Ph.D. Dissertation, Thesis

Cardiovascular effects of Tinuvin 770 in animal model

Dr. Sótonyi Péter

Semmelweis University, Department of Transplantation and Surgery

Tutor: Prof. Dr. Anna Kádár, MD, Ph.D., DSc.

Semmelweis University
Ph.D. School
8/2 Programme

Budapest
2003
1. **Introduction:**

Photostabilisers are plastic additives that can prevent light induced photodegradation of plastics. Hindered amines light stabilizers /HALS/ represents one of the most important groups of additives. A member of this group Tinuvin 770 /bis/2,2,6,6-tetramethyl-4-piperidiny/ sebacate/ is used world-wide to stabilise polyethylene, polypropylene, polycarbonate, polyurethane, polystyrene, polyamide, polyacetyl and acrylonitril polymer products. These polymers are components of plastics used in medical and food industries. Depending on the actual use of plastic products, they can gain access to the body via the gastrointestinal system, transdermally, or through the bloodstream. *In vitro* experimental studies show that Tinuvin 770 has a benzothiazepine /+/-cis diltiazem-like, extremely potent /IC\(_{50}\) values < 10 nM/ L-type Ca\(^{2+}\) channel-blocking effect. Its action is mediated through binding to the phenylalkylamine and benzothiazepine selective domain of the a1 subunit of the receptor. This compound also diminishes the 1,4-dihydropyridine-sensitive Ca\(^{2+}\) uptake. Interestingly, its structure and mode of action are not related to formerly known Ca\(^{2+}\) channel
blockers. Beyond the above mentioned effects, it is a more potent blocker /IC50 values \( \approx 200 \text{nM} \)/ of the neuronal nicotinic acetylcholine receptors. The significance of Tinuvin 770 content of medical industry products is underlined by fact that the molecule can come to direct contact with the circulation (haemodialysis treatment, infusion therapy, transfusion, heart-lung machine, ect.). It is apparent that the interaction between plastics and the human organism may cause a wide range of unexpected reactions like hypotension, nausea, vomiting, dizziness, sometimes loss of consciousness. Decreased blood volume, peripheral vasoconstriction and cardiac factors can be held responsible for the development of these clinical symptoms.

2. **Aim of the Study:**

1. **Analytical examination**: The purpose of this study was twofold: the development of an HPLC method for the detection of Tinuvin 770 from medical plastic materials such as haemodialysis membranes and the determination of the viability of its solid phase extraction from aqueous solution. In our study
we focused on the examination of Tinuvin 770 release from four commonly used haemodialysis membranes.

2. **In vitro cardiotoxicity**: Examination of direct cytotoxicity of Tinuvin 770 on isolated rat cardiomyocytes.

3. **In vivo cardiotoxicity**: Investigate the cardiotoxic effects of Tinuvin 770 *in vivo* on rat model.

4. **Haemodynamic study**: Describe the acute haemodynamic effect of Tinuvin 770 on dog model.

3. **Methods:**

1. Four different types of haemodialysis membranes were analysed: polysulphon, cuprophan, and two types of hemophans. A 4 grams aliquot of each membrane material was soaked in 100 ml of physiological saline solution. After 72 hours dipping isolation was carried out using solid-phase extraction (SPE). The LC-MS system was used in the positive ionisation mode, and we observed the protonated molecule ion (m/z 481).
2. Cardiomyocytes were isolated from Sprague-Dawley rats by Rajs. The cell culture was incubated with 0.9 % NaCl solution containing 25 nmol Tinuvin 770. Three different exposure time was applied (30, 60, 120 minutes). Light and electronmicroscopic analysis were performed. Creatine-phosphate and adenosine triphosphate were determined by standard enzymatic UV methods. For cytochemical detection of Ca\(^{2+}\) lead-acetate method was used.

3. Acute experiments were performed on 12 mongrel dogs (weight: 24?1 kg). After intravenous pentobarbital anaesthesia and intubation the dogs were ventilated with room air by a Cape CV2424 respirator. Arterial blood pressure (systolic, diastolic, mean blood pressure), left ventricular pressure, contractility (dP/dt) cardiac output were measured. Standard surface ECG recording was done to calculate the heart rate and PQ-intervals. In the first phase of the experiments the control incubation was performed using the vehicle (ethanol). Then 3.3, 6.6, 10, 33, 66 and 100 mg doses of Tinuvin 770 (T1-T6) were incubated for 15 min each. (The effective range of doses was defined in preliminary dog experiment: 1 mg – 100 mg.)
4. Fifty Wistar rats, weighing 250-330 grams, were selected into five groups /I – V/, with ten animals in each. The doses were defined on the base of the results of haemodynamic examination. The group I was the control. The Tinuvin 770 doses were injected IP 15 times during a five-week period /three times a week/ : 1 µg for group II, 10 µg for group III, 100 µg for group IV, and 1 mg for group V. Light and electronmicroscopic analysis were performed. For cytochemical detection of Ca^{2+} lead-acetate method was used. Alterations in adrenergic innervation were followed by the glyoxylic acid technique.

4. Results:

1. SPE isolation described previously results in excellent separation of this plastic additive. Under the given conditions the HPLC-MS peak of Tinuvin 770 were detected in both the standards and the samples. This peak was symmetrical, well resolved, and its retention time was reproducible. The HPLC
chromatogram demonstrates the satisfactory result achieved in the analysis of Tinuvin 770, and the obtained data allow qualitative and quantitative analysis.

