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ORIGINAL ARTICLE

GLUCOSE INTOLERANCE INDUCED BY OLIGEMIC BRAIN HYPOXIA: THE EFFECT OF TERGURIDE

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Summary: Two series of experiments were performed. In the first one experiments were carried out in Koletsky genetically hypertensive lean female rats and in the normotensive female rats of Wistar strain. Glucose intolerance was induced by oligemic brain hypoxia (4 hours of occlusion of both common carotid arteries followed by 44 hours reperfusion). Brain water content were used as a marker of brain edema.Changes in insulinemia and specific insulin binding were used as expression of regulative mechanisms participating in modification of glucose tolerance. The effect of terguride (trans-dihydro-lisuride) was tested.Brain hypoxia induced glucose intolerance in both strains of rat but brain edema was found only in the normotensive females. Both abnormalities were alleviated by terguride treatment. Basal glycaemia was not changed either by the brain hypoxia or by terguride treatment,except normoternsive female where brain hypoxia induced hyperglycaemia. The second series of experiments were carried out in the normotensive females. The arrangement of experiments was the same as in first series except omission of the final glucose tolerance test. Brain hypoxia causes increase in brain water content. The mentioned elevation of brain water content was alleviated by terguride treatmnet. Insulin binding to erythrocytes was not influenced by brain hypoxia. Terguride treatment shows decrease of insulin binding to erythrocytes. Brain hypoxia elevates insulinemia which was not alleviated by terguride treatment.

Key words: *Oligemic brain hypoxia; Brain edema; Glucose intolerance; Insulinemia; Insulin binding to erythrocytes; Wistar rats; Koletsky genetically hypertensive rats; Terguride*

Introduction

In our previous paper (2) we documented the abnormalities of glucose tolerance in the obese genetically hypertensive Koletsky (SHR/N-cp) rats as well as in their lean siblings. These genetically based abnormalities of the glucose tolerance were accompanied by alterations of insulin binding to erythrocytes and hepatocytes (8). Insulin binding was decreased in both obese as well as in lean SHR/N-cp rats when compared to the normotensive Wistar rats. On the other hand, the basal plasma insulin was elevated only in the obese animals.

In the other series of experiments (3) the ergopeptine terguride was found to be potent to alleviate the mentioned abnormalities. The study of insulin binding showed that long lasting terguride treatment elevated insulin binding to erthrocytes (4). These finding suggested a possible causal relationship between alleviation of glucose intolerance and the elevation of of insulin binding to erythrocytes.

It must be stressed that the above mentioned results we obtained in the animals where glucose abnormalities are based genetically.

In recent series of experiments we turn our attention to glucose tolerance abnormalities which are induced by brain oligemic hypoxia.

Since the time of Claude Bernard (12) it is known that the hypothalamic lesions cause hyperglycaemia and glycosuria.On the other hand, it was documented (1,11) that hyperglycaemia can be found in brain lesions which do not directly affect the hypothalamus.

The last mentioned data represents the starting point for our experimental arrangement,i.e., we have used not the local brain lesion but we induced brain ischemia invading all the brain.

Glucose tolerance abnormalities induced by oligemic brain hypoxia were submitted to the same ergopeptine, i,e., terguride,which showed beneficial effect in alleviation in glucose tolerance abnormalities based genetically (3).

Material and methods

Animals

Experiments were carried out in normotensive female rats of Wistar strain and in SHR lean females of Koletsky

type (10). After weaning at the age of 30 days the animals were kept in groups of four and supplied with water and pelleted diet ad libitum.

Occlusion of common carotid arteries

Occlusion was performed under Nembutal anaesthesia (45 mg/kg i.p.). The animal was fixed in supine posture, skin was incised in the ventrolateral neck region and the common carotid arteries were separated from the surrounding tissue bilaterally. Both carotid arteries were occluded for four hours by Yasargil Standard aneurism clip (Aesculap, Germany). Then reperfusion period(44 h) was started.

Glucose tolerance test

After finishing reperfusion period glucose tolerance test was performed. Blood was sampled to heparinized capillaries (from the retrobulbar plexus under light ether anaesthesia) before glucose loading (basal glycaemia), as well as 30,60,120 and 180 min after glucose loading. Glucose (3 g/kg b.w., in 30% solution) was applied intragastrically after 14 h starvation.

Measurement of brain water content

After finishing the glucose tolerance test (in the first series of experiements) or after finishing of reperfusion period (in the second series of experiments) the animal was decapitated, the brain was cut off at the boundary between the spinal cord and the oblongata and was removed from the skull. The brain was immediately weighted and placed in a hot air drying box. The drying was finished when the weight of dry brain remained 48 hours the same.

Insulin binding to rat erythrocytes

In second series of experiments where the animals were submitted to the same procedure as described above, except glucose tolerance test which was omitted,plasma was separated from approximately 3 ml of heparinized blood drawn by cardiac puncture under the light ether anaesthesia.

Erythrocytes were obtained by centrifugation in Ficoll gradient, and incubated in the presence of constant amount ^{125}I-insulin(33 pM) at 15°C 3 hours. Results were corrected for nonspecific binding. The details of the method were published previously (8).

Terguride treatment

The drug was applied in two daily doses (7.00 and 14.00) for four days before operation and for two days after operation.Terguride maleate was administered at a dose 0.1 mg/kg i.p.

Statistics

The data were analyzed by the Student t-test.

Results

In the first series of experiments (Table 1 and 2)

Table 1:

Group	Basal glycaemia mmol/l	Sum of glycaemia 30,60,120,180 min after glucose loading mmol/l	Brain water content %
NR Co	3.80±0.28(7)	23.71±1.38(7)	77.00±0.11(7)
Occlu	5.50±1.38(7)^{d}	35.15±4.73(9)^{d}	77.39±0.38(10)^{d}
Occlu±Ter	4.51±0.50(6)	26.46±2.31(6)^{d}	77.63±0.27(6)

Means + SEM are presented. Abbreviations: NR - normotensive rats of Wistar strain, Co - control animals, Occlu - bilateral occlusion of carotid arteries,Ter - terguride treatment. Number in bracketts = number of animasls per group. Statistical significance: a = P<0.10, b = P<0,05, c = P<0,02, d = P<0,01.

Table 2:

Group	Basal glycaemia mmol/l	Sum of glycaemia 30,60,120,180 min after glucose loading mmol/l	Brain water content %
SHR Co	5.12±1.1(9)	35.37±3.19(9)	77.60±0.33(9)
Occl	5.23±0.83(14)	47.58±7.32(14)^{d}	77.66±0.25(14)
Occl+Ter	5.50±0.61(11)	37.95±6.22(11)^{d}	77.69±0.30(11)

Means + SEM are presented. Abbreviations: SHR - lean genetically hypertensive rats of Koletsky (10) type.The other abbreviations are the same as in Table 1.

Basal glycaemia

Considering the control animals, the occlusion shows elevation in the normotensive female rats.Occlusion remains without effect in SHR/N-cp lean females Taking into account occluded animals without drug, terguride treatment does not show any effect.

Glucose tolerance

Taking into account control animals, occlusion shows elevation in both strains.

Considering occluded animals without drug, terguride shows decrease in both strains of occluded animals.

In this place it is worthwhile to note strain dependence of glucose tolerance in the females rats.

SHR/N-cp lean females show significantly increased the sum of glycaemia 30,60,120 and 180 min after glucose loading (i.e., there is expressed genetically based glucose intolerance) in comparison with normotensive females (normotensive:x=23.71+1.38(7) versus hypertensive: x=35.37+3.19(10) P<0,01.

Brain water content

Considering the control animals, the occlusion shows elevation only in normotensive females.

In the second series of experiments (Table 3)

Table 3:

Group	n	Basal glycaemia mmol/l	% of insulin binding to erythrocytes	Brain water content %	Insulin pmol/l
NR-F Co	8	4.68±0.88	6.43±1.34	77.72±0.15	92±18
Occl	7	5.01±1.16	7.20±1.30	78.14±0.28[c]	166±80[b]
Occl+Ter	9	4.51±0.55	5.23±2.23[b]	77.50±0.18[d]	175±111

Means + SEM are presented. Abbreviations are the same as in Table l.

Basal glycaemia

Considering the control animals, occlusion shows no effect.

Terguride treatment shows no effect in the occluded animals.

When compared the control normotensive females in the first series of experiments with those in the second series, then in the second series the females show elevated basal glycaemia.

Insulin binding

Occlusion shows no effect.Terguride treatment shows decrease in occluded animals.

Brain water content

The occlusion induced elevation. Terguride treatment in occluded animals shows decrease.

Insulinemia

Occlusion shows elevation of plasma insulin. Terguride in the occluded animals remained without effect.

Discussion

In our previous papers (3) we documented that terguride treatment shows alleviation of glucose intolerance based genetically in SHR/N-cp obese rats of Koletsky (10) type and in their lean siblings. The mentioned drug induced alleviation of glucose intolerance was accompanied by decrease of insulinemia (4) and by increase of insulin binding to erythrocytes (4). These data suggested a possible participation of insulinemia and insulin binding to tissue in the regulative mechanism of glucose tolerance.

On the other hand, in our previous paper (5) when the effect of dehydroepiandrosterone(DHEA) on the glucose tolerance was monitored, we found in SHR/N-cp lean males that decrease of sum of glycaemia 30,60,120 and 180 min after glucose loading is accompanied by decrease of insulinemia but insulin binding to erythrocytes was not influenced by DHEA.

In our recent series of experiments similar results were obtained. Very profound glucose intolerance induced by oligemic brain hypoxia was not accompanied by any chnges in insulin binding to erythrocytes (see Table 3) and the terguride treatment which alleviated the mentioned glucose intolerance (see Table l,2) is accompanied, paradoxically, by decrease of insulin binding to erythrocytes(see Table 3). When we consider the changes in insulinemia and in insulin binding to tissues as the participants in regulative mechanism of glucose tolerance then our previous (5) as well as our recent data suggest that in glucose tolerance can take part more than one regulative mechanism.

It is valuable to admit one notion to the terguride effect on glucose tolerance in the SHR/N-cp lean females. In table 2 we documented that the last mentioned animals show glucose intolerance which is based genetically. Genetically based glucose intolerance is alleviated by terguride (3).

Oligemic brain hypoxia induces in these animals the superponated glucose intolerance. In table 2 we documented that terguride alleviates this superponated glucose intolerance but the genetically based glucose intolerance was not influenced by terguride. At recent time we are not able to solve this very difficult problem.

The effect of brain hypoxia on the brain water content and the following effect terguride represent the other open question. On one side, when water content was studied in the occluded animals which were submitted to glucose tolerance procedure (i.e., the animals were repeatedly anaesthetized with ether)terguride shows no effect on brain water neither in normotensive females where brain hypoxia indiced elevation of brain water content, nor in the genetically hypertensive females where brain hypoxia showed no effect in brain water content.

On the other hand, when brain water content was studied in the occluded animals which were not finally submitted to the glucose tolerance procedure (i.e., the animals were anaesthetized with ether only once when the blood was sampled by cardiac puncture - see the second group) terguride treatment shows profound alleviationg effect on water brain content in the occluded animals (see Table 3). Goodman and Gilman (7) documented that barbiturates show antiedematose effect.

