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THE EFFECT OF INTENSIFIED NASAL BREATHING ON THE COUGH REFLEX INTENSITY IN GUINEA PIGS WITH OVALBUMIN INDUCED RHINITIS

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Abstract

The role of enhanced nasal breathing (potential microaspiration of nasal inflammatory content) in cough intensity was investigated in animal model of allergic rhinitis.

52 male TRIK strain guinea pigs sensitized with ovalbumin were recruited for the study. Animals were anaesthetized with urethane (1g/kg) and body temperature was maintained by means of external heating. A metal cannula was inserted into the thoracic cavity through the 5th intercostal space on the right side. 26 controls (C) were challenged with intranasal saline and experimental animals (E) were challenged with intranasal ovalbumin to induce rhinitis. All animals, which were divided into 4 groups, were inhaling humidified and tempered air with 2% CO2 for 10 minutes. The 1st group of C (n =13) was inhaling air enriched with CO2 through the tracheotomy; the 2nd (n = 13) group of C was inhaling through the nose with intact trachea. The 3rd and the 4th groups of E were arranged in the same manner. Tracheotomy was then completed in all groups. Laryngeal and tracheobronchial mucosa were stimulated separately to induce coughing. Cough intensity was evaluated from expiratory intrapleural pressure changes.

Intensity of laryngeal cough was gradually increased from the 1st to the 4th observed groups (10.9 ± 2.04 vs 14.4 ± 3.01 vs 24.8 ± 4.4 vs 43.3 ± 4.1 kPa). Significant differences were found between the C and E groups and between the 3rd and the 4th groups of E. Similar results were obtained for tracheobronchial cough (13.2 ± 2.1 vs15.8 ± 2.25 vs 27.6 ± 4.21 vs 31.1 ± 4.1 kPa) but difference between the 3rd and the 4th groups was not significant.

Conclusion: Intensity of cough is significantly enhanced by allergic process in the nasal mucosa. Nasal intensified breathing could enhance only laryngeal cough, tracheobronchial cough is not affected by this phenomenon.

Key words: cough, rhinitis, microaspiration, cough plasticity

INTRODUCTION

“Postnasal drip syndrome” due to various diseases of the nasal and paranasal cavities is one of the most frequent cause of chronic cough in immunocompetent adults who have a normal or almost normal X-ray of the lungs, do not smoke and do not use angiotensin-converting enzyme inhibitors (1, 2). It is likely that secretions not only mechanically stimulate airway nerve–endings mediating cough, but the inflammatory cells and mediators present in nasal discharge could sensitize them.

The cough sensitivity is increased in awake guinea pigs with experimental allergic rhinitis (3) and in pollen-sensitive patients suffering from seasonal allergic rhinitis, as well (4). On the other hand, Choudry and Fuller (5) reported that patients with post-nasal drip, suffering from dry – unproductive cough do not have increased cough reflex sensitivity, but the group of patients with post-nasal drip was not identified by the pathologies, which were responsible for this syndrome. O€Connel et al. (6) reported that subjects with upper respiratory tract infection had increased capsaicin cough sensitivity than during baseline testing and after the recovery.

There is no clear experimental or clinical evidence which mechanisms are responsible for either stimulation or sensitization of laryngeal and tracheobronchial nerve–endings mediating cough reflex during rhinitis. One of the hypothesized mechanisms responsible for heightened cough reflex sensitivity is microaspiration of nasal secretion into the lower airways due to labored breathing through the nasal cavity, which patency is due to inflammatory process enormously decreased (7). It is well known that air flow through the nasal cavity is turbulent due to

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nasal turbinate system, and this phenomenon is necessary for optimal function of the nasal cavity (warming, humidification and filtration of inspired air), that were reviewed elsewhere (8). Inflammatory processes due to different causes usually result into congestion of the nasal mucosa and nasal hypersecretion. Increased air velocity through the partially blocked nasal passages and nasal discharge could be condition for aerosol production during labored inspiration. Nasal discharge contains mediators released from inflammatory cells that could be breathed in, and modulate irritability of afferent mucosal nerve-endings in the larynx and trachea, as well.

