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CONTENTS

REVIEW ARTICLE

CLINICAL UTILITY OF CARDIAC TROPONIN I AND CARDIAC TROPONIN T MEASUREMENTS

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Summary: The measurement of CK-MB remains the test of choice for confirmation or exclusion of AMI and probably will remain the test of choice for routine diagnosis in the near future. Nowadays determination of cardiac troponin T (cTnT) and cardiac troponin I (cTnI) as a method relatively expensive and time-consuming should be restricted to clinical settings that really require their high specificity.

Key words: *Cardiac troponin T; Cardiac troponin I*

Introduction

Soon after the first reports (18,30,44) on serum aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK) activity increase in patients after acute myocardial infarction (AMI), by 1962, the World Health Organization (WHO) included the increase in cardiac enzymes into its criteria for diagnosis of definite AMI (11).

To meet clinical requirements an ideal marker of myocardial injury would (4):

1) be found in high concentration in myocardium
2) not be found in other tissues, even in trace amounts or under pathological conditions
3) be released rapidly and completely after myocardial injury
4) be released in direct proportion to the extent of myocardial injury and
5) persist in plasma for several hours to provide a convenient diagnostic time window but not so long that recurrent injury would not be identified.

Creatine kinase MB isoenzyme (CK-MB) has emerged as the biochemical marker of choice and has become the „gold standard" of biochemical diagnosis of AMI. Recently, CK-MB activity assays have been increasingly replaced by CK-MB mass assay utilizing monoclonal-anti-CK-MB antibodies (10). Although new method for measuring CK-MB isoenzyme considerably improved the analytical sensitivity of CK-MB, there are fundamental problems with CK-MB as a myocardial marker that cannot be solved even by the most sophisticated methods. The CK-MB content of normal myocardium is small, CK-MB is not a heart-specific marker, and CK-MB is detectable in the reference populations (4).

To improve biological specifity, several research groups focused their interest on the use of myofibrillar proteins as markers of myocardial damage. Myofibrillar proteins of striated muscle are expressed as tissue-specific isoforms and consequently these antigens may be differentiated by immunologic methods. Cardiac myosin light chains, cardiac beta-type myosin heavy chains, cardiac alpha-actin, cardiac tropomyosin, as well as cardiac troponin C are coexpressed in slow-twich skeletal muscle fibers (15,16,38,42). Only two candidates for heart-specific markers remain: cTnI and cTnT.

Biochemical aspects

Troponin is a thin-filament associated complex of the myocyte. This complex regulating calcium-dependent interaction of myosin and actin controls muscle contraction. There are three individual components in the complex, each of a single polypeptide chain. They are named after their function. Troponin T (TnT) binds the complex to tropomyosin, troponin C (TnC) binds calcium and then undergoes conformational changes inducing progressive change in the troponin I molecule that inhibits actin and myosin interaction in the absence of calcium (22). Troponin I and troponin T exist in three different isoforms with an unique structure, that is encoded by three different genes: one for slow-twich skeletal muscle, one for fast-twich

skeletal muscle, and one for cardiac muscle (37,45). cTnI has an extra 31 amino acid residues at the N-terminus and its amino acid sequence shows about 40% dissimilarity from both other isoforms (45). cTnT differs only by 6-11 amino acid residues from its skeletal muscle isoforms (37). The troponin C subunit is not currently explored as a cardiac marker because troponin C has only a fast-twich and a slow-twich/cardiac isoform (38). While the molecular mass of cTnI is approximately 24 kDa, the molecular mass of cTnT is about 37 kDa, therefore it is expected to be released rapidly from the injured myocardium (14, 47).

Isoforms of cTnI and cTnT during development

Cardiac troponin I is not expressed during early fetal development in humans. Though the predominant fetal cardiac troponin I isoforms is the slow skeletal TnI and complete transition to cardiac troponin takes place in humans only after birth (after the ninth postnatal month) (12,41), sufficient data exist to presume that cardiac TnI remains the only troponin isoform expressed in the myocardium even during chronic disease process (13,41). It is believed that cTnI is not expressed in human regenerating skeletal muscle but currently no published data exist to confirm this hypothesis.

The specificity of cardiac troponin T for myocardium during ontogenesis has not been fully delineated. Skeletal muscle troponin T and cTnT are coexpressed in fetal heart muscle, with the skeletal muscle form being supressed during ontogeny and reexpressed in stressed human heart (6). In contrast to cTnI, cTnT is expressed in fetal skeletal muscle and is reexpressed in adult rat skeletal muscle after injury and denervation (40).

In patients with left ventricular failure, heart biopsies also show downregulation of troponin gene toward the fetal form. The increased expression of the TnT4 isoform are associeted with a reduction of myofibrillar ATPase and may be an adaptation to the heart failure state, rather than contributing to the cause of failure (7).

Analytical aspects

Since 1989 several generations of enzyme-imunoassays specific for cardiac TnT and for cardiac TnI have been developed (14,47). While the specifity of the first generation of cTnT or cTnI assay using polyclonal antibodies ranged from 97 to l00 % (17,25), the new improved assays with monoclonal antibodies show no crossreactivity with skeletal troponins (28,31). Although these commercially available assays (cTnT - Boehringer Mannheim, Germany and cTnI - ERIA Diagnostics Pasteur, Marnes-la-Coquette, France) are fully automated, they have a turnaround time of over 90 minutes which is too long for emergency determination. That's why more rapid, qualitative, bedside immunoassay for cardiac troponin T using 150 µl of whole blood were developed (8). The basic principle is as follows: cTnT combines with both biotinylated anti-TnT antibodies and gold-labeled anti-TnT antibodies to produce a red or purple line that is read visually within 20 minutes. The positive result is obtained in patients with 0.2 g/l or more of cTnT in their blood (8).

Clinical aspects

Cardiac troponin T

Examination of healthy individuals showed that the normal range of cardiac troponin T is between 0 and 0.1 µg/l (21,32). There are numerous studies correlating the measurement of cardiac troponin T against creatine kinase MB isoenzyme in patients with acute myocardial infarction (21, 32, 34, 39, 50).

Cardiac troponin T concentrations in patients with confirmed AMI showed a roughly biphasic release kinetics, with the initial peak (9.6 - 22.7 µg/l) closely paralleling that of the mass concentration of CK-MB during the first 24 h after the onset of symptoms. The second peak occurs on about the fourth day after admission (21,32,50). Despite a biological half-life of cTnT of 2 h, the diagnostic time window of cTnT is unusually wide, ranging from a few hours to several weeks after the acute episode (32).

The biphasic release profile of cTnT is probably the result of intracellular compartmentation. Katus et al. (26) showed that there is an unbound cytosolic troponin T in ultracentrifuged homogenates of myocardial tissue of different species ranging from 0.013 to 0.036 mg/g wet weight (approximately 6% of the total troponin T). The appearance of troponin T within the first few hours after myocardial infarction is the consequence of a rapid loss of this cytoplasmic pool superimposed on the prolonged myofibrillar degradation that results in a long plateau effect several days after the onset of pain.

The sensitivity of cTnT for the diagnosis of AMI is very high but is time-dependent. The diagnostic sensitivity of cTnT was 50 % after 4 h and 100 % from 10 to 120 h after the onset of pain. Sensitivity of TnT on the seventh day after admission was 84 % (32). Even in patients with small myocardial necrosis, the time course of cTnT concentration contrasted more strongly with the normal range than did myoglobin, CK and CK-MB (32).

Comparison of early and late rises in troponin T concentration following acute myocardial infarction may provide valuable information about infarct size, reperfusion, and response to thrombolytic therapy (27). In patients with early reperfused AMI, a marked peak in troponin T serum concentrations was found at 14 hours after the onset of pain. This early troponin T peak was absent in patients with AMI reperfusion occurring later than 5.5 hours after the onset of pain and in patients with nonreperfused AMI. By contrast, the kinetics of troponin T release after the first day after AMI were unaffected by reperfusion. Troponin T remained elevated in all patients 14 - 21 days after the onset of pain.

Cardiac troponin T vs cardiac troponin I

Mair et al. (33,34,35) compared systematically cTnI with cTnT time courses in a large cohort of AMI patients and concluded that their kinetics of release into systemic circulation are very similar. There are only three important differences: 1) the larger cytosolic pool for cTnT, quoted at 6 % vs the smaller 2.8 % cytosolic pool for cTnI. 2) cTnT was elevated longer than cTnI and was significantly more sensitive on the 7th day after AMI. 3) the biphasic character of cTnT release, which leads to second peak about 4 days after AMI, is more pronounced than that of cTnI.

Recent reports on increased cTnT concentrations in the absence of elevated cTnI levels in patients with chronic maintenance hemodialysis for renal diseases or myopathies (19,23,29,43) suggest that cTnI may be more cardiac-specific in these pacients. However, a final judgement is currently not possible, because in these studies, the old cTnT assay with some residual crossreactivity with skeletal TnT was used. Another possible explanation of this findings is that cTnT is released from poorly perfused skeletal muscle in some critically ill patients (4).

Prognostic significance of cardiac troponin T and cardiac troponin I

The classic role of biochemical markers of myocyte damage in patients admitted with acute coronary syndromes has been retrospective confirmation of myocardial damage. Because cTnI and cTnT do not normally circulate in the blood and are more (for example cTnI 13 times) abundant in the myocardium than CK-MB on a weight basis, the signal-to-noise ratio associated with cardiac troponins is much more favourable for detection of minor amounts of cardiac necrosis missed by other biochemical markers. This ability is very significant because several studies (5,9, 24,36,46,48) have indicated that patients with acute cardiac ischemia in whom myocardial infarction was ruled out, but in whom the cTnT value exceeded a defined cutoff, were at increased risk for frank AMI or cardiac death. Although different cutoffs were used in these studies, metaanalysis by Wu and Lane (49) clearly demonstrated that patients with increased cTnT have fourfold increased odds for an adverse event compared with those patients for whom cTnT was negative.

Based on the available data Hamm and coworkers (24) suggested new classification of patients with unstable angina into a low-risk troponin T negative group and high-risk troponin T positive group [increased cTnT and cTnI concentrations in serum are found in about 30 % of patients with unstable angina Braunwald class III - (39)].

Detection of myocardial damage in neonates

To our knowledge, the data about the diagnostic performance of troponin T in neonates are very seldom. Our study (1) indicates that under physiological conditions almost no myocardial troponin T is present in cord blood of healthy term neonates (<0.1 μg/l) but the concentration increases in neonates exposed in utero to infusion tocolysis with betasympathomimetics (2). The study of Genser et al. (20) as well as our results (3) demonstrated that severe birth asphyxia may also result in myocardial necrosis detectable using cardiac troponin T measurement. Although the specificity of cTnT during ontogenesis has not been fully defined the available data seem to show that cardiac troponin T could become a suitable criterion in laboratory diagnosis of neonatal myocardial damage but this finding deserves further investigation.