On the other hand, ether increases intracranial pressure when the edema is induced by intracranial pathologic process(9).We documented that ether maximally elevates the brain water content after 4 h occlusion of both common carotid arteries (6).On the other hand, ether decreases brain water content immediately after frequently repeated anaesthesia.Moreover, ether elevates brain water content after two days after repeated anaesthesia(6).

Taking in mind the last mentioned data, it cannot be a priori excluded that the different effect of terguride treatment in the occluded normotensive females in the first and the second series of experiments is done by the different regime of ether anaesthesia.Before definite conclusion this question must be submitted to special series of experiments.

Before termination of discussion it cannot be omitted the most difficult question, i.e., what represents the sub-

stantial differences in the regulative mechanism of glucose tolerance when the terguride induced changes are accompanied by elevation of insulin binding and decrease of insulinemia (see SHR/N-cp lean males - 4) and when the same changes in glucose tolerance but induced by DHEA (5)are not accompanied by any changes in insulin binding and/or insulinemia. Moreover, which regulative mechanism are taking part in the induction of profound glucose intolerance induced by oligemic brain hypoxia, where no changes in insulin binding and elevated insulinemia was found and where the alleviation of this type of glucose intolerance by terguride treatment is nay even accompanied by a descrease of insulin binding to erythrocytes (the recent paper).

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ORIGINAL ARTICLE

SOME SERUM ACTIVITY MARKERS OF AIRWAYS INFLAMMATION IN DIFFICULT-TO-CONTROL ASTHMA PATIENTS

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Summary: The main aim of the present study was a search for a characteristic serum marker of inflammatory activity in the airways of asthmatics with difficult-to-control disease. Therefore, serum levels of interleukin-4 (IL-4), serum low-affinity Fc Epsilon Receptor II (sFcER II), Interferon-gamma (INF-gamma) Immunoglobulin-E (IgE), Interleukin-2 (IL-2), serum Interleukin Receptor 2 (sIL-2R) and Intercellular Adhesion Molecule-1 (sICAM-1) were measured in 2 groups of asthmatics: 1-26 patients with difficult-to-control asthma (DTCA), 2-22 asthmatics, minimally symptomatic (MSA).
Results: No significant difference in either measured parameters between the DTCA and MSA group in peripheral blood samples was found. Conclusion: The above mentioned serum markers of T- and B-cell activation as well as the serum ICAM-1 level are not sensitive enough to determine the type, activity and severity of the inflammatory process in the asthmatic airways.

Key words: *Difficult-to-control asthma; Interleukin-4; Interleukin-2; Soluble Fc Epsilon Receptor II; Soluble Interleukin-2 Receptor; Intercellular Adhesion Molecule-1*

Abbreviations used in the study

IL - Interleukin
INF - Interferon
TNF - Tumor necrosis factor
IgE - Immunoglobulin E
FcER - Fc epsilon receptor
LT - Leucotrien
PG - Prostaglandin
PAF - Platelet activating factor
Th - T-helper lymphocyte
APC - Antigen presenting cell
GM-CSF - Granulocyte-macrophage colony stimulating factor
ICAM - Intercellular adhesion molecule
VCAM - Vascular adhesion molecule
LFA-1 - Lymphocyte function associated antigen-1
VLA-4 - Very late antigen 4
BAL - Bronchoalveolar lavage
BHR - Bronchial hyperreactivity

The study was approved by local ethical commitee in April, 1994.
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Introduction

There has been an increase in the prevalence and the severity of bronchial asthma all/over the world, this fact being true especially for the atopic type of asthma (20,24,38,41). According to the study by Vondra (57), such an increase does exist in the Czech Republic as well. The reasons for such an increase in atopy prevalence are not quite clear. Whilst hereditability forms the basis of the definition of atopy, it is clear that the enviroment must have had a major influence on phenotype (34,38,48,63). Longitudinal cohort studies will be required to establish which of the many possible factors are critical to the development of allergy and subsequent asthma. The ultimate aim shall be to identify avoidable factors and to establish prevention protocols (61,62).

The view of asthma has changed radically over the last decade, due to the development of new molecular-biologic methods and by the ability to study cells and mediators directly via the fiberoptic bronchoscope (22,53). Asthma has been proven to be a chronic inflammatory disorder of the airways. The cellular constituents involve mast cells, eosinophils which are typical of allergic disorders. Major inroads have been made into the immunological mechanisms

driving the inflammatory response with T.cells taking primacy, and B-cells (via IgE) providing a trigger stimulus involving common allergens. Molecular technology has allowed a myriad of mediators to be identified as contributing to both the acute and chronic aspects of the disease, resulting in bronchial hyperreactivity and smooth muscle contraction too much and too early in response to various „triggers“ (specific - allergens, nonspecific -cold, exercise, smoke) (21).

The pathogenesis of the chronic inflammation of asthma is complex and still only partially understood. Once the inflammation has become established, it appears to be maintained by a tissue - driven response, (41). The aetiology of asthmatic airway inflammation is thought to be the development of a specific immune response in the airways. In allergic asthma, the allergen is captured and processed by antigen - presenting cells (APC) which are dendritic cells in the airways mucosa, other less efficient APCS in the airways include monocytes, epithelial cells, B - lymphocytes and fibroblasts. Following the capture of the antigen, the APC migrate to the regional lymph nodes in the airways, where they present the allergen, in association with the major histocompatibility antigen class II molecules, to lymphocytes.

A characteristic of allergic asthma is the selective development of CD 4+ lymphocytes of the Th 2 - type. This selective development of Th 2 - type lymphocytes in asthma is promoted by Interleukin - 4 (IL-4) and inhibited by IL-12. These allergen - specific lymphocytes enter the circulation and selectively home in to the submucosa and mucosa of the airways. Through the production of various cytokines, these lymphocytes direct the inflammatory reaction into the characteristic airways inflammation of asthma (27). Interleukin -4 appears to be an important cytokine in the development of allergic inflammation, not only because of its role in switching T-helper into Th2 lymphocytes, but because it promotes differentiation of B-lymphocytes to produce IgE (31,32).

The immunoglobulin isotype switch to IgE is dependent on three cytokines: IL-4, INF-gamma and IL-10. IgE secretion by B-lymphocytes requires an additional T-cell derived signal that is provided by a cognate interaction between B-cells and a membrane structure on the B-lymphocytes termed CD 40 (7,43,45,46).

The second cytokine that is critically important in the regulation of IgE synthesis is INF-gamma (1). Interferon gamma functions as an inhibitor of allergic responses through its capacity to inhibit the effects of IL-4 on B-cells. IgE production in atopy represents a combination of excessive IL-4 production occuring in the relative absence of Interferon - gamma (28,39).

Interleukin-10 is produced by IL-4 producing T-cells and inhibits the synthesis of INF-gamma by other T-lymphocytes.

In contrast to the Th2-subtype of lymphocyte, Th 1-cells differentiate in the presence of a different range of antigens associated with the delayed type hypersensitivity response. Th 1 cells generate predominantly Interferon-gamma (INF-gamma), IL-2 and TNF-beta (12,13,15,16).

The production of cytokines by Th-lymphocyte subtypes is shown in Table 1.

A summary of cytokine actions in allergies is presented in Table 2.

Table 1: Different types of cytokines produced by Th1 and Th2 - cells are described (according to Borish, 1992).

Type 1 helper cells	Type 2 helper cells	Both
Interferon gamma Interleukin 2 Tumor necrosis factor-beta	Interleukin- 4 Interleukin- 5 Interleukin- 6 Interleukin-10 Interleukin-13	GM-CSF Interleukin-3

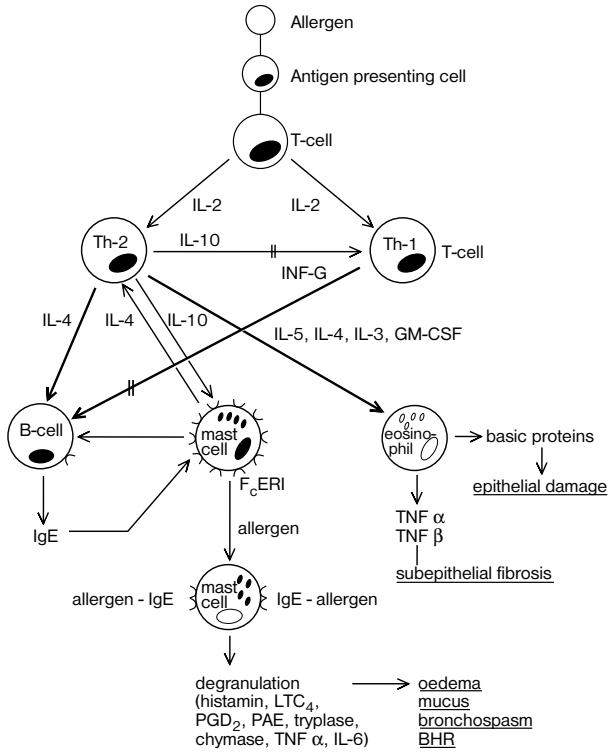
Table 2: In this Table main events dealing with the allergic airway inflammation, diffent cytokines involved and their activity are enumerated (according to Borish, 1992).

	cytokine	activity
IgE regulation	IL-4	IgE isotype switch
	IL-2, IL-5, IL-6	synergize with IL-4
	interferon gamma	inhibits IL-4
	IL-10	inhibits interferon-gamma
Eosinophilia	IL-3, IL-5, GM-CSF	eosinophilopoietins
Mast cell development and activation	IL-3, IL-9, IL-10	mast cell growth factors
	hematopoietic stem cell factor connective tissue activating peptide (CTAP)	
Inflammation	INF-gamma, GM-CSF, TNFs, IL-1, IL-4, IL-6, IL-8	neutrophil-activating factors
	GM-CSF, TNFs, IL-1, IL-3, IL-5	eosinophil-activating factors
	INF-gamma, GM-CSF, macrophage-CSF, TNFs, IL-1, IL-2, IL-3, IL-4	macrophage-activating factors

Either locally produced or circulating IgE antibodies bind to high-affinity IgE receptors (FcERI) on mast cells and circulating basophils, and to low-affinity IgE receptors (FcERII) on eosinophils, monocytes, macrophages, lymphocytes, dendritic cells and platelets (Fig. 1) Cross-linking of the IgE receptors by allergen on the surface of these cells leads to their activation, and the release of various preformed and newly formed mediators in the intercellular spaces

of the airways. The attraction, activation and prolonged survival of eosinophils in the airway submucosa and mucosa are caused by the production and secretion of IL-3, Granulocyte - Macrophage Colony-Stimulating factof (GM-CSF), IL-5, RANTES and Tumour Necrosis Factor - alfa (TNF-alfa) (15,16,17,18,19,55).

Figure 1: Illustrates schematic pathophysiologic patterns of allergic mucosal inflammation and its consequences in the airways of asthmatics (see text for further details).



The movement of circulating eosinophils and other inflammatory cells into the airways is controled by „priming" of the cells by cytokines, local production of chemokinetic and chemmotactic factors, and expression of adhesion molecules on inflammatory cells, endothelium and extracellular matrix (ICAMs, VCAMs, LFA-I, VLA-4) (7,9,23,29, 54,56).