Here we address the question: whether intensified nasal breathing during experimental allergic rhinitis could enhance cough response in this model of experimentally induced cough.

**AIM**

The purpose of this study was to assess the effect of intensified nasal breathing on mechanically induced cough in anaesthetized guinea pigs with ovalbumin induced rhinitis.

**METHODS**

**Animals**

All experiments were approved by Jessenius Faculty of Medicine Ethical Committee and were in agreement with Strasbourg Declaration. Animals were housed in approved animal holding facility maintained at a room temperature 21–22°C, humidity 60–70%, ventilation, 12 h light–dark cycle and had free access to water and standard animal food.

Male TRIK strain guinea pigs (n = 52), weighing 300 – 400 g were obtained from Department of Experimental Pharmacology, Slovak Academy of Science, Dobra Voda, Slovak Republic and used after at least 1-week adaptation.

**Model of experimental allergic rhinitis**

All animals (n = 52) were sensitized with ovalbumin (10 mg) administered intraperitoneally together with aluminum hydroxide (100 mg) in saline (1 ml i.p) (9). Twenty-one days after successful sensitization was confirmed by skin prick test (intradermal injection of ovalbumin 25 µl of 200 µg.ml⁻¹) on the skin of the back. Only sensitized animals with marked erythema and edema were involved in the study. In the first phase of our experiment one half of animals (n = 26) was challenged with intranasal ovalbumin to ascertain if allergic nasal response is present (sneezing, nasal discharge and worsening of breathing). If present, experimental allergic rhinitis was taken as confirmed. Controls (n = 26) were challenged intranasally with saline.

**Cough reflex sensitivity in awake animals**

As we mentioned before, sensitivity of the cough reflex is significantly increased in rhinitic animals in comparison to sensitized animals without clinical manifestation of inflammatory process in the nasal cavity (3). Confirmation of such a result in our experimental group was necessary for the study that followed.

Awake guinea pigs were placed into the plastic cylinder (a part of plethysmographic box equipment) allowing the animal immobilization. Then the animals were placed individually in a body-plethysmograph (type 855, Hugo Sachs Electronic, Germany) consisting of a head chamber and a body chamber. The opening between the head chamber and body chamber was equipped with a plastic collar lining around animal neck to prevent communication between the chambers. Appropriate collar size was chosen for each animal to prevent neck compression, which could cause airway obstruction and/or mechanical stimulation of the upper trachea and larynx.

To expose an animal to aerosol, the head chamber was connected to a nebulizer (Pari Provokation Test I, Menzel, Germany, manufacturer’s specification: output 5 l.min⁻¹, particle mass median aerodynamic diameter 1.2 µm). A suction device adjusted to the same input 5 l. min⁻¹ was connected to the head chamber to maintain constant airflow through the chamber.
during aerosol administration. Respiratory changes in the airflow were measured using pneumotachograph (Godart, Germany) with Fleisch head (No. 1, Gould Godart Statham BV) connected to the head chamber and recorded directly with the moving pen recorder (Multiscriptor Hellige, Germany).

Respiratory sounds including cough and sneezing were recorded with a microphone placed in the roof of the head chamber and connected to a preamplifier and loudspeaker.

Cough challenges were performed using inhalation of saline and gradually increased concentrations of citric acid (CA) (Lachema) (0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mol.l⁻¹) for 30 seconds. One-minute intervals were taken between each tussive challenge. Cough was detected from the expiratory change of airflow interrupting basic respiratory pattern accompanied by a cough sound. Cough sound was recorded and analyzed for power spectra by Fast Fourier Transformation computer implementation COUGH2 (Prof. Lorand A. Debreczeni, St. Emeric Teaching Hospital, Budapest, Hungary). Use of this method made it possible to recognize the cough from the sneezing.

Sensitivity of cough reflex was evaluated from the number of coughs induced by an inhalation of gradually increased concentrations of CA. The number of coughs was counted during the 30 second of the citric acid inhalation and subsequent 1-min period.