Conclusions

To sum up, the measurement of CK-MB remains the test of choice for the confirmation or exclusion of AMI and probably will remain the test of choice for routine diagnosis in the near future. Nowadays determination of troponins as a method relatively expensive and time-consuming should be restricted to certain groups of patients:

1) The patients with concomitant skeletal muscle damage in whom the high specifity of troponins is really required. There are patients for example after defibrilation or after surgical operation.
2) Further determination of troponins can be useful in case of retrospective diagnosis of AMI because the diagnostic window od troponins is wider than CK-MB.
3) The greatest utility of assays for cTnT and cTnI may be in the area of risk stratification of patients with coronary artery disease.

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ORIGINAL ARTICLE

DIFFERENTIAL INHIBITION OF THE BRAIN ACETYLCHOLINESTERASE MOLECULAR FORMS FOLLOWING SARIN, SOMAN AND VX INTOXICATION IN LABORATORY RATS

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Summary: The female Wistar rats were intoxicated (i.m.) with sarin, soman and VX in doses equal to 1xLD_{50} and pontomedullar areas of the brain were prepared, homogenized, centrifuged and in these samples, acetylcholinesterase (AChE, EC 3.1.1.7) activities were determined. In the same samples, AChE was separated using polyacrylamide gel electrophoresis and AChE molecular forms were detected and densitometrically evaluated. In control animals, AChE was separated into four forms differing in their electrophoretic mobility and their quantitative content in the sample. The form with lowest electrophoretic mobility represent the main part of AChE activity constituting the whole enzymatic activity. Following intoxication with the nerve agents mentioned, the whole AChE activity in the pontomedullar area of the brain was decreasing in intervals of ten minutes (soman and sarin) or one hour (VX). The AChE activity at the time of death (or terminal stage) was represented 5-30 % of controls. Molecular forms of AChE were inhibited in different extent: the form with lowest electrophoretic mobility was diminished to zero level while the form with the highest mobility was practically unaffected, independently on the type of nerve agent. From quantitative expression of percentage content of the forms vs their activity we can imply that values of the total AChE activity represent the „mean" activity of the forms determined.

Key words: *Acetylcholinesterase; Molecular forms; Brain; Rat; Sarin; Soman; VX*

Introduction

The threat of the use of chemical weapons not only in military conflicts but also in terroristic attacks is not excluded at present as it was clearly demonstrated in Tokyo (27) and Matsumoto (44) cities (sarin attack in the subway). The very actual group of these chemicals are organophosphates (OP) including nerve agents. The most important nerve agents are represented by sarin (O-isopropyl methylphosphonofluoridate), soman (O-pinacolyl methylphosphonofluoridate) (these two compounds belong to so called G-compounds) and VX (O-ethyl S-2-diisopropylaminoethyl methyl phosphonothiolate) (V-compounds). Moreover, there are produced many organophosphorus compounds in the civilian facilities and used in industry, agriculture, medicine etc. Basic mechanism of action and antidotal treatment for these compounds are in principle the same and therefore some conclusions from this paper can be applied in the civilian medicine.

From the point of pharmacodynamics and therapeutic possibilities, soman represents the most serious poison: its toxicity is comparable with that of sarin and VX (7,8,15,16,35) but therapeutic efficacy of antidotal treatment with present and perspective drugs is not good enough (14,17,23,24,29). This is probably a reason for intensive research dealing with soman intoxication and its treatment.

Soman and sarin are quickly resorbed at all routes of administration including inhalation, percutaneous and oral administration (7) and inhibit cholinesterases (preferably acetylcholinesterase, AChE, EC 3.1.1.7) in the central and peripheral nervous system. Because of high lipophility of soman, it posses high affinity to brain AChE (1,8). Sarin is less lipophilic, however, its affinity to the brain AChE is also very high (8).

Soman and sarin are detoxified in the liver, plasma (21,37), according to some authors also in the lung (34) and therefore this part is excluded from the toxic effect. The losses of G-compounds in the organism are caused also by binding to nonspecific esterases and this part of soman and sarin is not able to cause toxic effect. It was assessed that only 1-3% from the dose administered of the both compounds inhibited AChE in the brain, i.e. 1-3% of the dose administered caused the basic toxic effect (7,22,26,34). Another factor (up to now not very elucidated) influencing soman and sarin poisoning is an existence of a depot in the organism from which the nerve agent can be released and causes new attack of intoxication. This depot was described for the skin, erythrocytes, muscles and lung (8,22). Bearing in mind very low portion of the dose administered causing

basic toxic effect (1-3%) , it is clear that releasing of a very small quantity of sarin and soman can influence significantly survival or death of intoxicated organism independently on the treatment.

On the other hand, V compounds are not detoxified in the organism (7). Probably this is a reason of higher toxicity of V compounds in comparison with G-compounds. The effect of V-compounds (especially VX) is prolonged in comparison with sarin and soman (42). The mechanism of action of VX is inhibition of AChE preferably in peripheral nervous system (8, 28). However, the inhibition of AChE in the brain parts was described to be selective and the most marked in the pontomedullar area of the brain (7,8).

The evidence supporting AChE as the primary site of the both OP and nerve agents action has been summarized by many authors (6,19,28,39). It includes the following observations: symptoms of OP poisoning are similar to those of the AChE inhibitor physostigmine; the *in vivo* LD50 for a variety of OP correlates well with the inhibition efficacy to AChE determined *in vitro;* and ChE reactivators (e.g. oximes), anticholinergics (e.g. atropine) and spontaneously reactivating AChE inhibitors (e.g. carbamates) can reduce OP toxicity. However, despite the model's pragmatic success, a variety of data are inharmonious with AChE inhibition as the only important biochemical event in OP intoxication.

Thus, basic mechanism of action of nerve agents - similarly as for other OPs - is an intervention into cholinergic nerve transmission via irreversible inhibition of AChE and other hydrolases (6,28,39). Monitoring of cholinesterase changes - their development during the intoxication is at present the best reflection of a severity of OP poisoning as well as a reaction to antidotal therapy.

Both enzymes (AChE and butyrylcholinesterase, BuChE, EC 3.1.1.8) exist in multiple molecular forms (2-5,12,25,30,36,38). The activities of these forms are also influenced by many factors. The function of these forms is not known at present, however, their presence in the membrane structures at physiological conditions was demonstrated (5). There are a few data describing the changes of AChE molecular forms following intoxication with highly toxic OPs (25). Some experiments were performed with relatively less toxic OP (4,10,30).

Molecular forms of AChE showed different sensitivity to inhibitors in vitro (4,7,31) and in vivo (11,13,30,40,41). Following DFP (40) and highly toxic OPs (our results, and 25), the form with high molecular weight was the most sensitive. Intoxication with Parathion and Neguvon (less toxic OPs) caused medium inhibition of some forms of AChE (7,13, 43). One can suppose that following determination of the whole AChE activity, a „mean" of activities of the forms could be determined: in case of death, some forms of AChE reached an unmeasurable level, some of them were unchanged: the whole AChE activity was about 10 % of controls. Thus, determination of AChE molecular forms can contribute to more precise diagnosis of OP intoxication.

This approach could improve the therapy of OP and soman poisoning and simultaneously it could contribute to better understanding of cholinergic nerve transmission and thus to better insight to pharmacology and neuropharmacology in general.

Comparison of changes of AChE activity and its molecular forms following soman, sarin and VX poisoning is the aim of this study.

Material and methods

Enzyme source

Female Wistar rats (Velaz, Praha) weighing 170-200 g were used. The animals were killed by bleeding from the carotid artery, the brains were immediately removed, washed with saline and frozen. Then they were thawed and homogenized in an Ultra Turrax homogenizer (Janke and Kunkel, Germany) in distilled water and 0.5 % Triton X-100 to make a 10% (w/v) homogenate. The homogenate was centrifuged for 60 min at 105.000 g and 4°C (MSE, 50 T.C., England) and the supernatant was used as a source of soluble AChE.

Disc electrophoresis

Electrophoresis of the samples (20 μl) in 7,5% polyacrylamide gels was carried out for 60 min at 240 V and 40 mA as it was described previously (5).

Determination of AChE activity

AChE activity in the gel was demonstrated with acetylthiocholine iodide (Lachema Brno, Czech Republic) as substrate by the method described earlier (4,5). Colorimetric assay for AChE activity in the samples without electrophoretic separation was carried out according to Ellman et al. (18), with acetylthiocholine iodide as substrate and 5, 5-dithiobis-2-nitrobenzoic acid (Serva, Heidelberg, Germany) as chromogen. AChE activity was expressed as μmol of substrate hydrolysed per min per g wet wt. tissue or as percent of controls.

Densitometric evaluation

Following staining the gels were scanned on a Vitatron densitometer (Sci. Instr. Eefde, Holland) and the activity was expressed in arbitrary units or as percent of controls.

Inhibition of AChE and its molecular forms by OPs in vivo

Female rats (Velaz) weighing 180-200 g were divided into groups (n = 6). The animals in control group obtained saline, the animals in experimental groups were injected with sarin, soman and VX compound in a dose approximately 1 x LD_{50} (sarin - 0.2 mg/kg, soman - 0.12 mg/kg and VX - 0.05 mg/kg). The animals in both control and experimental groups were killed at different time intervals and supernatants from the pontomedullar area of the brain were prepared as described. The whole AChE activity in samples

removed 1, 3,5 and 10 min (sarin, soman) and 1,5,15,30 and 60 min (VX) after intoxication was determined. Samples removed 5 and 10 min (sarin, soman) and 30 and 60 min (VX) after intoxication were used for electrophoretic separation. The first interval chosen represents developed intoxication, the second one is practically the time of death.

The handling of experimental animls was under the supervision of the Ethics Committee of the Medical Faculty, Charles University, Hradec Králové.

Statistical evaluation

The results were calculated as the means with their 95% confidence limits. The differences between groups were evaluated by analysis of variance. Homogeneity of experimental groups was tested by Bartlett`s test. All calculations were done on a Hewlett Packard 9830.

Results

Normal AChE activity and its molecular forms

AChE activity in the brain homogenate was found to be 6 μmol of substrate hydrolysed per min per g wet wt. of tissue. AChE activity in the supernatant - i.e. activity of the soluble AChE - was approximately 95% of the activity in the homogenate.

Following electrophoretic separation of soluble rat brain AChE, the presence of four AChE molecular forms was demonstrated. They were designated by arabic numerals 1-4. Their percentage distribution was as follows: the forms with the highest electrophoretic mobility (forms 1,2) comprised about 1/5 of the whole activity and the forms with the lowest mobility (forms 3,4) contained the remaining ones, i.e. these forms represent the main part (about 80%) of the whole AChE activity (Table 1).