The relative roles of the different adhesion molecules in the development of airway inflammation are not known. Monoclonal antibodies to Intercellular Adhesion Molecule -1 (ICAM-1), Lymphocyte Function Antigen -1 (LFA-1) and Very Late Activation -4 (VLA-4) molecules have been shown to inhibit the influx of eosinophils in the airways following allergen challenge (37,58).

An important characteristic of the chronic airway inflammation of asthma is that constitutional and secondary inflammatory cells may be involved in the maintenance of the chronic inflammation. It appears that the production of pro-inflammatory cytokines by sensitized lymphocytes, but also the expanded involvement of mast cells, eosinophils, constitutive cells, such as epithelial and endothlial cells, and fibroblasts, all apparently play a role in the maintenance of chronic inflammation that characterizes asthma (41,44).

The above-mentioned findings of chronic inflammatory changes in the airway mucosa even in mild forms of asthmatic patients, has led to a change in therapeutic strategy (3). The inhibitory effects of corticosteroids on cytokine production, on cellular responses to cytokines, such as expression of adhesion molecules, increased survival of inflammatory cells and enhanced release of mast cell mediators, now provide a scientific basis for the early use of corticosteroids as a first-line measure in the treatment of asthma (2,4). Furthermore, the effects of corticosteroids on the synthesis of arachidonic acid metabolites are well-known. With this change in the management strategy, a decline in morbidity and mortality associated with asthma has been proven (20,24,41). Despite these fundamental changes in the management of asthma, some asthmatics still remain „difficult - to - control" (DTC). (25).

Multiple reasons for the failure of modern treatment strategy in these patients can be identified: 1) non-compliance with treatment, 2) failure to adhere to indicated preventive measures (smoking, exposure to allergens), 3) concommitant disorders (sinusitis, nasal polyps gastro-oesophageal reflux, bronchitis, left ventricular failure, psychogenic factors), 4) inherently severe asthma from the beginning of the disease.

The investigation of the latest category of severe asthmatics represents the main topic of the present study.

Aims of the study

1) To measure the serum level of IL-4, INF-gamma, the soluble form of the low-affimty receptor on B-lymphocytes (sFcER II/CD-23) and of IgE in patients with difficult - to- control asthma (DTCA), as well as in a group of asthma patients with minimal symptomatology (MSA).
2) To examine activation markers of the T-and B-lymphocytes by measuring IL-2, soluble receptor of IL-2 (sIL-2R/CD-25), IL-4 and sFcER II (CD-23) in the peripheral blood of both asthmatic groups.
3) To investigate the serum concentration of intercellular adhesion molecule-1 (ICAM-1) as a marker of airway inflammatory activity, in both asthmatic groups.

Study groups

1) **Group of difficult - to - control asthma patients (DTCA)**
   The group was composed of 26 asthmatics in whom daily symptoms were present despite a complex anti-inflammatory and bronchodilatory therapy according to the International Treatment Strategy being instituted.
   There were 14 women and 12 men in the group, their mean age being 52.1 years (range 24-72 years) and the

mean disease duration was 19.8 years (range 2-60 years). Atopic asthma was diagnosed in 14 (53.8%) patients, 5 of them suffering from asthma since childhood. Sustained systemic corticoid treatment was necessary in 16 (61.5%) patients.

2) **Group of minimally symptomatic asthma patients (MSA)**
This group consisted of 22 asthmatic subjects, 13 women and 9 men, whose mean age was 50.9 years (range 22-73 years), and who had an average diagnosis of asthma of 15.1 years (range 3 to 55 years). The atopic form of asthma was found in 15 (68.1%) of subjects, it being present since child-hood in 3 of them.
Two patients had no sustained therapy (only occasionally was the rescue bronchodilatory drug necessary). Twenty subjects were on inhaled corticosteroids with a daily dose of 0.2-0,8 mg.
The symptom score was classified into 4 grades according to the international consensus: grade 1 being no dyspnoea, no cough, grade 4 representing dyspnoea and cough every day and night for the last year. Similarly, the average daily use of rescue ß-2 inhalations for the last year, was estimated. The best value of the forced expiratory volume in 1 second (FEV-1%) and of the forced vital capacity (FVC %) was measureed following two inhalations of fenoterol (0.4 mg) from a dosier-aerosol device.

Methods

After obtaining an informed consent, the patients were referred to the immunological laboratory for blood sampling. The patients were asked to abstain from all corticosteroid drugs for a period lasting at least 36 hours. Blood sampling for immunological examination took place in a period of 1 to 2 weeks following clinical and functional investigation.

1. Blood sampling
Peripheral blood was obtained by venepuncture of cubital vein. Blood sample was left at room temperature for 1.5 hours. After centrifugation serum samples were stored at -70°C until measurement. Samples were thawed only once.
2. Determination of the serum level of IgE, cytokines and soluble membrane molecules
Determination was performed by standard immunochemical method using comercially available diagnostic kits according to the instructions of manufacturers. Absorbance was measured by Titertek, Flow, UK spectrofotometer.

Total IgE: Immunotech, Marseille, France
IL-2 level: Quantikinine, RD Systems, Minneapolis, MN, USA
IL-4 level: Quantikinine, RD Systems, Minneapolis, MN, USA
Interferon gamma: Endogen, Inc., Cambridge, MA, USA
sIL-2R: Immunotech, Marseille, France
sICAM-1: Quantikinine, RD Systems, Minneapolis, MN, USA
sCD23: Immunotech, Marseille, France

Statistical evaluation

An unpaired T-test for the evaluation of differences between groups was used. In some parameters, the variability analysis with the Bonferroni method was applied. Statistical evaluation was performed using the software package BMDP.

Results

The clinical characteristics of both investigated groups are depicted in Table 3. No significant difference of age and of the asthma duration between both groups was found, whereas the symptom score, and the number of rescue ß-2 inhalations was significantly higher in the DTCA patients.

A significant difference in FVC and FEV 1 values between the study groups was estimated, both values being much lower in the DTCA patients cohort (Table 4).

Table 3: Clinical characteristics of two asthmatic patient groups under investigation are represented.

Group	N	Age (years)	Asthma duration (years	Symptom score (1-4)	Rescue beta-2 (N)
Difficult -to-control-asthma	26	52.1±14.0	19.8±14.8	3.17±0.50	7.8±2.9
Minimal symptomatic asthma	22	50.9±13.9	15.1±12.6	1.15±0.32	0.9±1.1
Statistical significance		n.s.	n.s.	p<0.001	p<0.001

Table 4: This Table describes functional characteristics of both asthmatic groups under study.

Group	N	Forced vital capacity (% predicted)	Forced expiratory volume in 1 second (% predicted)
Difficult -to-control asthma	26	79.2±16.7	59.3±18.8
Minimal symptomatic asthma	22	92.4±13.6	72.5±21.6
Statistical significance		p<0.005	p<0.02

Table 5 demonstrates the serum concentrations of IL-4, INF-gamma, sFcER II and IgE in both asthmatic groups, as

well as in 20 healthy subjects (blood donors). No significant changes in IL-4 values between both asthmatic groups, as well as between asthmatics and control subjects, was found.

While no significant difference between DTCA and MSA asthmatics in INF-gamma was apparent, its value was significantly higher in both asthmatic cohorts in comparison with healthy poeple.

The same result is true for the sFcER II serum concentrations.

The average serum IgE level is much higher in the DTCA asthma patient group than in the MSA and control subjects, but because of a very high standard deviation in the DTCA group, statistical significance could not be reached. This high standard deviation was caused by an abnormally high IgE value in one very severe atopic patient, being 7,104 IU/ml.

Table 5: Serum concentrations of interleukin-4 (IL-4), interferon gamma (INF-gamma), serum Fc epsilon receptor II (sFcRII) and immunoglobulin E (IgE) in examined asthmatic groups are described.

Group	N	IL-4 (pg/ml)	INF-G (pg/ml)	sFcERII (pg/ml)	IgE (IU/ml)
DTCA 1	26	14.4±2,3	4.45±1.05	4.78±7.15	473.9±1374.4
MSA 2	22	15.8±4.1	4.31±1.10	3.25±1.57	129.5±165.2
Controls 3	20	16.4±4.7	3.76±0.53	1.98±1.10	122.4±105.2
Statistical significance		n.s.	1-2 n.s. 1-3 p<0.01 2-3 p<0.05	1-2 n.s. 1-3 p<0.05 2-3 p<0.01	1-2 n.s. 1-3 n.s. 2-3 n.s.

DTCA - difficult - to - control asthma; MSA - minimal symptomatic asthma

Table 6 summarizes the results of serum IL-2, sIL-2R and of ICAM-1 concentrations in three groups under study. No significant differences in IL-2 were noticed between DTCA and MSA patients, as well as in sIL-2R between DTCA, MSA compared with control subjects.

Table 6: This Table presents the results of serum levels of interleukin-2 (IL-2), interleukin-2 receptor (IL-2) and intercellular adhesion molecule-1 (ICAM-1) in different patient groups under study.

Serum levels of IL-2, IL-2 R and ICAM-1 in groups under investigation

Group	N	IL-2 (pg/ml)	IL-2 R (pg/ml)	ICAM-1 (ng/ml)
DTCA 1	26	9.91±0.70	1130.8±350.9	437.8±128.3
MSA 2	22	9.84±0.91	1260.9±564.0	457.2±190.4
Controls 3	20	9.53±1.11	-	189.0±42.3
Statistical Significance		1-2 n.s. 1-3 n.s. 2-3 n.s.	1-2 n.s.	1-2 n.s. 1-3 p<0.001 2-3 p<0.001

DTCA - Difficult-to-control asthma; MSA - Minimal symptomatic asthma

As far as ICAM-1 concentrations are concerned, a highly significant increase in both asthmatic groups in comparison with healthy subjects was estimated.

When our asthmatic patients were divided into atopic and nonatopic groups without regard to their disease severity, no statistical difference in serum levels of all measured parameters between both groups could be found (Table 7 and 8).

Table 7: Serum levels of IL-4, INF-gamma, sFcERII and IgE in atopic, non-atopic asthmatics and healthy people are illustrated in this Table.

Serum levels of IL-4, INF-gamma, sFc E RII and IgE in atopic and non-atopic asthmatics

Group	N	IL-4 (pg/ml)	INF-gamma (pg/ml)	sFcERII (pg/ml)	IgE (UI/ml)
Atopics 1	29	15.1±4.2	4.14±0.8	3.79±2.9	464.6±1398.7
Non-atopics 2	19	15.0±1.9	4.60±1.0	4.70±8.4	125.4±201.4
Controls 2	20	16.4±4.7	3.76±0.5	1.98±1.1	122.4±105.1
Statistical Significance		1-2 n.s. 1-3 n.s. 2-3 n.s.	1-2 n.s. 1-3 p<0.05 2-3 p<0.01	1-2 n.s. 1-3 p<0.001 2-3 p<0.05	1-2 n.s. 1-3 n.s. 2-3 n.s.

Table 8: This Table demonstrates the differences of serum IL-2, IL-2R and ICAM-1 between atopic, non-atopic asthmatics and healthy blood donors.