Then the guinea pigs (n = 26) were intranasally challenged with ovalbumin (75 µg in 0.015 ml of saline) to induce rhinitis. Just after the development of nasal symptoms (sneezing, nasal discharge, nasal rubbing and crackles - approximately in 15 minutes) cough reflex sensitivity was determined. The cough sensitivity in the control group of animals (n = 26) was determined by the same manner after intranasal administration of saline.

Cough responses to different concentrations of CA were compared between control (i.n. saline) and (i.n. ovalbumin) group of animals with rhinitis.

**Mechanically - induced cough in anaesthetized animals**

Animals (n = 52) were anaesthetized by intraperitoneal administration of urethane (Riedel de Haen, AG, Germany, 1.1 mg.kg⁻¹ i.p) and placed in the supine position on the heated operating table. Body temperature was continually monitored and maintained at 37 – 38°C. The trachea and the larynx were explored, and tracheotomy was performed in a half (n = 26) of animals. A plastic cannula (4 mm of external diameter) allowing spontaneous breathing was introduced into the trachea between the 7th and the 9th tracheal rings. A metal interpleural cannula was introduced into the right hemithorax via the incision of anterior thoracic wall at the 5th intercostal space and connected to electromanometer (Electromanometer HSE, Hugo Sachs Electronic) for recording of interpleural pressure.

Twenty-six controls were challenged with intranasal saline (0.015ml), experimental animals (n = 26) were challenged with intranasal ovalbumin (75 mg in 0.015 ml of saline) to induce rhinitis in 15 minutes. The 1st group of control animals (n = 13) was breathing through the tracheotomy air enriched with CO₂, the 2nd group of control animals (n = 13) was breathing through the nose air enriched with CO₂ with intact trachea. The same experimental design was used for two subgroups of experimental group of animals. All animals were inhaling humidified and tempered air with 2% CO₂ to enhance ventilatory drive for 10 minutes. After 10 minutes of intensified breathing the tracheotomy was completed in all subgroups of animals.

Cough was evoked by mechanical stimulation of the laryngeal and tracheobronchial mucosa separately. Coughing was induced from the tracheobronchial tree by insertion of a 0.3 mm diameter nylon fiber through the tracheal cannula, until its tip was judged to be near the carina and main bronchi. Repeated caudal advancements of the fiber to touch the carina and nearby airways walls were carried out over periods of 7 s. To evoke coughing from the larynx a similar procedure was carried out with the nylon fiber inserted cranially into the cervical trachea until its tip could be pushed against the laryngeal mucosa.

Tracheobronchial and laryngeal cough were analyzed separately. The number of cough efforts (NE) during the cough bout, intensity of the cough bout (ICB = sum of all positive
Deflections of interpleural pressure during all cough efforts in the cough bout and average intensity of cough effort (ICB/NE ratio) were used to quantify cough (9). These parameters were compared among four subgroups designed in our experiment.

**Experimental protocol**
For details and timing of separate stages of the study see scheme.

**Statistical analysis**
Data for the number of cough efforts are expressed as a median and interquartil range. Data represented intensity of cough bout and, average intensity of cough efforts are expressed as arithmetic mean ± S.E.M (standard error of mean). For statistical analysis the Friedman test and the nonparametric multiple comparison testing by Dunn’s test were performed. P< 0.05 was considered to be significant.
RESULTS

Model of allergic rhinitis

Clinical symptoms of rhinitis have occurred in all sensitized animals that were challenged intranasally with ovalbumin within 15 minutes after the challenge. These nasal symptoms involved sneezing, nasal discharge, nasal rubbing and nasal crackles. Some of the animals have showed signs of labored breathing - paradoxical movements of abdominal wall during inspiration (due to congestion of nasal mucosa).

Cough sensitivity in awake animals

The number of coughs during inhalation of all concentrations of citric acid in control and rhinitic groups was [2.5 (0 - 4.5) vs 6.5 (3 - 9.5), p < 0.05]. Differences in cough response between controls and rhinitic animals were found during inhalation of citric acid of 0.1; 0.2; 0.4 and 0.8 mmol/1 (Fig. 1).