Table 1: Percentual distribution of the AChE forms according to densitometric evaluation

form	1	2	3	4
%	8.0±2.4	10.1±2.8	33.9±3.5	48.0±3.1

Inhibition of the total AChE activity

The course of intoxication as well as a decrease (i.e. inhibition) of AChE activity was very fast for sarin and soman - within ten minutes the brain AChE activity reached the zero level (Fig. 1). On the other hand, following intoxication with VX, the time course of AChE inhibition was prolonged and reached to unmeasurable activity within one hour (Fig. 2). It was possible to calculate the half-lives of AChE inhibition using semilogarithmic transformation where the dependence of AChE activity changes vs. time gives the straight lines (Fig. 3).The half-life values were as follows: 24.1 ± 3.8 (VX), 3.1 ± 0.56 (sarin) and 2.2 ± 0.48 min (soman), respectively.

Fig. 1: Changes of AChE activities following intoxication with soman (S1) and sarin (S2). The results are means only, for errors see Table 2.

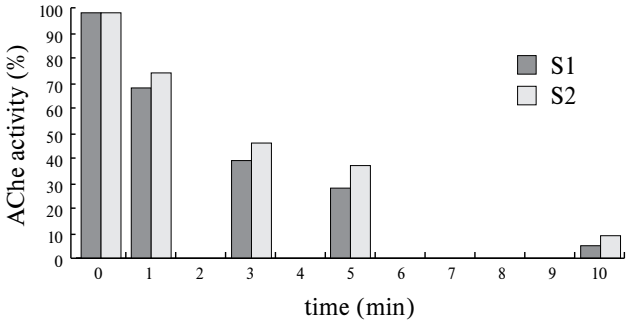
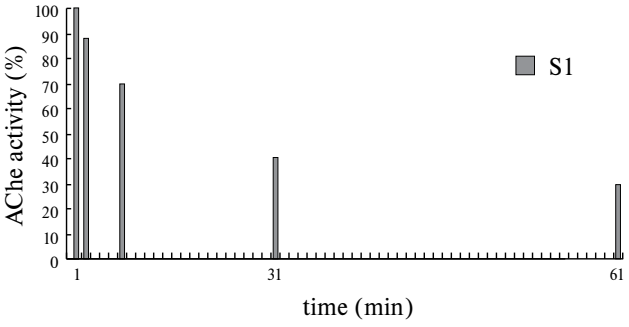


Fig. 2: Changes of AChE activities following intoxication with VX (S1). The results are means only, for errors see Table 2.



Inhibition of AChE molecular forms

The changes of AChE molecular forms were studied at time of developed intoxication and at time very near to death. These changes are summarized in Table 2.It is clear that the most sensitive was the form 4 having the lowest electrophoretic mobility. Its activity is reaching the zero at the time of death. On the other hand, the forms with highest electrophoretic mobility (forms 1 and 2) were relatively resistant to the inhibitory effect of OPs used (Table 2).

Table 2: Changes of total AChe activity and its molecular forms following sarin, soman and VX poisoning

time	Total AChe	form 1	form 2	form 3	form 4
	SARIN	**8**	**10**	**34**	**48**
min	**% control**	**% control**	**% control**	**% control**	**% control**
5	39.0±1.4	93.3±6.8	35.5±4.5	70.0±4.9	9.2±2.5
10	10.0±1.8	87.7±4.6	18.7±5.4	14.0±3.2	3.0±1.8
	SOMAN				
5	25.0±3.5	95.5±5.4	31.2±4.0	44.0±2.6	8.2±3.9
10	5.2±1.1	80.3±7.6	13.0±5.1	9.3±0.6	1.5±2.1
	VX				
30	40.2±4.1	94.3±5.8	70.0±4.9	62.2±4.1	13.2±4.9
60	29.7±2.4	94.3±5.3	64.8±5.0	39.8±3.4	5.3±2.2

For this reason, a distribution profile of different forms was drastically changed and the main part of the whole activity was represented by forms 1 and 2 as it is demonstrated in Fig. 4. However, this change was relative only because the calculation of percentage distribution was made using the values decreased.

Fig. 3: Changes of AChE activities following intoxication with sarin and soman. Semilogarithmic transformation.

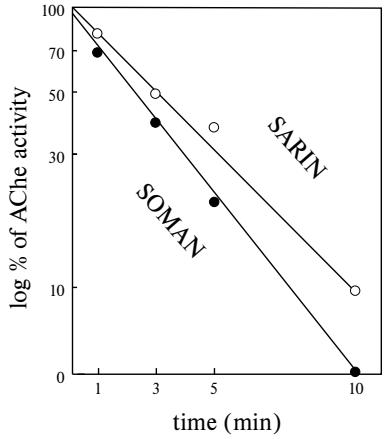
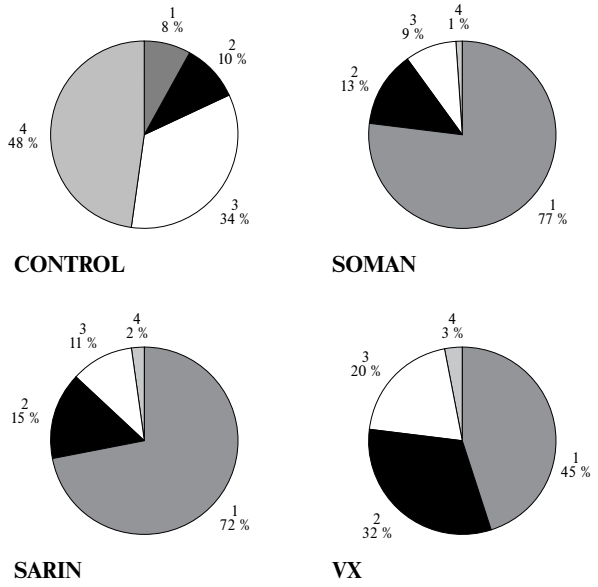


Fig. 4: Changes in relative distribution of the AChE forms following sarin, soman and VX intoxication.



When the values are expressed as the activity for each form as 100%, it is possible to compare real values of AChE activity determined for the unseparated enzyme and calculated (as weighted means) values of different molecular forms (Table 3). It is demonstrated that real and calculated values are in very good agreement differing from 0.1 (minimum) to 6.1 (maximum) %. It means that really determined AChE activity is corresponding to distribution of AChE molecular forms and therefore, the whole activity is a „mean" of the activities of AChE molecular forms.

Table 3: AChe activity and its molecular forms following sarin, soman and VX poisoning: real and calculated values

time	Total AChe		form 1		form 2		form 3		form 4	
	SARIN		8		10		34		48	
min	REAL.	CALC.	% cont.	% total	% cont.	% total	% cont.	% total	% cont.	% total
5	39.0	39.1	91.3	7.3	35.5	3.6	70.0	23.8	9.2	4.4
10	10.0	15.1	87.7	7.0	18.7	1.9	14.0	4.8	3.0	1.4
	SOMAN									
5	25.0	29.6	95.5	7.6	31.2	3.1	44.0	15.0	8.2	3.9
10	5.2	11.3	80.3	6.4	13.0	1.3	9.3	3.2	1.5	0.7
	VX									
30	40.2	41.9	94.3	7.5	70.0	7.0	62.2	21.1	13.2	6.3
60	29.7	30.0	94.3	7.5	64.8	6.5	39.8	13.5	5.3	2.5

Discusion

It appears from our results that inhibition of the brain AChE by G compounds (sarin and soman) is very fast reaching to 50% activity within minutes. For VX, there is a delay and decrease of AChE activity was observed after more than twenty minutes. The half-lives are very dependent on the dose of the agent administered, on the species and other factors and therefore it is difficult to compare our results. In general, inhibition of AChE in vivo is faster for G-compounds in comparison with V-compounds (1,8,25).

From our results describing multiple molecular forms of AChE can be concluded that AChE in the brain exists in molecular forms. These forms were observed also by other authors (3,5,12,13,31,40,43). These forms are different for various species. However, the electrophoretic mobility of AChE components from the rat, rabbit, mouse and human brains suggested that there are generally two types of AChE forms having high and low molecular weight. One BuChE and two AChE bands in the rat hippocampus after electrophoresis in polyacrylamide gel were observed (41). The distinction between the two AChE forms is difficult without electrophoresis. They differ in electrophoretic mobility and they can be well differentiated by electrophoresis.

Subcellular localization of AChE suggested that in nerve ending particles and microsomal fractions 2 - 4 AChE forms are present, in the mitochondrial fraction only one was detected (4). The microsomal form absent in the mitochondrial fraction is the most sensitive to OPs in vivo. From previous studies is known that high molecular form of AChE has the lowest K_m value (2) and the highest decrease of this component after deafferentation was also observed (3). These results suggested that this form of AChE would be very important for normal cholinergic nerve transmission. It arises the question on existence of the forms under physiological conditions. Using thermal dena-

turation, it was demonstrated that they are not artifacts formed during homogenization or other treatment of the brain tissue (5). These findings were also described by other authors (2,5,23,25,33,41,43). The overall data show that catalytic activity of AChE molecular forms is different and their inhibition by various inhibitors may be heterogeneous. This heterogeneity was demonstrated for AChE phosphorylating inhibitors as well as for inhibitors with different binding sites for the enzyme.

The results with another type of inhibitor - 7-methoxytacrine (7-MEOTA) fit well with our previous findings indicating a greater sensitivity of slowly migrating molecular forms separated by polyacrylamide gel electrophoresis (10). In fact, it has been demonstrated recently that slowly migrating forms of cortical AChE correspond to G_4 forms separated by sedimentation analysis (43). On the other hand, recent data indicate an almost equal sensitivity of G_4 and G_1 forms of both soluble and membrane-bound whole brain AChE to this type of inhibitor (31). It is not excluded that the reversible inhibitors such as 7-MEOTA modify its interaction with the active site resulting in a preferential inhibition of G_4 forms. It is of interest that the introduction of a heptyl group into physostigmine modified its interaction with the AChE molecular forms, heptylphysostigmine showed a stronger inhibition for G_1than for G_4 forms while in the case of the parent compound similar inhibition of the 2 forms was observed (31).

The data of 7-MEOTA are different from those obtained for DFP and paraoxon showing similar IC_{50} values for G_4 and G_1 forms (43). These findings have been confirmed for membrane-bound AChE (31). This is not surprising since the interaction of OP compounds (and physostigmine) with the active site of enzymatic molecule is different from that for 7-MEOTA-type compounds. In fact, OP compounds inhibit AChE by phosphorylating the esteratic serine in the catalytic site. On the other hand, acridine derivatives bind to the hydrophobic area close to the active site of AChE simultaneously affecting its catalytic center via an allosteric mechanism (20,32,38).