Serum concentrations of IL-2, IL-2 R and ICAM-1 in atopic and non atopic asthmatics

Group	N	IL-2 (pg/ml)	IL-2 R (pg/ml)	ICAM-1 (ng/ml)
Atopics 1	29	10.0±0.9	1215.4±510.4	448.9±148.1
Non-atopics 2	19	9.8±0.7	1171.5±386.2	446.5±185.6
Control 3	20	9.5±1.1	-	189.0±42.3
Statistical Significance		1-2 n.s. 1-3 n.s. 2-3 n.s.	1-2 n.s.	1-2 n.s. 1-3 p<0.001 2-3 p<0.001

Discussion

It is quite clear, that application of fiberoptic bronchoscopy with endobronchial (eventually transbronchial) biopsy has allowed a direct appraisal and investigation of mucosal cellular events of airways inflammaton in asthmatic patients (5,22,26). However this investigation, being invasive, cannot be repeatedly used in routine clinical practice. Nearly the same is true for the investigation of samples, taken from bronchoalveolar lavage (BAL) fluid. These methods are mainly applied in research studies.

Recently, a method of induced sputum cell counts, using hypertonic saline solution inhalation, has been pro-

posed as a non invasive alternative to BAL in clinical practice for investigation of various cells, coming from the periphery of the airways, the results being comparable to that of bronchoalveolar lavage (49). Despite its noninvasiveness, technical equipment requirement and longtime duration (45-60 minutes), makes this method less convenient for daily clinical use.

Because of the difficulty in routinely obtaining samples from the lung, there has been considerable interest in potential blood-born markers of airways inflammation. Some changes have been shown in T-lymphocyte activation markers and cytokines in peripheral blood, particulary in more severe asthma. However it is not quite clear what is their relationship to events in the airways (2,36). Bearing this in mind, the evaluation of our measurements will be discussed in the following paragraphs.

Interleukin - 4 (IL - 4)

Interleukin-4 is not only responsible for B-cells isotype switching in favour of IgE production, but it also may account, at least in part, for selective eosinophil recruitment at sites of allergic tissue reactions. Dr. de Vries (12) had been unable to find circulating IL-4 in patients with very high IgE serum levels, probably because the IL-4 is released locally and acts by cell-to-cell contacts in a paracrine or autocrine way. However, Matsumoto measured higher IL-4 serum levels in atopic asthmatics in comparison with nonatopic subjects, but no significant correlations with IgE or sFcER II (CD_{23}) levels were found (35).

IL-4 serum concentrations in our asthmatic patients were not increased either in severe or mild degrees of the disease in comparison with healthy control subjects. It appears that the measurement of IL-4 in peripheral blood cannot be a useful marker of inflammatory reaction in the asthmatic airways. Interestingly enough, IL-4 serum concentrations were not of discriminatory potency between atopic and nonatopic subjects as could be expected.

Soluble low-affinity FcER II (CD-23)

Soluble Fc epsilon receptor II is a fragment of FcE RII expressed mainly on B-cells, its expression being poor on normal B-cells. Interleukin-4 enhances this expression while INF-gamma inhibits the effect of IL-4 on FcE RII expression. IgE production and FcE RII expression on B-cells are two closely related events (35). FcE RII is both an autocrine and a paracrine B-cell stimulatory factor and soluble FcE RII (sFcE RII) may thus possibly be a parameter of B-cell activation.

An increase in sFcE RII in 77 asthmatics was found by Matsumoto, but no difference between atopic and nonatopic subjects was present and no significant relationship to IL-4 and total IgE was shown (35). In Billery#s work on 48 atopic subjects an elevation in percentage and absolute values of B-cells expressing FcE RII by flow cytometry was measured. Allergics to pollens had an elevation only during the season (6). There was no correlation between sFcE RII and IgE levels, eosinophilic cationic protein (ECP) or symptom score. According to their view, the upregulation of FcE RII in atopics reflects allergen exposure and cannot serve as a diagnostic marker of atopy out of the exposition period. The evaluation of CD_{23} isn't useful in monitoring allergic patients because it lacks correlation with clinical symptoms. Similarly, de Amici in 19 adults with allergic asthma bronchiale could not find any correlation between sFcE RII and asthma score (11).

An increase of sFcE RII in 55 atopic asthmatics in comparison with healthy people was found by Rogala, but no relationship to total IgE, to allergen specific IgE or to IgG_4 could be estimated (47).

The results of our measurements of sFcE RII are very similar to the above mentioned data. Moreover, in atopic subjects a significant correlation with IgE was found (Table 9). An increase in sFcE RII may by a marker of immunopathologic events in the airways of asthma patients but the lack of relationship to the disease severity indicates that its measurement is of no importance for the practicing clinician.

Interferon - gamma (INF-gamma)

Interferon-gamma was discovered in 1957 by Issacs as an antiviral protein, produced by cells infected by viruses (31). Since then, other mechanisms of its action (antiproliferative,immunomodulatory) have been demonstrated. Interferon-gamma is known to antagonize the efect of IL-4 on IgE synthesis in the presence of CD-4 T-cells. The administration of recombinant INF-gamma to patients with hyper-IgE syndrom was followed by a 50% decrease of the IgE blood level, without influencing other immunoglobulin types (7). Peripheral blood monocytes of atopic asthmatics were shown to produce less INF-gamma, following phytohemaglutinin stimulation. When recombinant INF-gamma was added, the INF-gamma was increased to normal values (33,39,64).

INF-gamma serum levels in asthmatics have shown different results. While in Soliman's work on atopic asthmatics no significant changes of INF-gamma compared to controls were found (52), Kimura was able to demonstrate a significant increase of INF-gamma in severe forms of asthma, in comparison with mild asthmatics (28).

A significant increase of the INF-gamma serum level in both groups of our asthma patients could be evaluated as a T-cell activation marker in the inflammatory process of their airways wall. On the other hand, no difference in its concentration between mild and severe asthmatics makes this finding of no useful purpose for treatment strategy changes in difficult-to-control asthmatics (e.g. recombinant INF-gamma application).

As shown in table 9, a significant positive correlation between INF-gamma and IL-4 in all asthmatic groups was calculated. The importance of this finding is not clear to us, and no data explaining this relationship in the available literature were found.

Table 9: In this Table, calculated significant correlations between various measured serum parameters in different asthmatic groups are shown.

Significant correlations between different measured serum parameters

Group		
Difficult-to-control asthma	IL-4 x INF-gamma r=0.900 p<0.05	
Minimal symptomatic asthma	IL-4 x INF-gamma r=0.871 p<0.01	
Atopics	IL-4 x INF-gamma r=0.919 p<0.01	sFcERII x IgE r=0.948 p<0.001
	IgE x ICAM-1 r=-0.535 p<0.05	IL-2 x ICAM-1 r=-0.495 p<0.05
Non-atopics	IL-4 x INF-gamma r=0.861 p<0.001	

Immunoglobulin-E (IgE)

Immunoglobulin-E was first isolated from serum in 1966 by Ischizaka (8). Its highest concentrations are found in atopic poeple, who are characterized by a persistent IgE-mediated response to common environmental allergens (1,7). Atopy, which contributes to diseases such as asthma, eczema and allergic rhinitis, is defined as a disorder of the IgE response to common allergens, such as pollen, animal dander, house dust mites and fungi. These diseases are frequently detected by a raised total IgE serum level, and positive skin tests to common aeroallergens. Despite the high prevalence of atopy in population studies (about 30-40%), the incidence of manifested allergic disease (asthma bronchiale, rhinitis, eczema) is present in only 5-10% of them (8,16,17). A number of variables have been shown to affect both IgE serum levels and bronchial hyperresponsiveness. Smoking has been shown to lead to an increase in total IgE serum levels. The effect of age, environmental factors undoubtedly was proven to influence the basal values of IgE (17,18).

The IgE plasma levels in nonatopics are usually normal or only slightly elevated.

The above mentioned findings are in accordance with our results. IgE serum concentrations were highest in the group of severe asthmatics and in patients with atopy. The absence of statistical significance was due to one excessive IgE value in one patient with severe atopy. This extreme concentration was not false result because repeated measurements were in a similar range. The IgE serum level positively correlated with sFcE RII concentration, while the correlation of IgE with sICAM-1 was found in a significantly negative value (r= -0,535 P< 0,05). No similar estimation for this correlation in the available literature was found.

One of our primary aims for studying these serum parameters in severe asthmatics was the identification of patients characterized by high IL-4, high IgE, high sFcERII and low INF-gamma. The intention was, to treat these patients by administering recombinant INF-gamma to block the influence of IL-4 on IgE switching in B-cell. Unfortunately, not one patient with this laboratory phenotype was estimated. In between, further progress in the therapy of atopic asthmatics has been demonstrated, e.g. by using specific anti-IL-4 or anti-IgE antibodies (12,14).

Interleukin-2 (IL-2) and s IL-2 R

Interleukin-2 is an essential growth factor for T-cells (28). IL-2 acts in an autocrine fashion to stimulate T-cell proliferation and also serves to regulate immunoglobulin production and the growth of cytotoxic T-lymphocytes and Natural Killer Cells (1,16). Upon activation of the T-lymphocytes by antigens, an increase of the HLA-DR antigen, of the alpha subunit of the IL-2 receptor (CD-25) and of VLA-1 are found. IL-2 R on the T-cell surface is appearing 24 hours after antigen challenge and is present there for several days. Interleukin-2 provides a necessary signal for the transition of the activated T-cells from the G 1 to the s-phase of the cell cycle. During activation, the lymphocytes secrete glycoproteins related to their surface proteins, including the sIL-2 R (40,50).

While the determination of activated T-lymphocytes in peripheral blood provides information only about the immunological situation in this compartment, the determination of sIL-2 R gives an overview of the general situation of the allergic situation of the allergic inflammation in specific organs (50).

Increased plasmatic concentration of sIL-2 R were found in acute asthmatic attack and its decline correlated with an increase in FEV-1 and a decrease of symptoms following treatment by corticosteroids (1,2,10,29). A segmental allergen provocation test in asthmatics is followed by an enormous increase of sIL-R in BAL fluid (55,56). The concentrations of IL-2 and sIL-2 R were higher in the BAL fluid of symptomatic patients with bronchial asthma than in those of asymptomatic patients (40). Serum s-IL-2R was elevated even in 77 minimally symptomatic adult asthmatics in comparison with 75 control subjects (35). No difference in sIL-2R levels between atopic and non-atopic subjects could be demonstrated (35). Quite different results were described by Walker (59). In his work, the sIL-2 was significantly elevated only in non-atopic asthmatics.

According to Kips and Midander, no uniform relationship exists between s IL-2R and allergic airways inflammati-

on as well as between s IL-2R and severity of asthmatic symptoms (29,36).

Our results of serum IL-2 and s IL-2R levels are in accordance with the above cited findings, these parameters not being able either to discriminate between the different severity of asthmatic symptoms or to differentiate atopic and nonatopic asthmatics. Therefore, we don't recommend the measurement of these parameters in the serum of asthmatic patients as an activity marker of allergic airways inflammation.