Figure 1. Cough response to gradually increased concentration of citric acid in awake guinea pigs. Empty columns represent cough response of control animals, dark columns represent cough response of animals with rhinitis. Number of coughs is expressed as median and interquartil range (q), * p < 0.05.

Cough intensity in anaesthetized animals

The number of cough efforts was gradually increased in studied subgroups of animals (controls - the 1st subgroup was breathing through the tracheotomy and the 2nd subgroup was breathing through the intact upper airways; and rhinitic groups (the 3rd subgroup was breathing through the tracheotomy and the 4th subgroup was breathing through intact upper airways) starting from the 1st to the 4th subgroups [2 (1-2) vs 2 (2-3) vs 4 (2-4) vs 4 (4-6)] after laryngeal stimulation and after tracheobronchial stimulation [3 (2-3) vs 3 (2-4) vs 5 (2-5) vs 5 (3-6)], as well. Significant differences were found between the control groups and the experimental groups (p < 0.001) for laryngeal cough and (p < 0.05) for tracheobronchial cough (Fig. 2).

The intensity of the cough bout (ICB) was gradually increased from the 1st to the 4th subgroups during laryngeal provocation (10.9 ± 2.04 vs 14.4 ± 3.01 vs 24.8 ± 4.4 vs 43.3 ± 4.1 kPa), and during the tracheobronchial stimulation (13.2 ± 2.1 vs 15.8 ± 2.25 vs 27.6 ± 4.21 vs 31.1 ± 4.1 kPa), as well. Significant differences were found between the control groups and the experimental groups (both p< 0.001), during laryngeal stimulation, (p < 0.05) during tracheobronchial stimulation, and between the 3rd and the 4th subgroups (p < 0.05) during laryngeal stimulation. Difference found between the 3rd and the 4th subgroups during tracheobronchial stimulation was not significant (Fig. 2).
Average intensity of cough effort (ICB/NE ratio) was not affected during tracheobronchial cough (5.5 ± 0.61 vs 5.01 ± 0.84 vs 6.64 ± 0.46 vs 6.81 ± 0.56 kPa), however average intensity of laryngeal coughs gradually increased from the 1st to the 4th observed subgroups (5.4 ± 1.14 vs 5.3 ± 0.91 vs 7.39 ± 0.55 vs 9.1 ± 91 kPa). Significant differences were found between the 1st and 2nd subgroups in comparison to the 3rd and the 4th subgroups (p < 0.05).

Figure 2. Intensity of mechanically-induced cough in anaesthetized guinea pigs. The figure explore number of cough efforts (NE), intensity of cough bout (ICB) and average intensity of cough effort (AI) for laryngeal and tracheobronchial cough, in control subgroups (1, 2) and rhinetic subgroups (3, 4). Empty columns represent cough response of those animals who were breathing through the tracheotomy, dark columns represent cough response of those animals who were breathing through the nose. NE is expressed as median and interquartil range (q), ICB and AI are expressed as mean and S.E.M. * p < 0.05, ** p < 0.001.
DISCUSSION

There is no clear experimental evidence which mechanism is responsible for either stimulation or sensitization of laryngeal and tracheobronchial nerve-endings mediating cough reflex during rhinitis (3). One of the hypothesized mechanisms of sensitization of these nerve-endings could be microaspiration of nasal secretion into the lower airways.

The aim of the present study was to test the assumption that nasal intensified breathing (that could enhance process of creation of aerosol in the nasal cavity and probable microaspiration of nasal content into the lower airways) could also affect mechanically-induced cough.

As we previously reported, sensitivity of the cough reflex in animals with rhinitis is heightened in comparison to controls (3). This phenomenon was tested in awake animals that were coughing after tussive stimuli of chemical origin (capsaicin, citric acid). To test the hypothesis that we address about intensified nasal breathing and the effect of this phenomenon on coughing, we designed an experiment with the possibility of by-passing the nose, for which general anaesthesia was necessary. It is well known that anaesthetized guinea pigs do not cough after administration of tussive substances (10). So we should use a model of mechanically-induced cough in this study. But first of all, we ensured that experimental animals in our study had significantly heightened cough reflex sensitivity to citric acid, during the period of acute rhinitic symptoms. This finding is consistent with the data reported previously.