As regards the data on AChE molecular forms, they confirm previous findings indicating a more pronounced sensitivity of G_4 forms, as compared to G_1 forms, in brain of rats injected with paraoxon (43). Somewhat lower inhibitory effects of the same dose of paraoxon (0.25 mg/kg s.c.) as well as a somewhat lower contribution of G_4 forms to total AChE activity in untreated rats were observed in another experiments (11) in comparison with those reported by Volpe et al. (43) and may depend on regional differences (cerebral cortex and whole brain).

In the case of brain AChE, as has been pointed out (43), G_4 and G_1 forms represent distinct pools in the cell, the former being mainly associated with membranes, with its catalytic site exposed to the extracellular space, and the latter confined to the intracellular compartment.

It appears from our results that following intoxication with nerve agents studied the highest sensitivity for high molecular AChE form was observed. Determination of the whole AChE activity is partly misrepresenting because of different distribution of AChE molecular forms in the sample. Following determination of the whole activity, a „mean“ activity containing activities of the forms is determined. It can be concluded that in studies requiring high sensitivity (e.g. the studies of antidotal action), AChE molecular forms could be of choice for more detailed information on functional stage of AChE - important marker of cholinergic nerve transmission.

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ORIGINAL ARTICLE

THE EFFECTS OF SUBCHRONICAL EXPOSURE TO SO_2 ON BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN GUINEA PIGS

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Summary: The effects of subchronical exposure to SO_2 (400ppm, 3 hours daily, 28 days) on biochemical and hematological parameters were investigated in guinea pigs. Mostly no significant changes in the values of biochemical parameters and no significant changes in hematological parameters were found. The levels of investigated ions (K^+, Na^+, Cl^-, Ca^{++}, Mg^{++} and phosphates), proteins (albumines, globulines, total proteins), enzymes (LD, ALT, AST, CK) and other biochemical parameters (urea, creatinine, bilirubin) were not significantly different between groups, with the exception of a significantly higher ALP concentration in the exposed group as compared with controls (2,17 μkat and 1,85 μkat, respectively). It can be concluded that a subchronical exposure to sulphur dioxide mostly did not induce any definite changes in biochemical and hematological parameters in guinea pigs.

Key words: *Sulphur dioxide; Subchronical exposure; Biochemical parameters; Hematological parameters*

Introduction

Environmental pollution is one of the most overwhelming contemporary problems and sulphur dioxide is one of the most common atmospheric pollutants in the Middle Europe region. Sulphur dioxide and various particles are emitted into the atmosphere by the burning of fossil fuels and smelting of metals. Many of these particles can promote the conversion of SO_2 to the more irritant sulphuric acid (1,10). Inversion, fog, and cold temperatures also support the negative consequences of exposure to SO_2 (2).

The influence of exposure to SO_2 on the respiratory tract has been demonstrated in various studies (4, 11, 13). Sulphur dioxide is a soluble gas which is readily absorbed in the nose and upper respiratory tract. SO_2 dissolves in the fluid lining the airway with the production of sulphite and bisulphite ions. These may react with low molecular weight disulphide groups in proteins. Excretion of sulphur absorbed as SO_2 occurs via sulphate, sulphate is produced by the conversion of sulphite being catalyzed by oxidative enzymes (5). Until now, only limited informations about the effects of SO_2 on the other body systems are available (genotoxical effects of SO_2 and increase of lung cancer mortality have been described - 6, 14). The aim of the study was to investigate the effects of the subchronical exposure to SO_2 on the biochemical and hematological parameters in guinea pigs.

Materials and methods

Male guinea pigs (BFA) with an average weight of 500g were used. The standard laboratory conditions were respected. The handling of experimental animals was under the supervision of the Ethics Committee of the Medical faculty, Charles University, Hradec Králové.

Two groups of animals were used. The experimental group (n=12) of male guinea pigs with an average weight of

500g was subchronically exposed to SO_2 (400ppm, 3 hours daily, 5 days in a week) for 28 days. Exposure to SO_2 was realized in special chambers, in each one 8 animals could be exposed together. The control group (n=11) was „sham" exposed to the air in the same time.

Exposures were conducted in 0,278 m^{-3} stainless steel and glass exposure chambers designed by Drew (3). The supply air was filtred and controlled for temperature and humidity, and flow rate 100 L/min was established by the main exhaust pump. Sulphur dioxide for calibration (Linde Technoplyn) was used for exposures. Nominal and analytical concentrations were determined daily. Analytical concentrations of sulphur dioxide were determined by spectrophotometric method described in unificated methodologies for determination of harmful compounds in air (7).

The noninvasive polygraphic cardiac recordings of systolic time intervals were measured using a polygraph Biomedica C6b (Italy) in ketamine anaesthesia (150 mg/kg i.p., Narkamon 5%, Léčiva, Czech Republic) at the beginning of the experiment (7 days before the exposure) and then weekly in the 7th, 14th, 21st and 28th day of the exposure (3 hours after the end of the exposure to SO_2 or after the „sham" exposure). Index PEP:LVET was calculated on the basis of simultaneous recordings of the electrocardiogram, phonocardiogram and carotid pulse waveforms (these results are not demonstrated in this paper).

At the end of the experiment (i.e., after the last polygraphic measurement, on the day 28 of the exposure), anesthesia was enhanced by urethan (20% solution, 0,65 ml/100g i.p.) and blood samples were taken from *v. cava caudalis* for biochemical and hematological analysis. After the sacrifice of the animal, the tissues (heart, muscle, lungs, liver) were taken for histological examination. Biochemical parameters (in plasma/serum) were determined with standard biochemical methods using an automatic analyzer Hitachi 717, Japan. Hematological parameters were determined using analyzer Coulter T890, U.S.A.

Statistical evaluation of values was performed using an unpaired t-test (comparison of different groups) for the level of significance ($p \leq 0.05$). Values are expressed as mean ± S.E.M.

Results

Biochemical parameters:

Almost no differences in biochemical parameters (Tab. 1) between the SO_2 exposed and control group were found, although in most of them the tendency towards an elevation could be observed in the former group. The levels of investigated ions, proteins, enzymes and other biochemical parameters were not significantly different, with the exception of a significantly higher ALP concentration in the exposed group as compared with controls (2,17 μkat and 1,85 μkat, respectively).

Table 1: Biochemical parameters

Parameter	SO_2	Sham
glucose (mmol/l)	9,31±0,37	8,13±0,79
sodium (mmol/l)	136,6±21,04	134,5±60,69
potassium (mmol/l)	9,65±0,84	9,13±1,00
chloride (mmol/l)	104,77±0,76	102,89±1,02
calcium (mmol/l)	2,53±0,05	2,40±0,03
magnesium (mmol/l)	1,36±0,06	1,35±0,03
phosphate (mmol/l)	2,77±0,15	2,44±0,13
urea (mmol/l)	13,07±0,67	12,06±0,56
creatinine (μmol/l)	54,15±2,41	55,67±2,58
uric acid (μmol/l)	108,31±7,38	99,33±7,76
bilirubin (μmol/l)	0,00±0,00	0,00±0,00
LD (μkat/l)	6,31±1,09	7,53±1,07
ALT (μkat/l)	1,22±0,08	1,11±0,10
AST (μkat/l)	2,31±0,32	2,32±0,46
CK (μkat/l)	32,68±11,38	26,96±8,82
ALP (μkat/l)	2,17±0,10*	1,85±0,09
cholesterol (mmol/l)	1,01±0,073	0,99±0,09
triglycerides (mmol/l)	0,95±0,22	0,75±0,05
protein (g/l)	46,41±1,05	45,86±0,86
albumin (%)	30,92±0,63	31,13±0,52
ealb	0,59±0,01	0,59±0,01
α1 globulin	0,03±0,00	0,02±0,00
α2 globulin	0,26±0,01	0,27±0,01
βglobulin	0,07±0,00	0,14±0,07
γglobulin	0,05±0,01	0,04±0,00
a/g quotient	1,45±0,04	1,37±0,11

LD lactate dehydrogenase
ALT alanine aminotransferase
AST aspartate aminotransferase
CK creatine kinase
ALP alkaline phosphatase
a/g quotient = albumin/globulin quotient
* statistical significant difference ($p \leq 0.05$) between groups

Hematological parameters:

No significant differences were found in hematological parameters (white blood cells count and white blood picture, red blood cells count, hemoglobin, hematocrit and thrombocytes count) between the guinea pigs exposed to sulphur dioxide and the control group and the observed non-significant changes did not exhibit consistent trends (Tab. 2).

Table 2: Hematological parameters

Parameter	SO_2	Sham
leucocytes (10^9/l)	4,45±0,36	4,16±0,21
erythrocytes (10^{12}/l)	5,38±0,16	5,53±0,09
hemoglobin (g/l)	140,36±5,27	145,90±3,73
hematocrit (ratio)	0,43±0,01	0,43±0,01
MCV (fl)	79,10±0,82	79,00±0,79
trombocytes (10^9/l)	573,9±129,70	560,70±45,57
eosinophils (%)	0,27±0,14	0,50±0,31
basophils (%)	0,09±0,09	0,10±0,10
monocytes (%)	5,36±1,42	5,90±0,86
lymphocytes (%)	57,5±44,26	62,60±4,91

MCV mean cellular volume

Discussion

Almost no significant differences in the values of biochemical parameters and no significant differences in hematological parameters were found after the exposure of guinea pigs to SO_2 (400ppm, 3 hrs daily, 28 days). Therefore, it can be concluded that a subchronical exposure to sulphur dioxide mostly did not induce any definite changes of parameters studied in guinea pigs. These results are in accordance with the conclusions of the investigation of the subchronical effects of SO_2 on the cardiac function (where only mild changes were found, 8). Previous experiments (investigating the effects of an acute exposure to SO_2) revealed, on the other hand, more frequent and - though mild - significant changes in the followed-up biochemical and hematological parameters as well as in parameters of cardiac function. The influence on the respiratory system following acute exposure to SO_2 (disposition to artificially induced cough and airway reactivity to histamin were significantly enhanced - 12) has also been demonstrated. On the base of mentioned differences between subchronical and acute exposure to SO_2 an adaptation of the organism to some effects of SO_2 exposure in guinea pigs can not be excluded.