Intercellular adhesion molecule-1 (ICAM-1)

The recruitment of leucocytes to sites of inflammation involves a well co-ordinated sequence of events, in which several cell adhesion molecules and chemotactic cytokines play an active role (21,30). Initial low affinity selectin-dependent vascular rolling is followed by leucocyte activation by endothelial-derived chemoattractants (e.g. IL-8, monocyte chemoattractant peptide-1-MCP-1) and a transition to beta-integrin-dependent high affinity leucocyte adherence cytokine-mediated upregulation of the Ig superfamily of adhesion proteins, ICAMs and vascular cell adhesion molecules (VCAMs), leading ultimately to transendothelial migration.

ICAM-1 is a 80-115 kD glycoprotein with a large cell distribution. Its increased secretion is stimulated by various cytokines (e.g. IL-1, TNF-alfa, INF-gamma). ICAM-1 serves as a ligand for beta-2 leucocyte integrins LFA-1 and MAC-1 and enhances adhesion of leucocytes provided with these receptors to the tissue expressing ICAM-1 (30).

Within 6 hours of segmental allergen challenge of the sensitized asthmatic airways, a marked endothelial upregulation of E-selectin and ICAM-1 occurs accompanied by an influx of leucocyte function associated antigen-1 (LFA-1) leucocytes comprising neutrophils and eosinophils (37).

Epithelial cell expression of ICAM-1 in asthmatics has been compared with controls. While Montefort found no difference in ICAM-1 expression between asthmatic and control epithelium (37), Vignola demonstrated that ICAM-1 expression was increased in asthmatics and that it correlated with clinical score and lung function (44,54). A soluble form of ICAM-1 (sICAM-1) has been detected in the sera of patients with bronchial asthma (42,51). Its increased concentrations were found both in atopic as well as in non atopic asthmatics. Serum concentrations of sICAM-1 were higher during asthma attacks than in the same patients during remission (51). Serum sICAM-1 levels were also lower during treatment with oral prednison (42,51).

On the other hand, biopsies from patients with severe „steroid resistant" asthma have revealed a marked upregulation of ICAM-1 and VCAM-1 in the absence of allergen exposure and while taking high doses of corticosteroids. According to Holgate, the examination of ICAMs may offer a possible biomarker of asthma activity (21). It has been shown that pretreatment of monkeys with a monoclonal antibody specific for ICAM-1 attenuates the eosinophil infiltration and airway responsiveness to inhaled methacholine (58). These studies suggest that ICAM-1 may by pivotal in the pathogenesis of airway hyperresponsiveness and asthma.

Based on the potential importance of cell adhesion molecules in allergic and inflammatory reactions, various approaches are being developed to develop specific antagonists (60). While inhibition of adhesion molecules is an attractive new approach to the treatment of inflammatory disease, there may be potential dangers in inhibiting immune responses, leading to increased infections and increased risk of neoplasia (3).

As far as our measurements of sICAM-1 are concerned, significantly higher levels in both asthmatic groups compared to controls, may be a marker of permanent allergic inflammation in the airways of these patients without relationship to clinical asthma severity. The upregulation of ICAM-1 in our patients was independent of their atopic profile. The significant negative correlation to IL-2 and IgE concentrations is hard to explain.

Because of the lack of differentiation between mild and severe asthmatics, we consider the estimation of sICAM-1 in peripheral blood as an non-sensitive marker of airway inflammation.

Conclusions

1) The group of difficult-to-control asthmatics cannot be differentiated by a specific cytokine profile in peripheral blood samples from minimally symptomatic patients.
2) Some increased levels of T-and B-cell activity markers (INF-gamma, sFcER II) and of sICAM-1 even in minimally symptomatic asthma patients, point to permanent chronic inflammatory process in their airways.
3) No patient of the difficult-to-control asthma cohort with a laboratory phenotype of high IgE, high sFcER II, low INF-gamma and high IL-4 serum concentration could be found, so as to allow the causative therapy by recombinant interferon-gamma.
4) Despite some interesting results observed in investigations of the above mentioned parameters, we do not consider their estimation in peripheral blood samples of severe asthmatics helpful in therapeutic strategy changes.

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ORIGINAL ARTICLE

SURGERY OF THE THYROID GLAND

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Summary: Since the year 1987 to 1996 all kinds of thyroid surgeries were performed at the ENT Department. Altogether 604 patients underwent 655 surgeries. Total lobectomy or total thyroidectomy represented the most common procedures. Due to a gentle surgical technique, which we call „preparation", good results were achieved in the morbidity of laryngeal recurrent nerve (permanent palsy in 0,6%), and in perioperative or postoperative bleeding (0,3% of wound revisions, 0,5% of blood transfusions). Postoperative hypoparythyroidism was found in 4,7% patients. There is no correlation between postoperative hoarseness and laryngeal recurrent nerve palsy. Laryngeal endoscopy immediately after surgery is the most valuable diagnostic procedure.

Key words: *Thyroidectomy; Surgical technique; Complications of thyroidectomy*

Introduction

Thyroid gland surgery was performed at our Department only occasionally until the year 1987. The patients treated for postoperative recurrent nerve palsy at the Department of Phoniatry stimulated our interest in the possibilities how to minimize their numbers. Since November 1987 all types of thyroid gland surgery were adopted step by step in our Department. We have started with unilateral lobectomy mostly for adenomas or cysts, later we have performed total thyroidectomy for cancer and even later for some cases of thyreotoxicoses of Graves-Basedow's autoimmune type. The number of operated patients rose every year (Table 1). Our surgical strategy is being based on:

- Operation should be sufficient for treatment of the disease (complete removal of the lesion)
- The extent of the surgery should avoid the need to return to the previously operated area in a future case of disease recurrence when reoperation is mandatory (9).

Operations on the thyroid were performed to reduce its function (thyrotoxicosis), reduce its volume (non-toxic goitre), or treat malignancy. It is essential for the surgeon to be familiar with the anatomy of the thyroid and parathyroid glands. Acquaintance with the blood and nerve supplies to the larynx and their anatomic variations is necessary for a safe thyroid surgery. Anatomical study and training of the surgical procedure at the Department of Anatomy is recommendable. Extremely close cooperation with an endocrinologist in the indication, preoperative preparation and selection of the optimal method and postoperative care is essential (5,6).

Methods

Between November 1987 and the end of December 1996 a total of 604 patients underwent different types of thyroid surgery (Table 1.). We record a constant yearly increase of surgeries. Altogether there were performed 655 operations.

Table 1: Thyroid gland surgery

Year	Females	Males	Total
1988	15	1	**16**
1989	25	3	**28**
1990	42	3	**45**
1991	36	3	**39**
1992	41	5	**46**
1993	59	8	**67**
1994	65	6	**71**
1995	108	16	**124**
1996	138	30	**168**
TOTAL	**529**	**75**	**604**

Preoperative preparation

Most of the patients are referred for surgery by the endocrinologist. Biochemical tests include estimation of se-

rum thyroxin (T3,T4), serum calcium, TSH, and thyroid antibodies, are routinely performed. Also the preoperative x-rays of the thorax is performed regularly, CT or MRI is done only in selected patients where technical or surgical problems are to be expected. Scanning with radio-isotopes is used in patients with suspected „hot nodule" or with nodular goitre. Sonography was performed in all patients. This examination is essential today and completes the preoperative assessment. Ultrasound is of a great value in distinguishing cystic from solid lesions. Fine needle biopsy (cytology) is used in cases of nodular lesions due to its high sensibility to detect malignancies. Estimation of the calcitonin level is done in patients with medullary carcinoma and in all their relatives.

Special preoperative medication is used in thyreotoxicosis only. Carbimazol is being administered to achieve euthyroid state. Iodine (Lugols solution, sodium iodine) is being used for 10-14 preoperative days to reduce vascularity and friability of the gland. No preoperative medication is given in patients with suspicion of carcinoma, especially in patients with nodular goitre.

The operation

All operations are performed under general endotracheal anesthesia in supine position. The neck is extended by placing a sandbag under the shoulders. The upper part of the table is raised to reduce tissue congestion. Local infiltration of Xylocaine with epinephrine is used to reduce bleeding of the skin incision.

Thyroid lobectomy

Total lobectomy is the most frequent surgery on the thyroid gland (Table 2). This is performed in patients with solitary nodules. The skin incision extends from one sternomastoid muscle to the opposite one, two fingers above the sternal notch. To divide the platysma electrocautery is used. The upper and lower skin flaps are then elevated. The fascia over the strap muscles is incised in the midline and the strap muscles are retracted laterally.

Horizontal incision of the sternohyoid muscle is used in cases of large thyroid glands. We use mainly the lateral approach based on mobilization of the lobe from the lateral side. The middle thyroid vein is interrupted when found. Superior pole vessels are transsected, as well as the inferior vein and lobe is thus mobilized and delivered into the wound. The last step is represented by dissection and mobilization of the posterior surface of the lobe with localization of the recurrent laryngeal nerve, parathyroid glands and their blood supply. After dissection of the parathyroid glands with their blood supply intact, all terminal branches of the inferior thyroid artery are transsected. Care should be taken in the region of the tracheo-esophageal groove to identify the recurrent laryngeal nerve. Transsection of the thick fascial suspension of the lobe to the cricothyroid membrane (Berry's ligament) is the last step of the procedure. This posterior suspensory ligament should be cut carefully, under direct vision. Recurrent laryngeal nerve is kept in view all the time. We prefer its visual identification but sometimes this is not possible. In these cases we perform dissection very close to the surface of the lobe or we incise the capsule and continue subcapsularly. Berry's ligament is an important structure in relation to the recurrent laryngeal nerve. Loré considered this ligament as one of the most important structures in thyroidectomy (3). The medial approach, as described by Loré, we use mostly in smaller lesions, but generally less frequently than the lateral one. After transsection of the isthmus and superior displacement of the inferior pole of the lobe, its posterior suspensory ligament is divided and the recurrent laryngeal nerve is identified. We carry out meticulous dissection using delicate retractors, dissectors and Deschampess needles for ligation of the vessels prior to their transsection. This procedure we call „preparation technique". Such a technique consumes more time than the conventional one, but offers better results in preventing laryngeal recurrent nerve palsy or damage of the parathyroid gland vessels. We avoid rough handling and inadvertent clamping tissues during the dissection and then transsection of the tissue between hemostats. This technique we call „resection". We have had an opportunity to use the nerve stimulator for easier identification of the laryngeal recurrence nerve. This device is helpful but not in each case.

The operation technique of the total lobectomy is grounding for other surgeries.

Total thyroidectomy

After the first side completed the same procedure is performed on the opposite side. If it is possible we start usually on the left side and remove all parts of the thyroid in one block.

In case of some difficulties we start with transsection of the isthmus and each lobe is dissected separately. Subtotal thyroidectomy is performed by leaving a small remnant of the tissue but with the same technique as a total procedure. We use the same nomenclature as Dvořák (2).

Subtotal thyroidectomy

When resecting nodular goitre, the margin of the remnant is given by the normal tissue. Most conventional are remnants of the posterior parts at the both sides. Nevertheless, sticking to our surgical rule „not to return to the previously operated area" we rather perform a total lobectomy on one side and subtotal on the other. In case of recurrence, reoperation is performed on the latter side. Otherwise, we leave adequate remnants of the superior parts of both lobes connected with the superior thyroid vessels.

Retrosternal goitre

Retrosternally positioned goitres are usually easily shifted in the neck by traction and gentle dissection from above, once the vessels have been secured. In cases of excessive size of retrosternal protuberations, cooperation with the thoracic surgeon is necessary.