We have found that animals with rhinitis, nevertheless they were breathing through the tracheotomy or through the intact upper airways, have heightened intensity of cough reflex. The cough response was significantly increased when coughing was provoked both from the larynx and from the trachea, as well, but there are several differences between laryngeal and tracheobronchial cough. Intensity of laryngeal cough could be entirely explained by increased frequency of cough and parallel enhancement of average intensity of cough effort. On the other hand, increased intensity of tracheobronchial cough could be ascribed only to increased cough frequency. Why it is so, we have not convincingly explained. Although there are recent papers concerning central mechanism responsible for generation of cough pattern, they do not explore, either discuss possible differences in regulation of pattern for both the laryngeal or tracheobronchial cough (11).

But from our results it is clear that cough response during rhinitis is increased both in awake animals that were coughing to chemical stimuli and in anaesthetized animals that were coughing to mechanical stimuli. There is one possible explanation of such a result.

We could suppose that afferent nerve-endings in the nasal mucosa were stimulated by the process of allergic inflammation of nasal mucosa, via liberation of a number of mediators responsible for the early phase of allergic response. These mediators are believed to stimulate afferent nerve-endings (12, 13). There are recent papers discussing mechanism of plasticity of the cough reflex termed central sensitization (14, 15). It means modulation of cough response by afferent stimuli arising from nasal cavity, esophagus, etc. Although it is well established that cough-mediating afferent nerve fibers are supplied by the vagus nerve, the identity and precise central projections of the cough fiber(s) are not known. Even less information is available on central integration and regulation of cough (15). The simplest explanation of the phenomenon described in this study is that some of the central terminals of nasal afferent neurons affect the activity of the secondary sensory neurons of the cough pathways. This may occur either via monosynaptic or polysynaptic connections. Alternatively, that the trigeminal afferents influence the activity of the nucleus tractus solitarii (16).

Except central sensitization of the cough reflex, there could be recruited mechanisms responsible for peripheral sensitization. It means, mechanisms affecting neurophysiological characteristics of nerve-endings mediating cough in the larynx and more peripheral airways. Especially -irritability of these nerve endings and cough threshold levels to standard tussive stimuli. The process of sensitization of nerve-endings mediating cough could be caused by transportation of a little amount of nasal content (inflammatory mediators, cells, and their products) into the lower
airways. It is well known that inflammatory mediators (histamine, kinins, metabolites of arachidonic acid, etc.) could modulate sensitivity of afferent nerve–endings in the airways and could cause a phenomenon of hypersensitivity/hyperreactivity of the airways (17).

Whereas, the nasal cavity was by–passed in a half of animals, we could not exclude the possibility of transportation of a little amount of nasal inflammatory secretion into the lower airways by mucociliary clearance of the upper airways. General direction of mucociliary clearance from the nose is toward the naso– and hypopharynx. It is not probable that beating of the cilia could transport the mucus from the nasal cavity into the larynx, because cilia of laryngeal mucosa are beating freely in the oral direction to transport the mucus into the hypopharynx (18).

One additional factor should be taken into consideration. General anaesthesia may cause a depression of swallowing reflex. Abnormal swallowing also could be seen after fixation of trachea and tracheotomy. The mechanisms responsible for abnormal swallow are not entirely known, however, several causes have been proposed: decreased laryngeal elevation due to fixation of the trachea and obstruction by the cuff that leads to esophageal obstruction, causing secretion to stagnate above the cuff in the proximal esophagus and hypopharynx (19). There may be a possibility of transportation of a little amount of secretion into the airways, especially into the larynx. But the heightened cough response arose both from the larynx and the trachea, so we prefer the hypothesis of central sensitization of cough pathways/center.

This mechanism of central sensitization could be very important in pathogenesis of cough during rhinitis. We have found that stimulation of nasal mucosal afferents with capsaicin could enhance coughing evoked by chemical and mechanical stimuli in animals (20) and in human healthy volunteers, as well.

