leads to an increase of AST activity in the serum and this correlates with the moderate enlargement of portal connective tissue with inflammatory cells.

It is interesting that the high concentration of $TPPS_4$ in the kidneys has no influence on the glomerular filtration rate or tubular function (no elevation of urine waste of NAG). The accumulation of $TPPS_4$ in the tubular cells can be explained by the low or absent activity of the enzymes which metabolise this drug or by its slow excretion into the urine. Proximal tubuli excrete porphyrines including $TPPS_4$. Several porphyrine derivatives with widely different lipophilicity, including polar $TPPS_4$, were located in extranuclear granules or in desintegrated lysozomes of light-exposed cells (Berg *et al.* 1990). The lipophilicity can be related to the slow excretion from the tubuli into the urine.

We can conclude that this high dose of $TPPS_4$ (25 mg/kg b.w.) was not nephrotoxic and there were no changes in glomerular filtration rate and urine waste of

NAG as an indicator of tubular damage. Compared to other organs studied the highest concentrations of $TPPS_4$ were found in the kidney. The fact that we found no fluorescence in hepatocytes 24 h and 21 days after administration of $TPPS_4$ could be explained by the increased activity of degrading enzymes.

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Reprint requests
T. Zima, M.D., Ph.D., First Institute of Medical Chemistry and Biochemistry, First Faculty of Medicine, Charles University, Kateřinská 32, 120 00 Prague 2, Czech Republic.