Once haemostasis is satisfactory, the neck can be closed. We use the suction Redon drain (for 24, 48 or 72 hours, depending on the amount of blood suctioned; collection of 10 ml or less indicates the drain removal). The muscles are approximated in the mid-line with interrupted or continuous absorbable sutures. The skin is usually sutured with intradermal continuous suture. Laryngoscopy is performed at the end of the surgery immediately after endotracheal tube removal. We use McIntosh laryngoscope, in difficult cases there are better results with a 25 degree, 2.7 mm endoscope (Richard Wolf, Knittlingen, Germany). Also a fibrolaryngoscope may be used.

Results

In 604 patients who underwent different types of thyroid gland surgery, 655 operations were performed. The female to male ratio was 7:1. Reoperations were done in patients with preoperative diagnosis of benign lesion (nodular type mostly), but with postoperative final diagnosis of carcinoma. This secondary completion of the total thyroidectomy had the same morbidity as the primarily performed total thyroidectomies (1).

Hemorrhage is usually peroperative or within a short period of time postoperatively. The wound had to be reopened early after operation in two patients (0,3%). Reoperations revealed the source - the superior thyroidal artery in both cases. Blood transfusion was needed in 4 patients (0,5%). Hemorrhage during operation is minimized by a gentle dissection technique using a bipolar coagulation and dividing vessels between ligatures (Table 2).

Hypoparathyroidism

The serum calcium is usually low after the total thyroidectomy even if all parathyroid glands were saved. Hypocalcemia in these cases is present mostly without a clinical manifestation. This hypocalcemia is transient due to blood supply damage, not to the parathyroid gland removal. Transient hypocalcemia was found in 43 patients. Treatment according to symptoms rather than estimation of serum calcium was applied in 36 patients (by calcium supply in 11 patients and in 25 of them dihydrotachysterol was added) (Table 2). Treatment lasting for more than 1 year very likely proves a permanent hypoparathyroidism (14 patients).

Recurrent Laryngeal Nerve Palsy

Laryngeal nerve is at risk of damage in total thyroidectomy on both sides, as well as in the subtotal thyroidectomy. In the subtotal thyroidectomy the risk is different, in the classic procedure (2) there exists the risk on both sides, but smaller one when compared with total thyroidectomy. In our modification with one-sided total and the other-sided partial procedure, only the side with total lobectomy is at risk. In the modification with remnants of tissue left on the superior thyroidal vessels, both laryngeal recurrent nerves are at risk. Partial thyroidectomy should lack the risk totally. In 604 patients with 655 operations, 961 laryngeal recurrent nerves were at risk of damage (Table 2). Postoperative laryngoscopy was impossible in the usual way in 15 patients (2,4%) only. Immobile vocal cords or evident discoordination of vocal cords movement were revealed in 102 cases (10,6%) from 961 cases, where laryngeal recurrent nerve was at risk (100%). From this group of patients the recurrent nerve palsy was evident in magnifying laryngoscopy the day after operation in 92 cases (9,6%). In three patients palsy has not been recognized immediately after operation, but as late as the next day. From 102 cases of laryngeal recurrent nerve palsy recognized immediately after the operation, 6 patients suffered for more then 6 months and are considered to have a permanent palsy (0,6%). We compared the number of palsies and number of patients suffering from hoarseness after operation (Table 3). Hoarseness was more often then palsy. There were 242 patient who suffered from hoarseness following thyroid gland surgery, while only 102 patients had a laryngeal recurrent nerve palsy. Palsy could be connected with hoarseness (70 cases) but not in each case, 32 patients with palsy were without voice impairment. Postoperative excavated vocal cord on one side of the larynx which confirms superior laryngeal nerve palsy was diagnosed in 12 patients (1,2%).

Table 2: Postoperative complications

Complication	**No**	**%**
Hemorrhage: patients at risk	**605**	**100**
Postop. revision needed	**2**	**0,3**
Blood transfusion	**3**	**0,5**
Parathyroid glands at risk	**298**	**100**
Hypoparathyroidism temporary	**43**	**14,4**
Hypoparathyroidism permanent	**14**	**4,7**
Recurrent nerve at risk	**961**	**100**
Vocal cord preoperative	**1**	**0,1**
Palsy postoperative	**102**	**10,6**
Temporary	**90**	**9,4**
Permanent	**6**	**0,6**

Table 3: Hoarseness and recurrent nerve palsy

	Palsy	Hoarseness	Total
Hoarseness	**70**	**172**	**242**
Palsy	**32**	**70**	**102**
Total	**102**	**242**	

Postoperative care

After the operation the patient is placed in the recovery unit until fully conscious. The patients after total thyroidectomy, especially due to thyrotoxicosis, stay at the unit until the next morning, exceptionally longer.

The suction drain (Redon) is removed between 24 hrs and 72 hrs, usually after 48 hrs. Thereafter a light dressing may be used to cover the incision. The intradermal stitch is removed usually on the 7th postoperative day. The patient is sent back to be seen by the endocrinologist.

Discussion

The operation technique is based on the conception of total surgery (8), cooperation with an endocrinologist is mandatory. As to the radicality of the procedure we state two rules: 1.- surgery should be fully sufficient for the treatment or removal of a lesion and 2.- must be extensive enough to avoid the need to a future return to the previously operated area if recurrence of the disease occurs and reoperation is necessary. For these reasons we commonly perform total lobectomy or total thyroidectomy. Other indication for the total thyroidectomy are some Graves-Basedows thyrotoxicoses and most of the thyroid papilocarcinomas (with iodine accumulation).

Radioactive Iodine-131 therapy is beneficial in those patients whose primary tumors or metastatic deposits concentrate radioactive iodine. After total removal of the thyroid gland the patient develops hypothyroidism and the concentration of the iodine in the thyroid tissue remnants, including metastases, is more effective. Thus the total thyroidectomy or completion of total thyroidectomy (1), plays an important role in our surgical policy (7). This radical therapy represents the standard in Czech Republic and excellent results advocate this procedure which is not generally accepted everywhere, but in the USA (4).

Our results, as go for permanent postoperative recurrent nerve palsy and perioperative bleeding, are comparable or better than data in literature (2, 7). The gentle dissection technique using small dissectors, special forceps for bipolar coagulation, and the ligation of vessels prior to their transsection are most probably the main reasons. Furthermore, we separate the branches of the inferior thyroid artery in the thyroid gland capsule, not in its trunk.

The recurrent nerve is being identified and displayed. This dissection procedure is time consuming but perioperative bleeding and especially the damage of the nerve can be thus minimized. Direct laryngeal endoscopy either with Mc Intosh laryngoscope or with fibrolaryngoscope immediately after removing endotracheal tube is very useful, mainly in patients after the total thyroidectomy. Endoscopy shows normal or pathological mobility of the vocal cords and in case of palsy we are able to start proper treatment for the safe postoperative recovery.

Hoarseness is highly unreliable for estimation of recurrent nerve palsy. Unilateral palsy is often asymptomatic and discovered only by laryngoscopy. On the other hand, hoarseness could be due to intubation with normal vocal cords mobility.

Conclusion

We conclude that thyroid gland surgery is a part of the neck surgery which needs a very gentle surgical technique. This technique we call „preparation“, in contradiction to „resection“ technique. Preparation procedure is time consuming but the results are better as far as the postoperative recurrent nerve palsy, hypoparathyroidism or perioperative bleeding are concerned. The surgeon should be familiar with the neck surgery, of course. Thyroid operations should not be available for the occasional surgeon.

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ORIGINAL ARTICLE

VISUAL FUNCTIONS IN APHAKIA AFTER SECONDARY INTRAOCULAR LENS IMPLANTATION

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Summary: 21 aphakic eyes of 21 patients corrected by glasses (A-G) were examined 1 and 6 months before and after secondary intraocular lens implantation (SILI). Visual acuity (VA) was tested using Snellen chart and computerized method with Landolt rings (CL). Contrast sensitivity (CS) was measured using computerized Contrast sensitivity system 8010 in spatial frequencies from 0.74 to 29.55 c/deg. Preoperative best corrected VA (BCVA) in A-G eyes was significantly lower in comparison with control group of the same age only using computerized method with Landolt rings. A reduction of BCVA by both methods at 1 month and its return to original values after 6 months were noted. Significantly lower values of CS were found in A-G patients before SILI compared to the control group of the same median age in spatial frequencies from 3.69 to 29.55 c/deg. After 1 and 6 months the values stayed on the preoperative level, except the frequency 29.55 c/deg, which increased significantly ($p < 0.01$) 6 months after SILI.

Key words: *Aphakia; Secondary intraocular lens implantation; Contrast sensitivity; Visual acuity; Landolt rings*

Introduction

Traditional aphakic spectacle correction is generally not acceptable because of induced anisometropia as great as 25 % - 35 % (7). Contact lens correction is satisfactory for many patients, but for those who cannot manipulate a contact lens because of tremor or arthritis, those whom inconvenience and the expense of multiple lens replacements become a burden, or those who are unable to tolerate a contact lens for other reasons, the aphakic eye is functionaly blind (2). For these patients and for those who require improved uncorrected vision and stereopsis for occupational or psychological reasons, the remaining treatment option is secondary intraocular lens implantation.

In our clinic SILI is made both in aphakic eyes after intracapsular or extracapsular cataract extraction in the past and in aphakic eyes after injury if there were no contraindications like chronic uveitis or disorganized anterior chamber. The patients before SILI were given complete ophthamologic examination including slit lamp, ophthalmoscopy, tonometry and biometry. Uncorrected VA (UNVA) and BCVA were tested using common Snellen chart. Postoperative examinations were on 1. day then on 1. week and 1. month by ambulant ophthalmologist.

It is questionable to which extent it is possible the VA tested by means of Snellen chart or on Landolt rings (1) hold for a sufficiently sensitive sign of potential discrete visual changes that we expected in A-G after SILI. For such a study the examination of CS is more suitable because it offers information on the resolving power of the eye at submaximal contrasts of the environment and on a larger area of the retina which is after Arden (1) more important.

We decided to complete the evaluation of the results of SILI in A-G eyes using computerized method with Landolt rings (9) and computerized method for CS.

Materials and method

1. 21 aphakic eyes of 21 patients corrected by glasses were examined before and 1 month after SILI, 18 eyes also after 6 months. Median age of the 10 women and 11 men was 70 years (range 50 - 83). BCVA before SILI was 6/12 and better using Snellen chart, only in one case was BCVA 6/15. In 18 cases were the eyes aphakic after intracapsular cataract extraction (ICE), in 3 cases after extracapsular cataract extraction (ECE), so in 18 cases were implanted lens into anterior chamber, in 3 cases posterior chamber lenses. In no eye clinically signigficant macular pathology was seen.
   As a control group were examined 20 eyes of 20 people with normal intraocular findings with no clinically significant macular pathology and BCVA 6/9 or better using Snellen chart. There were 10 women and 10 men with median age of 70 years (range 54 - 81 years).