The role of intensified nasal breathing in mechanically-induced cough was tested in this study. We supposed that intensified nasal breathing could enhance creation of the inflammatory aerosol in the nasal cavity and its aspiration into the lower airways. We do not have a convincing evidence for such processes in the nasal cavity following experimentally–induced rhinitis. But this hypothesis could be grounded, because it is probable that the increased resistance of nasal cavity due to edema of mucosa and vessel congestion requires more intensive inspiratory efforts. This in naturally intensified breathing was potentiated also by inhalation of carbon dioxide in this study. We have found that intensity of tracheobronchial cough after intensified nasal breathing was heightened but not significantly in the animals that were breathing through the nose in comparison to those who were breathing through the tracheostomy. But on the other hand, there was found a significant enhancement of laryngeal cough in those animals that were breathing through the nose in comparison to those who were breathing through the tracheostomy.

Based on these results we could suppose that intensified breathing could facilitate transportation of a part of nasal content (via aerosol) into the larynx, with subsequent enhancement of irritability of laryngeal nerve–endings as a consequence of peripheral sensitization. This finding is in agreement with previously published results (21) showing that cutting of the superior laryngeal nerves in the animals, abolished cough reflex sensitivity, significantly heightened during allergic rhinitis. On the basis of recently published data (21) and our present results we could suppose that laryngeal afferent nerve – endings may be responsible for the increased cough response in rhinitic subjects.

Conclusions
1. Mechanically–induced cough in anaesthetized guinea pigs with allergic rhinitis is significantly heightened in comparison to control animals, both provoked from the larynx and trachea. It means it showed the same tendency as did the chemically induced cough.
2. The process of central sensitization may contribute to enhancement of cough evoked both from the larynx and trachea in guinea pigs with experimentally induced rhinitis.
3. Intensified nasal breathing enhanced cough induced by mechanical stimulation of the larynx that may be caused by transportation of the mucus or inflammatory aerosol from the nasal cavity into the larynx with subsequent modulation of the sensitivity of afferent nerve–endings mediating cough. (mechanism of peripheral sensitization).
Additional factors responsible for coughing in patients suffering from rhinitis need to be further elucidated and defined.

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INFLUENCE OF DICYCLOMINE ON IN VITRO REACTIVITY OF URINARY BLADDER SMOOTH MUSCLE IN GUINEA PIGS

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Abstract

Introduction: Nowadays, new therapeutic approaches are sought for treatment of functional disorders of urinary bladder, which could prevent the decrease of patients’ quality of life. One of the possibilities of conservative therapy is the administration of anticholinergics. The aim of this study was to verify the in vitro action of dicyclomine (used in the therapy of irritant bowel disease) on urinary bladder smooth muscle in guinea pigs and to compare its effect with previously tested oxybutynin.

Methods: The reactivity of the urinary bladder smooth muscle was estimated by in vitro method using organ chambers. The smooth muscle strips were prepared from guinea pig urinary bladders and aerated under the tension in Krebs-Henseleit’s solution in the organ bath. The cumulative concentration-response curves to acetylcholine (10⁻⁸–10⁻³ mol.l⁻¹) were plotted before and after adding of dicyclomine at concentration of 10⁻⁶, 10⁻⁵, 10⁻⁴, and 10⁻³ mol.l⁻¹.

Results: Dicyclomine caused decrease of urinary bladder smooth muscle reactivity to acetylcholine. This decrease was statistically significant only at the concentration of 10⁻⁴ and 10⁻³ mol.l⁻¹ of dicyclomine.

Conclusions: Dicyclomine significantly influenced the reactivity of urinary bladder smooth muscle in guinea pigs to acetylcholine. By comparing the influence of oxybutynin we can conclude, that oxybutynin caused significantly stronger decrease of reactivity to acetylcholine than dicyclomine.