Acknowledgements

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ORIGINAL ARTICLE

FENFLURAMINE CHALLENGE TEST IN OBSESSIVE-COMPULSIVE DISORDER - FIRST RESULTS

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Summary: Obsessive-compulsive disorder is a chronic psychiatric illness, affecting up to 3% of the general population, to the midle of 60-th it was supposed to be untreatable. Antidepressant pharmacotherapy is one of the treatment alternatives today. We compared efficacy and safety of citalopram versus clomipramine (serotoninergic antidepressants) in 6 weeks in double blind therapy of obsessive-compulsive disorder. The second objective was to compare prolactin response to a fenfluramine challenge test before the treatment of patients and after 6 weeks of the treatment. In a sample of 14 patients we confirmed significant therapeutic response after 3 weeks of pharmacoterapy, better in obsession than in compulsion. We found low level of adverse effects in the first week of therapy - dry mouth, anxiety, nausea, somnolence, tremor, and sexual adverse events. There were no changes in the laboratory, test EEG, and ECG examinations. Fenfluramine challenge test showed statistically significant decrease of prolactine levels 1 hour after administration of fenfluramine. It was not observed after six weeks of the therapy. Statistically significant negative correlation between prolactine plasma levels at the 6-th hour after administration of fenfluramine and obsession item of YBOC Scale was showed after the 3-rd and 6-th week of the therapy. The correlation was not observed for compulsion item YBOC Scale. Side effects observed during and after the challenge test were anxiety and nervousness and gastrointestinal problems, lasted from 1 hour to 10 hours. These preliminary result could support the idea, that obsessions and compulsions have not necessary the same biological background. The challenge paradigm appears to be a possible way to clarify the pathogenesis of OCD. Our study will continue.

Key words: *Obsessive-compulsive disorder; Fenfluramine*

Introduction

Obsessive-compulsive disorder (OCD) is a common, chronic psychiatric illness, affecting up to 3% of the general population. In spite of the high relatively prevalence, relatively few patients seek the treatment.

There are two alternatives of the therapy. The first one is a psychotherapy, especially cognitive behavioral therapy, the second one is pharmacoterapy. From 1967 is known that antidepressant drug clomipramine may be more succesful than other agents (2).

Recent evidence has led to the hypothesis that abnormalities in serotonin metabolism play a significant role in the pathogenesis of OCD. Clomipramine was the first antidepressant with serotoninergic activity higher than other antidepressants in 60-th and 70-th (16). Today new potent antidepressants - serotonin selective reuptake inhibitors (S.S.R.I.) have demonstrated antiobsessional and anticompulsional efficacy similar to clomipramine in a number of studies (3,9,17,21).

The results of studies employing neuroendocrine challenge tests sugest that serotonin receptors may be altered in OCD too (5,6). The serotonin hypothesis receives support from a paper by Lucey et al. (12), who compared the prolactin response to d-fenfluramine, a serotonin releasing agent. The blunted prolactin response after d-fenfluramine was seen in the patients group.

Fenfluramine (dextro isomer of n-ethyl-alfa- methyl- 3-fluoromethyl-phenethylamine) is a indirect serotonin (5-HT) agonist in the brain, a stimulator of the release of prolactin from the anterior pituitary gland through a serotonin mechanism and an indicator of the ability of the activation of the central serotonin system.

Fenfluramine test is studied as a marker of obsessive-compulsive symptoms and a predictor of a therapeutic response to a pharmacotherapy in OCD (1).

In the double-blind study we compare citalopram (potent S.S.R.I) versus clomipramine (first and classical drug in this field) in the therapy of obsessive-compulsive disorder with the aim to evaluate their efficacy and safety. The objective is to compare prolactin response to a fenfluramine challenge test before treatment of OCD patients by serotoninergic drugs, after 6 weeks of treatment and in a small group after 6 months of follow up.

Material and methods

The study group consisted of 14 patients (9 male, 5 female, mean age 33,7 years, ranging from 22 to 52 years). The patients, who were at least 18 years old and met the ICD-lO criteria for OCD for 6 months or longer and scored minimum 18 at YBOC Scale, were considered for participation. Women who were pregnant, lactating, or not using reliable contraception were not be enrolled. Other exclusion criteria included significant concomitant physical disease, depression with 17 item HAMD total score more than 22, suicidal tendency, a history if seizure or organic brain disorder, substance abuse.

Patients who met inclusion criteria signed Informed Consent. Physical examination and medical work up (including ECG and blood laboratory tests) were provided. After one week of wash-out period the double-blind study was started. Psychiatric examination included YBOCS (Yale-Brown Obsessive-Compulsive Scale) (4), HAMD (17 item Hamilton Depression Scale) (7) and CGI (Clinical Global Impression). EEG examination was made in the first day of double-blind medication, before the first dose of the active drug. The next one was made after 21 days and the last one after 42 days. Side effect occurrence was observed daily in the first week, than in the rating days. Five patients continued study till the 6-th month, when these examinations were made again.

Fenfluramine challenge test and blood samples

The first fenfluramin test has been carried out day before the treatment was started. The patients were in fasting and nonsmoking state over the night, 60 mg of d-fenfluramine (Isolipan caps.) was given per os at 6 A.M. immediatelly, after the first blood sample.

The second test has been done after 42 days therapy. In five patients after 6 months too.

Blood samples for the assessment of prolactin levels were taken at 6, 7, 8, l0, 12 A.M. from a peripheral vein.

The patients were asked about feelings and adverse effects during and after the test.

The levels of prolactine have been assessed by immunoluminescence method (5).

Medication

Identical capsules containing either citalopram 20mg or clomipramine 75mg. Under double-blind condition one capsule has been served once daily at 12 A.M. According to the result of examination in day 21 it could (no change or worsening in YBOCS) be increased to two capsules a day (at 8 and 12 A.M.). The medicacion was started the first day after fenfluramine challenge test.

Results

14 patients were randomized to double-blind treatment with citalopram (N=7) or clomipramine (N=7). The code was not opened yet.

YBOCS evaluation showed significant improvement (scale score decrease) from baseline in the end of the third week of therapy. This decrease was greater in the end of the sixth week and a trend continued till the sixth month. Obsession item improvement is faster in the beginning, than compulsion one. See fig.l, 2, 3.

Fig. 1: Yale-Brown Obsessive Compulsive Scale (Y-BOCS); average total sample score (N=14/day 0 - 6th week/; N=5/6-th month/)

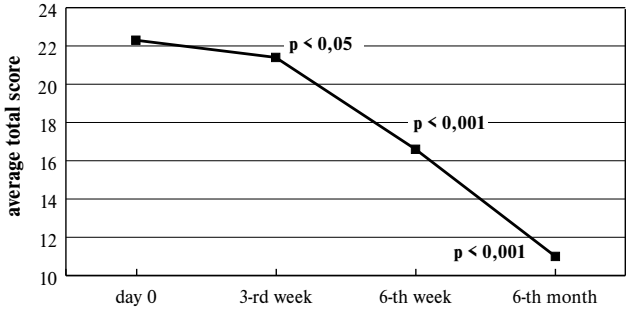


Fig. 2: Yale-Brown Obsessive Compulsive Scale (Y-BOCS); average obsession item score (N=14/day 0 - 6th week/; N=5/6-th month/)

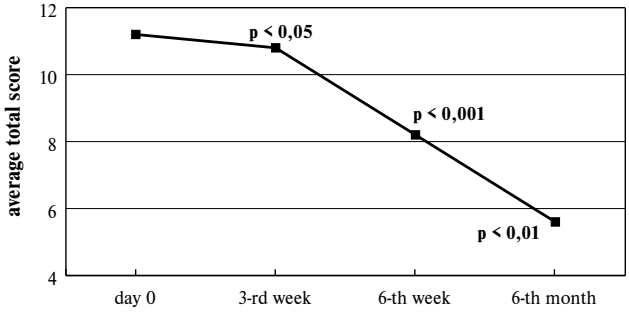
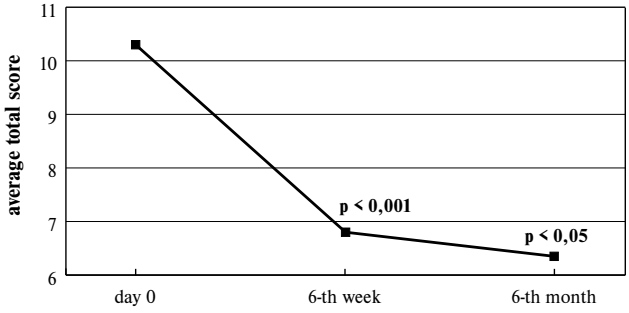


Fig. 3: Yale-Brown Obsessive Compulsive Scale (Y-BOCS); average compulsion item score (N=14/day 0 - 6th week/; N=5/6-th month/)



HAMD evaluation indicated statistically significant improvement in the end of sixth week, trend continued till month 6. See fig.4.

CGI scale showed significant improvement begining the end of the sixth week, in the case of 5 patients in follow up till month 6, see fig. 5.

Fig. 4: Hamilton depressive scale; average total sample score (N=14/day 0 - 6th week/; N=5/6-th month/)

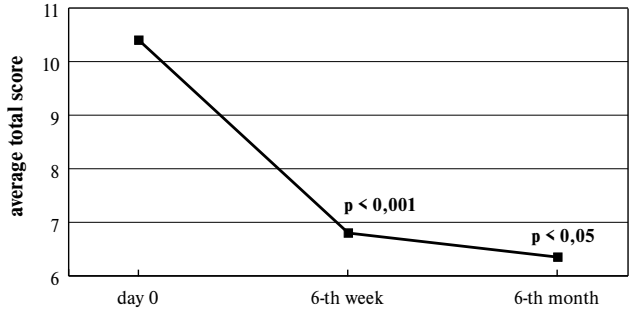
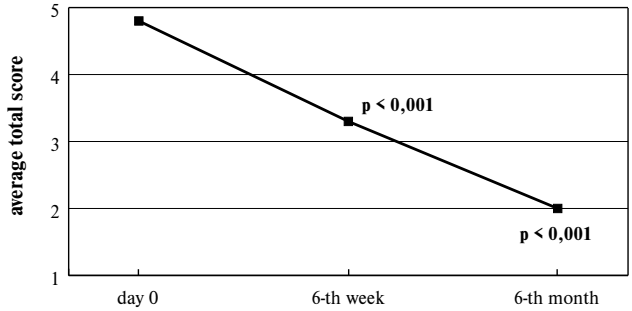


Fig. 5: Clinical global impression; average total sample score (N=14/day 0 - 6th week/; N=5/6-th month/)

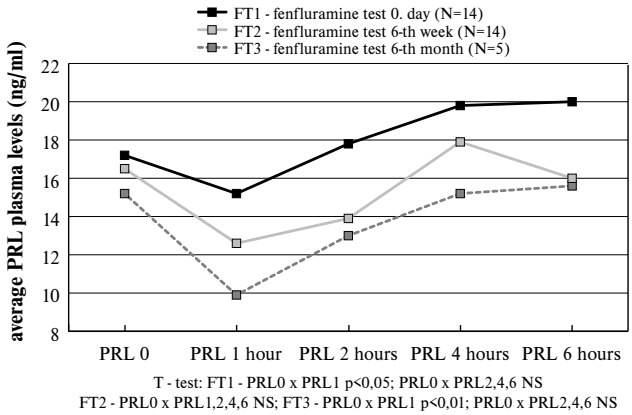


Side effects in the first therapy week were dry mouth (N=2), anxiety (N=2), nausea (N=2), somnolence (N=2), tremor and sexual adverse events in l case. After three weeks of therapy they were: dry mouth, anxiety, somnolence, tremor and sexual adverse events in one subject. In the sixth week dry mouth, tremor and sexual side effects lasted in l case.