2. UCVA and BCVA using Snellen chart and computerized method with Landolt rings were tested. CS was tested using computerized Contrast sensitivity 8010 system (Neuroscientific corp., Farmingdale, USA) in spatial frequencies from 0.74 to 29.55 c/deg.
   The distance for examination of VA using computerized method was 4 meters. The patient determined the position of the gap in Landolt rings that could be in one of four basic directions. After two right answers followed Landolt ring of a half size (means about three lines smaller). This preparatory phase continued till the subject did a mistake. Then a measuring phase started in which after two right determinations followed Landolt ring smaller by one line, after one wrong answer was Landolt ring about one line greater generated. In the end of the programm threshold VA was determined (9).
   The distance for examination of CS was 2.2 meters so that the range of spatial frequencies from 0.74 to 29.55 c/deg was achieved when the size of monitor was 5 x 3.5 angular degrees. An adjustement method with ascendent and descendent approach of the threshold contrast determination was used (8).

Results

Visual acuity

- BCVA using Snellen chart in the control group was 6/9 and better, in A-G patients 6/12 and better, in one case 6/15.
- BCVA in A-G eyes was significantly lower compared to the control group of the same median age only using computerized method with Landolt rings ($p < 0.05$).
- 1 month after SILI BCVA decreased by both methods, significantly only using computerized method ($p < 0.05$).
- after 6 months BCVA returned to its preoperative level using both methods (Tab.1, Tab.2, Fig.1).

In both terms after SILI no significant differences in VA between patients with anterior and posterior chamber lenses were detected.

Tab. 1: The number of eyes with BCVA 6/6, 6/9, 6/12, 6/15, 6/18 and 6/24 using Snellen chart in A-G eyes before SILI and 1 and 6 months after SILI.

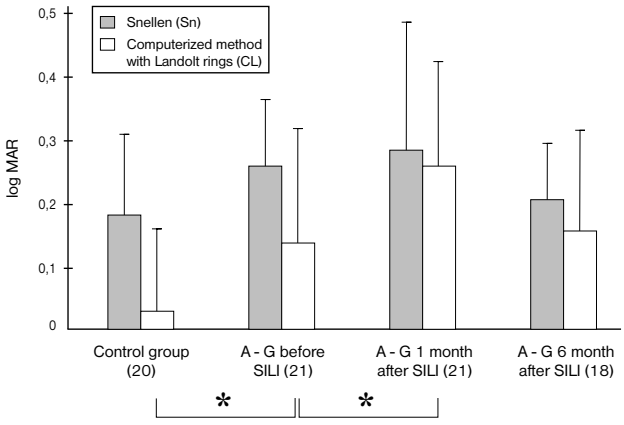
Group	BCV A					
	6/6	6/9	6/12	6/15	6/18	6/24
A-G before SILI (n = 21)	1	10	9	1	-	-
A-G 1 month after SILI (n = 21)	1	10	6	2	1	1
A-G 6 months after SILI (n = 18)	1	11	4	1	-	1

Tab. 2: Comparison of VA of control group and A-G patients before and 1 and 6 months after SILI using Snellen chart (Sn) and computerized method with Landolt rings (CL).

Group	log MAR (Sn)	log MAR (Cl)
Control group (n = 20)	0,18±0,13	0,03±0,13
A-G before SILI (n = 21) stat. significance	0,26±0,11 n.s.	0,14±0,18 *(p = 0,021)
A-G before SILI (n = 21)	0,26±0,11	0,14±0,18
A-G 1 month after SILI (n = 21) stat. significance	0,29±0,20 n.s.	0,26±0,17 *(p = 0,021)
A-G 6 month after SILI (n = 18) stat. significance	0,21±0,09 n.s.	0,16±0,16 n.s.

(n. s. ... nonsignificant differences)

Fig. 1: Visual acuity values (in log MAR units) using Snellen charts (Sn) and computerized method with Landolt rings (CL). The control group consisted of 20 persons, A-G group of 21 persons. (* ... $p < 0.05$)



Contrast sensitivity

- CS in aphakic eyes was significantly lower compared to the control group, especially in moderate and higher spatial frequencies (Fig.2).
- Nonsignificant changes of CS in both terms after SILI were noted except of the value in frequency 29.55 c/deg which increased significantly 6 months after SILI (Fig.3).

There were no significant differences in CS between patients with anterior and posterior lenses detected.

Fig. 2: Contrast sensitivity (CS) values in decibels (dB) for spatial frequencies of 0.74, 1.97, 3.69, 7.39, 14.77 and 29.55 c/deg. The differences in CS between the control group (black diamond) and A-G patients (white rectangle) before SILI are statisticaly significant at spatial frequencies from 3.69 to 29.55 c/deg. (* ... $p < 0.05$, ** ... $p < 0.01$, *** ... $p < 0.001$)

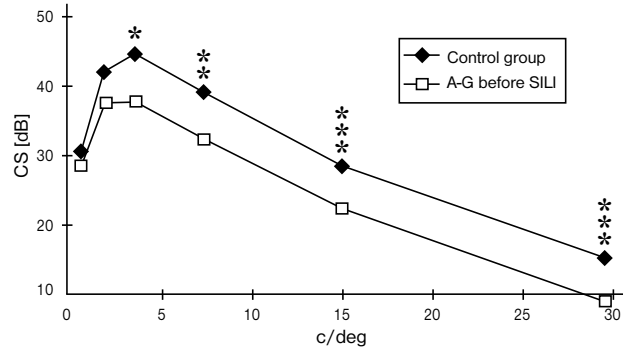
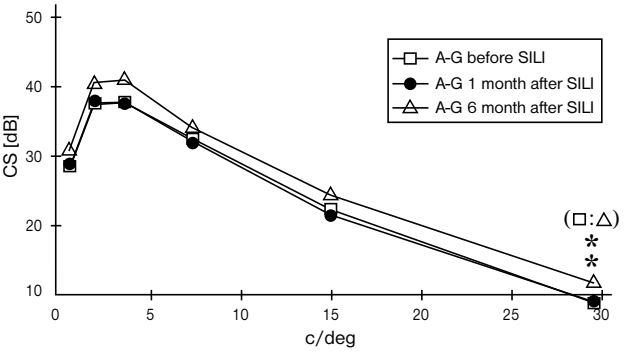


Fig. 3: The changes of CS in decibels (dB) after SILI were nonsignificant except of significant increase of the CS in frequency 29.55 c/deg after 6 months. (** ... $p < 0.01$)



Discussion

We noted significantly lower values of VA in A-G eyes compared to the control group of the same median age using computerized method with Landolt rings, significant reduction of BCVA 1 month after SILI only using computerized method and the return of BCVA 6 months after SILI to original values by both methods used. BCVA 20/40 and better have 80.9% of patients 1 month after SILI and 88.8% of patients after 6 months.

Our results are comparable to those of Durrie et al.(2) who described that 73% of the patients receiving intraocular lenses had postoperative BCVA equal to or better than their preoperative BCVA and 70% of the patients had VA 20/40 or better.

In this study statistically lower values of CS from 3.69 to 29.55 c/deg in A-G eyes compared to the control group of the same median age and no statistically significant changes of CS except of the frequency 29.55 c/deg after SILI are demonstrated.

Contrary to us Hejcmanová et al.(4) found nearly identical values in CS curve when comparing CS in A-G eyes with phakic eyes of the same median age using Vistech charts in spatial frequencies from 1.19 to 27.25 c/deg. Only in frequency 7.97 c/deg the aphakic group has a statistically lower CS ($p < 0.05$). The artephakic patients with a retropupillary lens have, when compared with a control group have lowered CS approximately by one third. Also Hess et al. (5) registered a reduction of CS in patients after ECE with anterior chamber lens and Howe et al. (6) saw reduction of CS after ECE with posterior chamber lens.

Identically with us Owsley et al. (9) found normal values of CS not only on ECE with retropupillary lens but also after ICE with anterior chamber lens. Also Furuskog and Nilsson (3) did not find even after repeated control examinations of the patients after ECE with retropupillary lens statistically significant differences compared to the control group of the same median age.

Conclusion

Our resuts indicate that SILI both with anterior chamber lens and posterior chamber lens is a very effective method for the treatment of aphakia from the functional point of view.

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HISTORICAL ARTICLE

THE HISTORY OF MILITARY TOXICOLOGY

Jiří Bajgar, Josef Fusek

Military Medical Academy, Hradec Králové; (Rector: doc. MUDr. S. Býma, CSc.)

COL Assoc. Prof. J. Bajgar, (1944) COL Prof. J. Fusek (1945)

Research on protection against CWA was very intense in the former Czechoslovakia. As early as pre-WW II, this research was conducted at the Military Technical Institute in Prague. In the fifties, the medical and technical topics were separated and the medical aspects were delegated to the Department of Toxicology (DTOX), Military Medical Academy (MMA), Hradec Králové. Military toxicology is a prime responsibility of the DTOX. The MMA was founded in 1951 and the DTOX, without experience or equipment, began its work on 1 September 1951 in one room of the civilian Medical Faculty of Charles University. Its total equipment on hand was one typewriter and two masks.

All the activities were focused to education, however, research work begun. Technical equipment was enlarged, e.g. Lange's photometer, oscilometer, Warburg instrument. The first head of the Department was Prof. MUDr. Zdeněk Fink, DrSc..

In the sixties initial scientific work focused on research into the mode of action of mustard gas, cyanide and nerve agents. The results of this research were classified secret and had little or no publicity and practically no outlet into scientific journals. One result of this research was the development and production of pralidoxime (PAM Spofa) which was introduced into the Armed Forces. In 1965, the lay syringe (LIS) for self-administration of atropine was introduced into the Czechoslovak Army. This particular research effort during the sixties and later into the seventies, resulted in the assessment of the maximal available doses of nerve agents for man.

By the end of the sixties and the Prague Spring, the first publications of the DTOX were seen in scientific literature. However, the name of the Department as well as the term „military" were excluded from the title. The Institute was designated as the Medical Research Institute for foreign journals only. On the other hand, in the Czech journals which were freely circulated abroad, the word „military" was present as well as other specifications such as military rank.

At this time, the Department was equipped with techniques on very sophisticated basis, e.g. fluorimeter Farrand, Auto Analyzer Technicon, ultracentrifuge MSE etc. The staff of the Department was also on high level able to cover the research of toxic substances from synthesis and analysis (Ing. F. Ornst, CSc.) later on through toxicity testing (MUDr. M. Krejcar, CSc.; MUDr. V. Hrdina, CSc.), including percutaneous route of administration (MUDr. V. Vondráček, CSc.), biochemical characterization in vitro and in vivo (MUDr. Tulach, CSc. and MUDr. A. Jakl, CSc.), histochemical evoluation (MUDr. R. Urban, CSc.) and development of antidotal treatment (Dr. J. Vachek). In the year 1968, Prof. Fink was named the Head of the whole institute (Purkyně Military Medical Research and Postgraduate Institute). New chief of the DTOX was Doc. MUDr. V. Vondráček, CSc. Prof. Fink was in this post till 1971. Then he had to leave the Army as a result of a political persecution during the so called „period of normalization" which started after the year 1969.

Although the political situation changed substantially after the Prague Spring, all activities of the DTOX more or less continued. During the seventies to eighties, COL. Prof. Ing. Jiří Matoušek, DrSc. was a new member of the DTOX staff. His work focused on development of new decontamination agents that resulted in a decontamination kit based on sorption mechanical principle (DESPRACH) for the Czechoslavak Army and Civil Defence. In the year of 1973, COL Prof. MUDr. V. Hrdina, CSc. was named the Head of the Department.