Key words: urinary bladder, contraction, oxybutynin, dicyclomine, smooth muscle

INTRODUCTION

An increasing prevalence of overactive bladder in the older population was described in various studies (1). Detrusor hyperactivity could be a result of several pathomechanisms, most probably with myogenic or neurological basis (2). Muscarinic receptors mediate normal bladder contraction, but also contractions of overactive bladder, so antimuscarinic drugs can block detrusor contractions in patients with bladder hyperactivity (3). There are also other ways to influence hyperreactivity, including drugs which have primary effects on membrane ion channels (Na⁺, Ca²⁺, K⁺), prostaglandin synthesis inhibitors (4) as well as agents modifying the activity of released mediators into the synaptic cleft. To sum up, also α-adrenoceptor antagonists, β-adrenoceptor agonists, vasopressin analogue, antidepressants like imipramine, botulotoxin, and capsaicin should be mentioned.

The antagonism of muscarinic receptors is the major mechanism of action of various drugs, used in many pathological conditions. Dicyclomine, as an agent used in the therapy of irritant bowel disease, seems to be usable (due to its potent antimuscarinic activity) also in overactive urinary bladder smooth muscle (5).

The aim of our study was to study the effects of dicyclomine on in vitro reactivity of urinary bladder smooth muscle in guinea pigs. Oxybutynin, which is able to decrease hyperactivity of detrusor by summation of effect of muscarinic receptors blockade and local anesthetic effect, is considered to be a conservative therapy standard of overactive urinary bladder in humans in Slovakia (6). In this study we compared its effect with that of dicyclomine.

METHODS

The reactivity of urinary bladder smooth muscle was estimated by in vitro method (7,8,9). 8 animals weighting 250-350 g were used. The preparations of urinary bladder smooth muscle...
strips (2 x 2 x 15 mm) from guinea pigs were mounted between two hooks and placed into a 30 ml organ chamber containing Krebs-Henseleit buffer of the following composition: NaCl 110.00 mmol.l⁻¹, KCl 4.80 mmol.l⁻¹, CaCl₂ 2.35 mmol.l⁻¹, MgSO₄ 1.20 mmol.l⁻¹, KH₂PO₄ 1.20 mmol.l⁻¹, NaHCO₃ 25.00 mmol.l⁻¹ and glucose 10.00 mmol.l⁻¹ in glass-distilled water. The organ chambers were maintained at 36.5 ± 0.5 °C and were aerated continuously with a mixture of 95% O₂ and 5% CO₂, to maintain pH 7.5 ± 0.1. One of the hooks was connected to a force transducer (TSR 10G, VÝVOJ Martin, Slovakia) and an amplifier (M1101 SUPR, Mikrotechna Praha, Czech Republic) and tension recordings were made on a Line Recorder TZ 4620 (Labotatorní přístroje Praha, Czech Republic). The tissue strips were initially set to 4 g of tension (30 minutes loading phase). After this period, the tension in each strip was readjusted to a baseline of 2 g (30 minutes adaptation phase). During both of the periods the tissue strips were washed at 10 minutes intervals. Thereafter, cumulative doses of acetylcholine (10⁻⁸ to 10⁻³ mol.l⁻¹, subst. Sigma-Aldrich) were added and a continual graphical recording of contractions was made. This recording was named “Control”. After 25 minutes of washing-up period, water solution of dicyclomine (subst. Sigma-Aldrich) was added into each chamber in order to reach the concentrations of 10⁻⁶, 10⁻⁵, 10⁻⁴ and 10⁻³ mol.l⁻¹. After 15 minutes period of incubation the amplitudes of contractions (g/100mg) of urinary bladder smooth muscle strips to the cumulative doses of acetylcholine (10⁻⁸ to 10⁻³ mol.l⁻¹) were recorded. These records were used for evaluation of the contractile responses (10).

A non-parametric ANOVA test was used for the statistical analysis. Results are presented as mean _ standard error of the mean (SEM). A probability level of p<0.05 was accepted as significant. All experiments were conducted in accordance with basic ethical norms and Helsinki Declaration of 1975, revised in 1983.

**RESULTS**

Addition of acetylcholine into the organ bath with urinary bladder smooth muscle strip in cumulative manner resulted in a dose-dependent increase of the contractile responses in controls. In the organ baths with dicyclomine in all of the concentrations, the contractile responses of urinary bladder smooth muscle were decreased. This decrease was statistically significant only at concentrations of 10⁻⁴ and 10⁻³ mol.l⁻¹ of dicyclomine (Fig.1).