Laboratory blood, EEG and ECG examination were not changed in the six weeks of study, neither in a group of five patients after 6 month of therapy.

Fenfluramine challenge test showed statistically significant decrease of prolactine levels in the 1-st hour after admission of d-fenfluramine. It was not seen ater six weeks of therapy (T-test), see fig.6.

Fig. 6: Fenfluramine test; prolactine (PRL) levels before and after 60 mg d-fenfluramine admission



Statistically significant negative correlation between prolactine plasma levels in the 6-th hour after the admission of d-fenfluramine and obsession item of YBOC Scale was showed after the 3-rd and 6-th week of therapy (multiple rergession analysis) as well.

Side effects during and after the test were observed: anxiety and nervousness in 2 cases, gastrointestinal problems in l case. They continued from l hour to 10 hours.

Discussion

Fenfluramine is a serotonin (5-HT) agonist agent. Because fenfluramine enhances the release of 5-HT and also inhibit re-uptake of 5-HT it stimulants both pre- and postsynaptic receptors. Challenge studies with fenfluramine in OCD are rare, but some available evidence indicates, that it could be a „window" to the functional status of 5-HT system in OCD (20). A blunted plasma prolactin response to fenfluramine challenge (more in female) was reported in patients group by Hewlett and Martin (8). Lucey and coworkers (12,14) found blunted prolactin response to fenfluramine challenge in patients group. Controversial results were reported by Hollander (9) and Price (19). They found identical prolactin response to fenfluramine in OCD patients and controls.

The abnormal prolactin response is not confirmed to OCD. Similar blunted responses to fenfluramine challenge have been reported in depressed patients (12,15). Considering the fact that the prolactin response to fenfluramine is mediated through 5-HT receptors - subtype 2A and 2C, it is conceivable that these receptor subtypes are involved in the pathogenesis of OCD (13). The notion of altered sensitivity is, not easily explained in therm of a super- or subsensitivity. A behavioral response to m-chlorophenylpiperazine, a $5\text{-HT}_{2A/2C}$ agonist could be indicative of supersensitivity of those receptors (6,l8). On the other hand in studies in which a blunted prolactin response to challenge with fenfluramine was found, these findings could be interpreted as an evidence for subsensitivity of the same subtype of receptors (10,ll). A reason for the lack of consistency in the behavioural and neuroendocrine results of challenge studies in OCD may be that the dysfunction possibly is confined to a specific subgroup of OCD patients, we could not diverse in exploration, whereas clinical studies have been on all OCD patients population (9,14,22). Other reasons for that fact could be the multiple influences to challenge test, for example sex, age, level of anxiety and/or stress (21).

Our results are preliminary, in a small sample of patients, it is reference from the first year of study. We consider interesting result of negative correlation of prolactine plasma levels and obsession item of YBOCS. Respecting small number of patients it could support the idea, that obsessions and compulsions have not necessary the same biological background. We can not answer the question of predictive value of fenfluramine challenge tests yet. The larger sample of patients is necessary.

Our results demonstrate that citalopram is as effective as clomipramine in the treatment of patients with OCD. We confirm safety of both drugs, there were low level of side effect and no laboratory, EEG or ECG changes. Results of fenfluramine challenge test do not show predicting value in a sample of 14 patients. The group of 5 patients followed for 6 months is too small to make any conclusions.

The challenge paradigm appears a way to clarify at least the part of the pathogenesis of OCD, although not many consistent findings have been reported yet. The papers described above do support the 5-HT theory of OCD.

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ORIGINAL ARTICLE

CHILDREN PAIN DURING DENTAL TREATMENT

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Summary: This research work was done on the set of 69 children and adolescents 6-14 years old at the children's department of the dental clinic, university hospital in Hradec Králové. We found their expectancy of dental pain inadequate to reality: 67 % children overestimated expected pain, 12 % underestimated it. It does not see that children feelings prior to very performance would signalize in advance how much unpleasant or painful the dental procedure is going to be. We have not found any significant difference in either understanding the instruction or sticking to them, or general cooperation of children. The average time interval of dental procedures fluctuated between 18 and 40 minutes, children were not given any anesthetics (with exception of two cases of extractions) which could be one of the causes of distress. From all the chidren 35 % experienced pain in the dental chair and were able to assess it by VAS and verbally characterize its quality. According to the view of children assessing the subjectively experienced pain intensity there exist two types of dental procedures: the first type being represented by painless but demanding patience procedures, the second group of painful treatment (making fillings or extractions). There were no statistical difference between girls and boys in their experiencing pain but there was some difference between girls and boys as went for an approach of health workers: these much more often tried to support girls.

Key words: *Child; Adolescent; Dental treatment; Distress; Pain intensity; Pain quality; Expectancy of pain; Social support*

Chalange

Dental treatment represent for most of our population quite an unpleasant and sometimes even uneasy matter. This attitude is being established and fixated by both the health workers and parents or non-professional public, as well. The painfulness of some dental treatment with negative emotional experience following some treatment are being much talked about ever since the childhood. There exist only few studies, which establish the ways how the children experience and manage various types of dental treatment. Experience of children fear from treatment procedures and strategies of coping with the stress situations in dentistry have already been described (3), as was the experience of anxiety and pain caused by dental treatment (1,2,4,5). This study of ours tries to answer the question if dental treatment typology according to demands and painfulness of treatment procedures for a child patient can be created, and if so, how these different types of dental treatment are experienced by children.

Set

Altogether 69 children within the age span 6 to 14 years who attended Department of paediatric dentistry of University Hospital in Hradec Králové were involved. From this number there were 34 boys (49%) and 35 girls (51%). Dental care was demanded from various reasons: preventive check-ups, fissure sealing, making filling and even extractions. This set has not been collected randomizely; inquiry was done one afternoon every 2 weeks. Only those children who attended the department mentioned above within 3 months period were involved.

As far as the sex of children is concerned, this set of ours is relatively homogenous. Chi-square test showed that boys and girls did not differ even in age stratification (value of chi-square criterion was 0,1957, p=0,9068).

Methods

In our research we used standardized interview, which followed an original questionnaire. Prior to the children questioning an agreement of parents, or in older children also their agreement, was obtained. The cooperation was refused by 1 child only, though his parents had agreed.

Interview was taking place in the waiting room and was done by an experienced high school graduate. Before a child entered the dental office we investigated: mental conditions (what are his/her usual feelings when attending

the dentist, what are his/her feelings today), anticipation (what is he/she expecting to be done by dentist and what pain does he/she expect).

After treatment accomplished the interview went on. We searched for the actual pain experience intensity and quality, strategy usually spontaneously used by children to manage pain and also a degree of support from the health workers (how much doctor and nurse tried to help him/her in pain managing).

The health workers were asked to identify the person who had actually treated (doctor, medical student), then to elucidate the character of treatment, state the length of treatment and assess the level of child cooperation.

Most of the followed variables were rated within the five level scale. In addition, the actual psychic state was further assessed according to nine level range of a sketched child face from joy to crying. Correlation of verbal and picture methods for finding out the actual psychic state was higher (Spearman's ro reached 0,61, $p<0,05$). The pain intesity was evaluated by children with help of visual analogue scale.

From statistical methods mostly non-parametric procedures: Chi square test, Mann-Whitney's test, Spearman's coefficient of rank correlation.

Aims and hypotheses

The main goal of this study was to describe and analyse mutual relations among these following variables: sex, age, type of treatment , children experience of stress situation, intensity and quality of children pain, pain expected, and pain experienced. Then we wanted to create the typology of dental treatment from the view of their painfulness for children and to map time demands of individual types of dental treatment and correlate these demands to experienced paedodontists and dental students.

Hypotheses:

H_1: Boys and girls will differ according to ways of experiencing and mastering the dental stress situations. Girls are more emotional and thus they will have less favourite indices.

H_2: Boys and girls will not differ in types of treatment. Dental disorders bringing children into the dental office do not depend on sex.

H_3: Children (regardless to sex) will differ in types of treatment according to their age. Each age group has its own sphere of dental disorders.

H_4: Individual dental treatment can be classified into four groups according to demands and painfulness for the patient. These groups will differ in their time demands, experienced pain intensity, chidlren's going through the whole situation, degree of cooperation of children and social support provided by dental staff.

H_5: Pain expectations of children prior to dental treatment will not be adequate to the pain really experienced. Most children will overestimate the pain, minority of them will undervaluate it and only a very small number of children will be correct in their expectations. Adequacy of the pain expected and that experienced will depend on the type of treatment, which means on the dental disorders bringing the child in dental office.

Results

First we would like to point out the differences between boys and girls in their experiencing the dental treatment and their ways of coping with stress situations on the dental chair. Details are given in Tab 1.

Tab. 1: Relation of sex to selected characteristics of children experiencing and behaving.

child versus	sex chi-square criterion level	statistical significance	
usual feelings prior to dental visit	4,3930	0,3554	-
actual feelings in the waiting room (verbally)	3,4403	0,4870	-
actual feelings in the waiting room (drawing)	6,1764	0,5193	-
expectations of unpleasant treatment	0,5301	0,9705	-
understanding to health workers instructions	2,2881	0,3185	-
following these instructions	2,0023	0,3675	-
cooperation with dental staff	2,3357	0,5057	-
social support from health workers	5,7576	0,0164	$p<0,05$

According to Tab. 1 the first hypothesis has not been confirmed. There are no statistically significant differences either in statements concerning facing the stress situations or in statements of health workers how the children cooperate on the dental chair.

The only statistically significant difference is represented by different behaviour of health workers to girls and

boys on the dental chair. Significance of this difference was studied also by Fischer exact bilateral test and difference was confirmed (p=0,0463). Health workers more often tried to calm down *girls* who were thus being helped in coping with the stress situation. In boys the manifestation of health workers' support was rarer. Personnel clearly supposed that „man has to stand something".

The second hypothesis has been confirmed. There was no difference between boys and girls as concerned the types of dental treatment done (value of chi-square was 0,1269; differences unimportant, as p=0,7216).

Even the third hypothesis has been confirmed. Children, regardless to sex, differed as for the types of dental treatment procedure (criterion chi-square was 15,8319 and differences were significant on level p=0,0147). Different types of treatment used to be also differently time consuming. As the complexity of dental treatment usually grows together with advancing age, also the time demands of treatment were bigger. Differences are statistically significant (chi-quadrate value of 25,4961; difference being statistically significant on level p=0,0126). Tab. 2 shows how much time a child of certain age category spent on the dental chair.