Education at the MMA, which was focused on military toxicology, began to consider more modern techniques of video, movies, computers, etc. More than 10 movies were produced at the DTOX. These dealt with, amongst other subjects, clinical laboratory diagnosis of organophosphate and other chemicals poisoning.

Some research works on intoxication with psychotomimetic compound, e.g., LSD-25, IDPN and BZ and their treatment were done and prospective antidotes were studied in a more detailed fashion. New concrete antidotes against

nerve agents (FOSAN, CHONOL I and II, RENOL) prophylactic antidote against organophosphates (PANPAL) and antidote (7-MEOTA) against psychotomimetic compound BZ were real results of this research which were finalized by the end of the eighties.

Modernization also was also seen in the means of administration; the autoinjector GAI containing atropine and obidoxime was developed for the Army in cooperation with the former GDR. After the so-called „velvet revolution", a new head of the DTOX was elected (COL doc. MUDr. J. Bajgar, DrSc.), and research dealing with further study of action of CWA and their antidotes was continuing in two research projects designated as OTRAL and SOMAN.

OTRAL described the toxic effect of the new nerve agent GV, with properties similar to G, as well as V. compounds, and GV's medical treatment by the usual antidotes, which is difficult. Cholinesterase activity in the blood following GV and other nerve agents (soman, sarin, VX) intoxication, corresponded to activity in the target organs and enabled us to assess the degree of inhibition corresponding to different symptoms.

Some aziridine neurotoxins were characterized using electrophysiological and behavioral methods including the method for testing of non-lethal effect (spontaneous motor activity). The suitability and sensitivity of the method was demonstrated using various derivatives of aziridines, nerve agents, mustard and BZ.

Further details on the action of antidote (7-MEOTA) against BZ intoxication, expecially different inhibitions of molecular forms of rat brain acetylcholinesterase (AChE) by 7-MEOTA and good tolerance following administration to healthy volunteers were reported.

SOMAN: Inhibition of AChE in the rabbit red blood (RBC) cells to zero activity by soman was described. A reinfusion of these erythrocytes to normal rabbits, with a corresponding decrease of up to 60 % of controls (caused by mixing with normal RBC) was demonstrated in these animals. All animals survived 24 h following transfusion.

An increase of DNA content in the liver was also demonstrated following from 1h to 3 days of soman administration. Synthesis and physico chemical properties of the ester and amide of 4-substituted 2-pyridinealdoxime and tetroxime were characterized. Their biological effects were compared with those of HI-6.

All compounds suppressed cholinergic and noncholinergic changes following soman poisoning, however, superiority of HI-6 and its ability to reactivate inhibited AChE in the brain was observed. The best prophylactic, 5 hours lasting effect was PANPAL (combination of pyridostigmine, benactyzine and trihexyphenidyle). The prophylactic effect was demonstrated when administered 2 hours before soman intoxication. Moreover, the subsequent antidotal treatment with HI-6 and benactyzine potentiated the prophylactic effect of PANPAL.

The last part described reaction of cyclodextrines (as potential decontaminants) with soman. A list of all publiactions is available.

After an accident in which Prof. Bajgar fractured two vertebra, COL Prof. MUDr. J. Fusek, DrSc. became the new head of DTOX and also the rector of the whole Academy. Other officers of the staff of the DTOX include: LTC MUDr. O. Krs, CSc. (histology); LTC doc. MUDr. J. Kassa, CSc. (biochemistry) and LTC Ing. J. Cabal, CSc. (decontamination). There are also very qualified and experienced civilian research professionals participating in research in the following areas: Dr. J. Vachek (antidotal treatment and development of antidotes); doc. RNDr. J. Patočka, DrSc. (enzymology); MUDr. J. Herink, DrSc. (electroencephalography); RNDr. M. Koupilová, CSc. (behavioral techniques) and Ing. J. Bielavský (synthesis of chemicals). Their contributions are of great importance for the final results in the many and varied areas.

There was also another research direction: in the sixties there were some rumors in the press concerning testing and weaponization of psychotomimetic agents in some NATO countries. As a reaction to this information, the Czechoslovak Army proposed a testing of potential psychotomimetic agents which might be identified for military use and experimental work was begun.

LSD-25 was tested as the first compound. Some changes in the content of catecholamines, acetylcholine and cholinesterase activity in the brain were observed, including the influence of LSD-25 on behaviour in laboratory rats and in some cases dogs. The effect of LSD-25 was tested on two groups of officer volunteers simulating the work of a commander and his staff during military operations. Members of the staff with LSD-25 administration were unable to work after 30-45 minutes following administration (60 μg/person, p.o.), and this lasted for a 3-6 hour period.

However, according to data in the literature, as well as an increasing amount of baseline data we were establishing, it was becoming clear that LSD-25 could represent a model for study of psychotomimetic effect rather than chemical warfare agent; a weaponized compound would not be LSD.

Therefore attention was focused on the group of anticholinergic as well as psychotomimetic effects. From this group of compounds, JB-336, Ditran, BZ and others were studied. Later on, BZ was found to be the most important and it was studied in detail. According to a character of this compound, physostigmine as a military antidote was first proposed. However, its side effects and relatively high toxicity were stimulating the drive to search for a new drug, preferably with reversible cholinesterase inhibitors.

Because of the known effects of Tacrin as a representative of this group, some other acridine derivatives were synthesized and characterized with the intent to find a substance less toxic than Tacrin.

Hundreds of compounds were synthesized and anticholinesterase activity was tested and tens of them were characterized toxicologically. The 7-methoxy derivative (7-MEOTA) of Tacrin was found to be the most effective against BZ intoxication. Moreover, its toxicity expressed as LD50 (rat, 258 mg/kg, i.m.) is lower than the toxicity of Tacrin (rat, 33.8 mg/kg, i.m.). Therefore it was decided on

the use of this drug as an antidote against BZ intoxication in the Czechoslovak Army. Shortened toxicity tests for single use of 7-MEOTA were performed at the DTOX of the Military Medical Academy (Hradec Králové) and the Army was equipped 7-MEOTA as an antidote against BZ (100mg tablets) in 1991.

Other experiment dealing with further study of the mechanism of 7- MEOTA were done. The results achieved demonstrated that this durg would be useful in the treatment of other conditions than just intoxication with BZ, e.g. tardive dyskinesias, Alzheimer's disease, prolongation of anaesthesia etc. For multiple chemical use of the medicament, a chronic toxicity study (1 year) was done at the Research Institute of Pharmacy and Biochemistry, VÚFB Rosice nad Labem, but it was not evaluated until the end of 1996. However, it was decided by the Ministry of Defence at the same time that the problem of treatment of civilian diseases is not in the scope of the Army and funding was stopped.

Approximately at the same time, the USA declared that all of their stocks of BZ were destroyed (CD/1074, 1991). The Czech Army now had an antidote against a non existing chemical warfare agent. This story was described and documented at the first Chemical and Biological Medical Treatment Symposium in Spiez, Switzerland in December 1994 by Professors Fusek and Bajgar (Antidote Against BZ Intoxication: a Story of 7-MEOTA).

More detailed data on this subject are given in the textbook of the Czech Military Medical Academy „History of the use of chemical weapons and negotiations on their prohibition" published by the MMA, Hradec Králové, textbook No 302, 1996.

Following velvet revolution in 1989, the new possibilities for further contacts over the world were opened. The Department was very active at the Organization for Prohibition of Chemical Weapons (The Hague): on the Task Force on Medical Treatment, the Department tabled 8 working papers. Dr. Bajgar was invited by the same organization to teach international inspectors for Convention on prohibition of chemical weapons (entered into force on 29 April 1997). He is also a member of Scientific Advisory Board on the Applied and Sciencies Analysis (USA). Department of Toxicology has been awarded (NATO Scientific Committee) by Linkage Grant dedicated to solve the problem of treatment of nerve agent poisoning, in cooperation with Military Medical Academy in München. The research results of the Department were published in more then 140 publications and cited more than 200 times (1993 - 1996) according to Institute for Scientific Information (USA). The members of the Department presented their results also in different scientific meetings, symposia and conferences, in some cases as chairs of sessions or invited speakers, e.g. 1st and 2nd C-Schutz Tagen, München, 1995 and 1997 (Fusek, Bajgar, Krs), 5th North American Congress of Clinical Toxicology Rochester, 1995 (Bajgar), 12th International Conference on Alzheimer Disease, Jerusalem, 1996 (Fusek), Workshop IITRI Chicago 1996, 1997 (Bajgar) etc.

From the results achieved, it can be mentioned following:

- synthesis of tetrahydroaminoacridine derivatives (hundreds). 7-Methoxyderivative - 7-MEOTA was chosen as the best for clinical and antidotal use. Development of tablets and injections was finished and the drug was tested on volunteers.
- synthesis of four medicaments for the treatment of nerve agents poisoning and four compounds for testing of neurotoxicity
- finalizing of development of mentioned antidotes such CHONOL I and II, FOSAN, RENOL, PANPAL and 7-MEOTA.
- synthesis of new reactivator HI-6, elaboration of method for its production and 1.5 kg of HI-6 was produced for the Czech Army.
- analytical determination of nerve agents was improved
- diagnosis of nerve agent poisoning was improved to achieve better sensitivity
- the method for neurotoxicity testing was developed
- new group of nerve agents (GV) was characterized
- the results were published in the Czech and foreign Journals
- in the period 1989-96, 6 textbooks were elaborated.
- 5 authorized attestations and 2 patents were obtained
- for education, two cinemas and nine movies were produced

List of the Heads of the DTOX:

COL prof. MUDr. Zdeněk Fink, DrSc.

Born 29 May 1919, died 15 September 1995. Head of the DTOX 1952-1968. Pharmacology of acetylcholine, study of its level following effects of some toxic compounds. Solving of vital functions during intoxication with psychtomimetic agents. Effect of parasympatholytics on isolated organs. One of the founders of military toxicology.

COL doc. MUDr. Vladislav Vondráček, CSc.

Born 26 December 1921, died 4 June 1973. Head of the DTOX 1968-1973. Study of effects of mustard and possibilities of its decontamination. Maximally permitted doses of nerve agents. Precise methodical approach.

COL prof. MUDr. Vratislav Hrdina, CSc.

Born 11 April 1926. Head of the DTOX 1973-1989. Study of catecholamine levels following intoxication with nerve agents and antidotal treatment. Study of delayed neurotoxicity.

COL doc. MUDr. Jiří Bajgar, DrSc.

Born 18 january 1944. Head of the DTOX 1989-1993. Study of effects of inhibitors and other factors on cholinesterases including their multiple molecular forms. Inhibition and reactivation of these enzymes in vitro and in vivo. Transfer of data from animal to man. Development of antidotes against nerve agents.

COL prof. MUDr. Josef Fusek, DrSc.

Born 12 May 1945. Head of the DTOX 1993-now. Study of effects of toxic compounds and drugs on isolated organs. Monitoring of physiological functions following intoxications and treatment. Solving the problems of psychotomimetic compounds and development of specific antidote - 7-MEOTA.

x Scientific degrees are introduced in Czech equivalents

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