2. Tinuvin 770 induced decline of rodshaped and viable cells after 30 minutes incubation period and this effect is more expressive after 60-120 minutes incubation process. Toxic injury of Tinuvin 770 to isolated cardiomyocytes induced by an early morphological alterations, plasma membrane belbbing and hypercontraction necrosis. After 90 minutes calcium aggregation was observed in mitochondrias and myofilaments

3. 1 mg of Tinuvin 770 intravenous administration showed any significant haemodynamic changes. After the injection of highest dose of Tinuvin 770 (100 mg) 75% of the animals consumed. Low dose of Tinuvin 770 (3,3-10 mg) cause slight depression of arterial blood pressure with decreased myocardial contractility. Higher dose of Tinuvin 770 (33,3-66,6 mg) lead an expressed fall of arterial blood pressure with additional intense decline of contractility. Cardiac output is relatively constant till the 33,3 mg dose. End diastolic left ventricular pressure stand on a higher value range between 6,6 mg and 10 mg. Heart rate
decreased and PQ interval increased only in higher dose of Tinuvin 770. EC$_{50}$ value = 0.137 mg/Kg body weight, and LD$_{50}$ value = 4 mg / kg body weight were defined.

4. Light and electron microscopic studies of the myocardium showed no pathological changes in group I and II. In group III, small disseminated haemorrhages were presented on the upper third of the interventricular septum at gross examination. On histology, slight, small, focal hypercontraction, interstitial oedema and myocytolysis were found. In group IV, histology showed large amount of red blood cells in the interstice, oedematous sarcoplasm with destruction of myofibrils. The remaining sarcoplasm lined the sarcolemma in an annular fashion. Two other alterations were also myocytolysis and hypercontraction bands was present. In group V, gross examination of the heart showed extensive haemorrhages. Disruptions of fibrils, the filaments were diverged, with overstretched sarcomers. The described histological alterations represent hypercontraction necrosis and damage in the inner mitochondrial membrane and reduced number of glycoprotein granules. Myocyte Ca$^{2+}$ intake was substantially increased with
calcium particles present in the mitochondria. Fluorescent microscopy demonstrated massive, irregular catecholamine release in the nerve terminals of the subendocardial regions.

5. Discussion:

The analytical haemodynamical investigations and the toxicological examinations provided the following results:

- The developed SPE procedure and the MS detection combined with HPLC separation are suitable methods for the analytical detection of Tinuvin 770.
- As the developed procedure has very high sensitivity (LOD=1 ng, LOQ=10 ng), it is suitable for detecting the presence of traces of Tinuvin 770 in minute quantities.
- Tinuvin 770 can unambiguously be detected in the membranes of haemodialysis units.
- Water based solutions (like 0,9% NaCl solution) are capable of eluting Tinuvin 770 from the membranes.
- Isolated cardiomyocyte cell culture system is a suitable model for studying individual cells without interfering exogenous affects.
Tinuvin 770 is capable of inducing irreversible cellular damage on isolated cardiomyocytes in very low concentrations (25 nM). This effect positively correlates with the length of the exposure and becomes irreversible after 120 min.

The toxic effect of Tinuvin 770 is related to its membrane injuring property combined with its pathologic effect on the calcium homeostasis.

The observed reduction of the cellular ATP level results in the impairment of cardiomyocyte relaxation, therefore hypercontraction necrosis develops.

Prominent haemodynamical effects of Tinuvin 770 have been detected in the canine models.

Lower doses (1-10 mg) of Tinuvin 770 reduced myocardial contractility, thus elicited mild blood pressure reduction.

Higher doses (10-60 mg) of Tinuvin 770 do not only reduce myocardial contractility, but also increases the peripheral vasodilation, therefore result a strong blood pressure reduction. Following the administration of 66,6 mg an increase of PQ distance on ECG and a reduced cardiac frequency was detected. The cardiac output was relatively steady up until reaching lethal doses.

The application of 100 mg results in circulatory collapse, 75% of the animals did not survive this dosage.

The subacute toxic effects of Tinuvin 770 were experimentally assessed in a group of rats with a chronic drug administration regime.
Dose dependent myocardial changes developed in the animals. Hypercontraction necrosis and myocytolysis were the main pathological phenomenon in animals treated with doses of 1 ug and 1 mg.

As cytochemical reactions proved, intracellular calcium concentration was dramatically increased following Tinuvin administration. The catecholamine content of the peripheral nerve endings was found to be increased at the same time. The regular structure of the end plate becomes disorganized.

The 1 mg dose was lethal in almost 30% of the animals, proving the toxic effect of chronic high dose Tinuvin 770.

Our results give emphasis to the importance of toxicological and clinical investigations on the effects of Tinuvin 770. The functional similarity of the molecule to known calcium channel blockers provides the rational for its putative circulatory effect during dialysis treatment. Tinuvin 770 therefore might participate in the development of dialysis related cardiovascular disturbance. Besides Tinuvin 770, there are countless synthetic compounds applied in plastic industry that comes to long lasting contact to the human organism. It might be necessary to implicate more comprehensive toxicological screening before industrial licensing as well as to introduce more thorough quality control for currently applied materials.
6. Publications:


7. Acknowledgement

The author wish to thank for Prof. Dr. Anna Kádár, Prof. Dr. Ferenc Perner, Prof Dr. Jeno Járay, for their advocacy and advice. I am very thankful for help to my colleges, friends at the Departement of Transplantation and Surgery, Department Vascular and Heart Surgery, Department of Pathology, Department of Forensic Pathology, and National Institute of Toxicology.

I would like to thank the my family’s love, care and tolerance during this difficult period. Thanks for my wife and my children.