Tab. 2: Duration of dental treatment with regards to age of children (cumulative frequencies in percentages).

age categories	duration of treatment in minutes						
	0-10	11-20	21-30	31-40	41-50	51-60	61-85
6-8 years	8,7	60,9	91,3	91,3	95,6	100,0	-
9-11 years	0	36,4	59,1	77,3	77,3	95,5	100,0
12-14 years	8,3	33,3	54,1	58,3	87,5	91,7	100,0

From Tab. 2 it can be seen that treatment procedures lasted (according to child age) for a relatively long time. This fact thus represents quite an important source of stress for children that is not always being mastered well. In the age category 6-8 years only 61% of dental treatment were completed within 20 minutes; in category 9-11 years about 60% of them lasted less than 30 minutes and in the age category 12-14 years about 60% of treatment did not take longer than 40 minutes. From these data we can conclude that relatively great part of children stayed in dental chair for much a longer time period. Maximum time interval in our set was 1 hour 25 minutes.

As far as the fourth hypothesis is concerned we postulated four groups of dental treatment (in detail given in tab 3). First group was represented by treatment that by dentists are considered as not painful at all. In the second group there belonged long lasting treatment, but also (according to dentists) not painful, though demanding a certain degree of child patience. The third group was represented by restorative procedures that most of the population consider to be painful.

In the last group there were included extractions without or with anesthesia that may be connected with painful experience for children.

Tab. 3: Taxonomy of dental procedures from the view of their possible painfulness.

1. Easy, not painful treatment: 91070 preventive check up 92216 topical application of fluoride 91030 clinical examination 91130 teething check up 92102 oral hygiene check up
2. Long lasting treatment, not painful but demanding the child patience: 92103 fissure sealing 92130 tooth reconstruction 92204 crown fracture reconstruction 92208 crown fracture reconstruction with composite materials
3. Restorative procedures: 92201 one surface filling 92202 two surfaces filling 92203 three surfaces filling 92241 root canal treatment 92242 root canal treatment of immature permanent tooth 92252 primary teeth restorations
4. Extraction with or without anesthesia: 91510 topical anesthesia 91520 local anesthesia 93101 simple extraction of a loose tooth 93111 extraction of tooth

First we were interested in if children expectations *prior* to the treatment itself marked a certain treatment, if subjective feelings in advance signalled the discomfort or pain connected with the very dental treatment. As the tab. 4. shows it does not seem to be any significant connection present. Children's feelings did not differ either generally or actually in the waiting room. There were *not existing* any important differences in children population as for understanding the instructions, their observing, as well as for cooperation of children with health workers, regardless to any dental treatment named within the four groups above, which represented rather a pleasant surprise. Level of child cooperation with health workers in variously painful treatment procedures was tested also by the help of analysis of dispersal but no significant differences were found (p=0,5965).

Tab. 4: Experiencing the four types of dental treatment by children

type of procedure versus	chi-square criterion level	statistical significance
common feelings prior to visit the dentistry	1,2059	0,871 -
actual feelings in the waiting room (verbally)	2,2785	0,6847 -
actual feelings on the waiting room (drawing)	4,3173	0,7426 -
health workers instructions understanding	2,5700	0,2767 -
instructions following	0,9479	0,6226 -
cooperation with health workers	2,6177	0,4554 -
social support form health workers	0,0523	0,8192 -

On the contrary, an unpleasant surprise was the fact found on the last line of tab. 4. It informs us that the health workers handled the child always in the same way, regardless to type of treatment . Saying in other words: they *did not differentiate* when supporting the child and helping him to master the situation, no matter what degree of pain this child may have experienced. The tab. 4. generally does not seem to support our fourth hypothesis.

Furthermore, we searched for what degree of our proposed typology of dental treatment was actually valid. How much it really differentiated the pain intensity which was being subjectively experienced by children in various types of treatment procedures. Results are given in tab. 5.

Tab. 5: Differences in intensity of pain experienced according to various types of dental treatment

type of treatment	No of children treated	intensity of pain experienced	standard error of mean
1. (easy)	19	3,31	3,57
2. (long lasting)	17	3,11	3,77
3. (making filling)	21	11,95	3,39
4. (extractions)	11	19,91	4,68

Table 5 shows that hypothesis of four types of dental treatment ought to be corrected. Man-Whitney's non-parametric test confirmed this suggestion (see tab. 6).

Tab. 6: Differences between the suggested types of dental treatment concerning the subjectively experienced children pain

compared types of dental treatment	values of T criterion	statistical significance
1. versus 2.	38,5	0,1351 -
1. versus 3.	88,0	0,0028 p<0,01
1. versus 4.	49,5	0,0034 p<0,01
2. versus 3.	56,0	0,0948 -
2. versus 4.	31,5	0,0390 p<0,05
3. versus 4.	72,0	0,4969 -

As for the children there were not existing four types of painfulness as we supposed, but two only. The first group contained the 1st and 2nd types postulated, second, more painful group of procedures consisted of 2nd type postulated (making filling) and 4th type (tooth extraction).

From the dentists point of view, as well as from the parents' one, there exist several interesting data showing how *time consuming* the individual types of dental care were (see tab. 7).

Tab. 7: Time demands of individual types of dental treatment.

type of treatment	No of children	average time of treatment in min.	standard deviation	time min max
1. (easy)	19	18,42	12,02	5 60
2. (longlasting)	17	35,88	18,58	15 85
3. (fillings)	22	40,45	13,88	20 70
4. (extraction)	11	14,09	13,00	15 60

Data given above are of practical importance as they illustrate the time demands in paediatric dentistry, that rather differ from time demands in adult patients. Nevertheless, the rate tariff of dental treatment considers these differences are non-existent. It is supposed that time needed for an adult cooperative patient and that for a child patient, who usually has to be persuaded to cooperate, is almost identical.

In this connection we have also found quite interesting differences in the length of treatment procedures either by well experienced doctors or by medical students. As our research was conducted in the Teaching Hospital during the morning hours most of the treatment were done by students. The average period of time needed for well-experienced doctors (nine persons) was 25 minutes (s=14,14), for students (59 persons) this time average reached 31,44 minutes

(s=17,50). If we consider the time interval needed by doctors to represent 100 %, then medical students needed about 26 % longer time. Also this finding shows the necessity of special regimen in scoring the educational dental departments. Neither this aspect is being considered by rate tariff.

There remains the fifth hypothesis concerning the relation between pain expected and pain really experienced by children. Pain was expected by 46 children, which means by 67 % of children from this set of ours. Pain really experienced was confirmed by 24 children (35 % of our set). The remaining part of the children group did not mention any pain.

It can be generally stated that the correlation between pain expected and that experienced on the dental chair is of a mean tightness. Spearman's ro reaches 0,46 and tightness of this relation is important on level $p<0,05$.

Differences between the expected and real intensity of pain were studied by index C_{21}. This index is characterized by following quotation:

$$C_{21} = \frac{\text{pain experienced - pain expected}}{\text{pain expected}} \cdot 100\ (\%)$$

What are the results?

In 63 % of our children set the expected level of pain has *not been confirmed*. From all the set 51 % of children experienced the pain *smaller* than expected. Reality for them was thus more pleasant, less demanding, than they had prepared themselves for, than they had expected.

In 37 % of children the expectations have been *confirmed*. Altogether 33 % expected that they would not experience any pain and it really was so. Three children (4 %) had estimated the pain intensity well, they experienced the same level of pain as they had expected. In these cases of right estimation we have not found any relation to age (children were 7, 8 and 13 years old).

Generally speaking, the fifth hypothesis has been proved. The expectations were usually far from reality. Most children overestimated the possible pain, then there is a group of realistic estimations and, finally, only a small group of children underestimating the pain.

We have also compared the set of children who underwent painful procedures (3rd type - making filling and 4th type - extractions) with number of children who declared the subjectively felt pain during all types of performances. The painful treatment was done to 32 children, subjective pain experience was stated only by 24 of them. Children most probably experienced the pain in various ways; some of them might be able to modulate it (see the 3rd and 4th types of treatment), other were more sensitive to the whole situation and felt a slight pain even during the treatment of the 1st or 2nd types that could not, according to dentists, be painful at all.

What does the pain experience depend on? Mostly on the type of treatment , though not explicitly. Correlation with the type of treatment is of a mean tightness (Spearman's ro is equal to 0,43, $p<0,05$). There may exist children who undergo even a more serious treatment procedure without the pain experience. On the other hand, there are children experiencing pain even during less serious intervention. Pain experiencing most probably depends also on the child age (connected with the specifics of their dental disorders bringing them to the dental office).

Nevertheless, it doesn't seem that pain experienced would correlate much with emotional states of children in a waiting room, as judged from the children faces drawings (Spearman's ro reaching 0,18 only).

Up to now we were interested in the *intensity* of the experienced pain only. But also the characteristics of the pain *qualities* are important. Taking no account of pain expected, the pain experienced during the dental treatment was admitted by 24 children (35 % from the whole set). We asked them to describe this pain.

From these 35 % of children 3 % of patients were not able to describe the pain verbally. The remaining 32 % at least tried to. About 10 % stated only the aspect of pain *(unpleasant, mild, normal, great, horrible)*. The remaining 22 % selected some sensoric aspects of pain: *it was tingling, hummimg like a humble* (2 children)*, pain was stinging, pricking like the bee sting or the needle prick* (4 children)*, it was stabbing* (4 children)*, it was a pressure pain* (6 children)*, burning* (*1* child)*, scratching* (1 child), *universal pain* (1 child).

Conclusions

This research work has concentrated on relations between the types of dental treatment, children's stress situations experiencing, the expected pain and the pain really experienced. Children from our set were not given any anesthetics (with exception of two cases of extractions). This lack of anesthesia represents the main difference from foreign dental sets of patients (Milgröm et al,1994 and others). In our set of children 6 - 14 years old we found:

1) There were no statistical differences between girls and boys either in their interpretations of experiencing stress situation, or in health workers reports concerning the children patients' cooperation.
2) There was some difference between girls and boys as went for an approach of health workers. These much more often tried to support and calm down *girls*, helping them to master stress situation. In the group of boys the social support from the side of health workers was rarer.
3) Boys and girls did not differ from the view of dental treatment performed. Dental complaints bringing children to the doctor did not differ accoridng to sex.
4) Children (regardless to sex) differed in types of treatment done according to age. Each age period brings a special complex of dental complaints.

5) Dental treatment in one child took tens of minutes. Such a treatment is thus to be considered for a stress situation that may not be succesfully mastered by each child.
6) It does not seem that children feelings prior to *very treatment* would mark a certain treatment procedure , that their subjective experience would signalize in advance how much unpleasant or painful the dental treatment is going to be. Children feelings do not differ either generally, or in the waiting room. We have not found any significant difference in either understanding the instructions or sticking to them, or general cooperation of children, no matter what type of treatment was done. Our conclusion that health workers *did not differentiate* in their social support of children should be considered as quite a strucking one. The workers did not change their approach, no matter how painful the treatment might have been.
7) The suggested typology of treatment procedures according to painfulness for a child has not been confirmed. From the view of children who assessed the subjectively experienced pain intensity there exist rather two than four types of dental treatment. The first type is being represented by painless and time consuming procedures , that are also demanding patience and children cooperation. The second group involves more painful treatment - making filling and extractions.
8) This research has brought new, unknown data concerning the time needed for individual types of treatment. The average time interval fluctuated between 18 minutes (simple, not painful treatment) and 40 minutes (making filling). Data found illustrate the time demands in paediatric dentistry, being so much different from the adult dentistry. This also is not being followed by rate tariff. This research of ours also confirmed the differences between well experienced doctors and medical students as far as it went for the consumed time. Medical students spend about 26 % time more, than experienced doctors. Neither this situation is being accounted for by rate tariff.
9) Pain expectancy in majority of children was inadequate to reality. Children mostly overestimated expected pain (67 % of all the children), the second group expected about the real intensity of pain (37 %) and only 12 % underestimated it.
10) From all the children 35 % experienced pain in the dental chair and were able to assess it by visual analogue scale. If asked to characterize the *quality* of pain most of the children did it from the sensorial aspect, minority of them used the evaluating aspect. The affective aspect was not used at all.

The method used for assessing the children pain proved to be acceptable during normal business hours of the dental department.

It brought the knowledge that - according to our opinion - it is able to enrich paediatric dentistry with new aspects.

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THE HISTORY OF DEPARTMENT OF UROLOGY AT MEDICAL FACULTY OF CHARLES UNIVERSITY IN HRADEC KRÁLOVÉ

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Assoc. Prof. P. Morávek (1942)

Foundations of the Department of Urology

The history of the Department of Urology in Hradec Králové is unequivocally connected with the name of Academician Jan Bedrna. The roots of its history can be traced to Bedrna's appointment as the Head of Surgical Department in Hradec Králové. Associate Professor Bedrna (pupil of Professor Petřivalský) had gradually grewn up to become an outstanding, rather versatile surgeon. From the very beginning of his medical career he was interested mainly in urology, especially in children's urology, as he expressed in his book „The Child's Urology". This book hasn't lost any of its value even after 45 years. Being a forethoughtful, progressive surgeon, Bedrna was wise enough to foresee the necessity of segregating the individual specialized surgical fields, and that is also why he established an independent urological ward within the surgical department of the hospital. This act represented laying the foundations of the independent urological department.

Bedrna was not only the pioneer of the idea of independent urology in Hradec Králové, but also propagator of the separation of urology as a medical branch, when being a member of the Czechoslovak Surgical Association in the early fifties. Following a lot of stern discussions a committee for urological problems was constituted in 1953 and two years later an independent section was founded with Professor Neuwirt as Chairman and Academician Bedrna as Deputy Chairman.

Shortly after World War II, in November 1945, the Faculty of Medicine was established in Hradec Králové as a branch of Charles' University in Prague.

An important point in the Faculty development was the year 1951 when it became a Military Medical Academy. While being controlled by the Army some new lecturers, such as Šváb, M.D (1951-1992), Stefan, M.D. (1952-1960, later Head of the Paediatric Surgery Department), Navrátil, M.D. (1954-1990), and Jeřábek, M.D. (1955-1992) were acquired for the Academy. When mentioning all these famous names, last but not least, I would like to remind the external postgraduate Zvara, M.D. (1953-1955), whose supervisor was Academician Bedrna himself. Professor Zvara later became Head of the Department of Urology in Bratislava.

The next step, significantly influencing the establishment of an independent department of urology, was represented by transformation of Military Academy of Medicine back into a civilian Faculty in the year 1958. Other highly educated lecturers started working there. In 1959 Konečný came to the department (later Head of the Urological Department in Havířov) and in the year 1961 Pokorný came.

Establishment of the first University Department of Urology in Bohemia and the initial period of its activities

The fast development of urology as a scientific branch, its high quality within the Surgical Department in Hradec Králové and, of course, the heritage of Professor Bedrna were decisive. On March the lst, 1962, according to the decision of the Minister of Health Service Plojhar, Th.D., with the agreement of the Minister of Education, the **first University Department of Urology** in Bohemia was founded.

Prof. J. Šváb, M.D., Ph.D. The first Head of the Department of Urology

Prof. Šváb, M.D. became the Head, Navrátil, Jeřábek, Konečný and Pokorný formed the staff.

In the year 1967 Konečný left the Department and new people, seriously interested in urology, came: Baše M., M.D. (1968-1973), Baše J., M.D. (1969-till now), Morávek, M.D. (1971-till now), Navrátilová, M.D. (1974-till now), Navrátil Jr., M.D. (1976-till now), Hiblbauer, M.D. (1976-1995)

and Zita, M.D. (1984-1995). At that time there were 31 hospital beds there. Associate Professor Pokorný, the senior lecturer, leaved in 1975 and became Head of the Urological Department in Plzeň.

When in the year 1983 Professor Šváb retired, his longtime colleague, Professor Navrátil, who also acted as the Dean of the Medical Faculty, became Head of the Department of Urology.

The following period of Department existence in the new building

The next stage began when the Department moved into a new Surgical pavilion in 1985. It was named after Academician Bedrna. Nowadays, our Department has 69 hospital beds (55 for adults, 14 for children). The Department of Paediatric Urology within the Department of Urology was established in 1986 and J. Baše became the leading surgeon. New doctors have come to the Department since that time: Novák, M.D. (1987-till now), Janoušková, M.D. (1987-till now), Rýdel, M.D. (1987-till now), Kutílek, M.D. (1987-till now), Šístek, M.D. (1990-1995) and Louda, M.D. (1992-till now).

The reason for such a staff expansion was a necessity to amplify the team because of the increasing number of beds and also as the out-patient care and complement were extended. Nowadays, at the Department there are special X-ray and endoscopical rooms, operating and ESWL rooms. The Department has been fully equipped with modern devices and appliances, the diagnostics and treatment are generally considered to be of a high quality and comparable with the best Czech and even European departments.

Department priorities in the field of medical care, pre- and postgradual tuition and publications activities

Moreover, there are some priorities in medical care and prevention of the Department of Urology in Hradec Králové, springing not only from recent days, but also from the previous times. In the year 1953 the first suprapubic transvesical prostatectomy with primary closing of the bladder in the Czech Republic was carried out in Hradec Králové. In 1956 the first ileocystoplastics in our country and in 1961 the first kidney transplantation in Middle Europe were performed. Our Department was among the first ones in this country where antireflux operations, operations for bladder exstrophy, resection pyeloplastics, kidney resections, nefrectomy with resection of the lower caval vein because of the tumorous thrombotic process and retroperitoneal and pelvic lymphadenectomy in the presence of prostate and testicle carcinoma have been performed. Lately, radical prostatevesiculectomy and orthotopic continuous derivation of urine with creating an ileal bladder after cystectomy were carried out. Also the Transplantation Centre has developed successfully. It was established in the year 1978 and since 1988 it has been working under heading of Navrátil, Jr., M.D. Even the modern treatment of urolithiasis (ESWL) is prosperous, too.

Parallelly to medical care, the Department is being fully involved in the pregradual tuition of students, postgradual lessons for doctors and scientific research, as well.

Three doctors, members of our Department, have become Professors (Šváb, Navrátil, Stefan), three Associate Professors (Pokorný, Morávek, Baše) and 9 doctors have reached the scientific titles Ph.D.

The scientific papers published by the Department workers have dealt with kidney resection, surgery of suprarenal glands, using the small intestine for replacing the bladder (Šváb), kidney cancer surgery, tumorous thrombotic process in the lower caval vein (Navrátil, Morávek, Baše J., Navrátil Jr.), treatment of testicle carcinoma (Baše), diagnostics and treatment of prostate cancer (Morávek), etiology of bladder cancer (Pokorný), transplantology and endoprothetic aids in urology (Navrátil Jr.), and surgery of serious inborn defects - exstrophy, epispady, etc. (Stefan).

The next period of Department activities after the change of the social system

The last stage of the life of our Department is being connected with a change of the social system in the year 1989. Prof. Navrátil, M.D. has retired, and after a short period of substituting, the senior lecturer Baše, M.D. took the office of the Department Head. Novák, M.D. became Head of the Department of Paediatric Urology. He had certain experience in paediatric urology and, moreover, which was very important, indeed, the previously retired Professor Stefan, M.D. has become his colleague and teacher. Paediatric Urology had always been Stefan's hobby and his decision to devote some time to the tuitioning and practising it again was positively accepted by hospital authorities. Prof. Šváb,

Medical staff on the Department of Urology in the year 1975.
Navrátilová - Baše - Morávek
Pokorný - Jeřábek - Šváb - Navrátil - Hofman

who had been working as a consultant till the year 1992, has left the Department for his native town Bardějov, where in 1996 he celebrated his 80th birthday anniversary. I am sorry to state that Jeřábek, M.D. who had been working as a retired doctor at the out-patient departments in Hořice and Nová Paka, suddenly died in 1996. Hiblbauer, M.D. and Zita, M.D. started their private practices in 1995. They were gradually replaced by new doctors: Veselský, M.D. and Prošvic, M.D. in 1994, Broďák, M.D. and Pacovský, M.D. in 1996. In Autumn 1995 the Associate Professor Baše left his post of the Head of the Department because of serious health reasons.

In 1996, as a result of competition proceedings, the Associate Professor P. Morávek, the senior lecturer of the Department, was appointed as the Head of Department of Urology in Hradec Králové.

Contemporary activities should not be evaluated prematurely. But the credits and aims of the present representatives are clear: not only to maintain the good name of the Department and its good position among other Departments of Urology in the Czech Republic (having been achieved mainly by their teachers), but also to go ahead in prosperity of the Department itself. Development of scientific research and teaching activities of the Department are being to be supported, as well. Nowadays, there is also an extra task concerning the economical prosperity of the Department. Purposive studies, postgradual sharing of knowledge, endeavour to gain experience by taking part in important sessions, congresses and trainings, both at home and abroad, represent, according to my opinion, the best means how to fulfil our goals and dreams.

Personally, I would further underline and stress the good working athmosphere based on fair and correct relationship between the colleagues. I do believe all these aims could be really reached.

I would like to express my gratitude to my first teacher of urology, to Prof. Šváb, M.D., Ph.D, who also provided me with the historical data. It was him who stood at the birth of the Department and whose efforts were remarkable.

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