![Fig. 1](image-url) Reactivity of urinary bladder smooth muscle in guinea pigs after adding of dicyclomine to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) with standard error of the mean (SEM). One asterisk represents statistical significance of difference with p < 0.05 (DIC-6 = dicyclomine at concentration of 10⁻⁶ mol.l⁻¹, etc.).
Figures 2 and 3 show the comparison of the urinary bladder smooth muscle reactivity to oxybutynin (6) and dicyclomine at concentration of $10^{-5}$ and $10^{-4}$ mol.l$^{-1}$ in guinea pigs. In both concentrations, oxybutynin caused significantly stronger decrease of reactivity to acetylcholine than dicyclomine.

![Figure 2](image1)

**Fig. 2** Comparison of the urinary bladder smooth muscle reactivity in guinea pigs after adding of dicyclomine (DIC, thick solid line) and oxybutynin (OXY, thin dashed line) in dose of $10^{-5}$ mol.l$^{-1}$ to cumulative doses of acetylcholine.

![Figure 3](image2)

**Fig. 3** Comparison of the urinary bladder smooth muscle reactivity in guinea pigs after adding of dicyclomine (DIC, thick solid line) and oxybutynin (OXY, thin dashed line) in dose of $10^{-4}$ mol.l$^{-1}$ to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) with standard error of the mean (SEM). One asterisk represents statistical significance of difference with $p < 0.05$. 
DISCUSSION

The urinary bladder and any impairment of its functioning can influence the behavior of the whole organism, as the ability to accumulate urine and consecutively release it, belongs to basic social needs. Any changes in this basic need can disturb its integration and social positioning and so could lead to significant decrease of quality of life. Therefore, it is very necessary to study the mechanisms participating in the urinary bladder activity and to be able to modulate it in case of disorder.

The problems of hyperresponsiveness or hyperreactivity of smooth muscle in various organ systems, like respiratory system, gastrointestinal tract, and skin are found very often (11). Similarly, the urinary bladder „stability” problems are very often, too. Švíhra et al. (2001) showed in their recent study that overactive, „unstable”, bladder incidence in population of Slovakia rises especially with age (1). The frequent voiding and bladder fullness sensations, sensation of not complete bladder emptying after voiding and later also impaired ability to accumulate urine – incontinence – are considered as typical symptoms of overactive bladder. And especially the incontinence can significantly impair the patients’ quality of life.

The parasympathetic nervous system, similarly to other organ systems, plays the major role in the regulation of the urinary bladder smooth muscle (12). Five pharmacologically different muscarinic receptors (M<sub>1</sub>-M<sub>5</sub>) are distinguished, with representation of receptors M<sub>2</sub> and M<sub>3</sub> in humans. Although M<sub>2</sub> receptor was found to be the predominant one, the major role in contraction responses plays the M<sub>3</sub> receptor subtype (13,14).

The role of postsynaptic M<sub>2</sub> receptors is expressed as the ability to act against beta-adrenergic receptors, whose activation enables the urinary bladder smooth muscle relaxation and urine accumulation (15,16). Besides, M<sub>2</sub> receptor stimulation is associated with an activation of non-specific cation channels and inactivation of potassium channels (17,18). To conclude this, M<sub>2</sub> receptors are responsible for a direct contraction of the smooth muscle during voiding, until postsynaptic M<sub>2</sub> receptors prevent the relaxation of the smooth muscle evoked by sympathetic activation. These two effects are synergical in order to expel the urine from the urinary bladder effectively (19).

An abnormal stimulation of muscarinic receptors is responsible for the contractile properties of the urinary bladder smooth muscle in diseased state (overactive bladder). Muscarinic M3 receptor antagonists have therapeutic potential for the treatment of disorders associated with altered smooth muscle contractility or tone. These include irritable bowel syndrome, chronic obstructive airways disease and urinary incontinence. Dicyclomine is a potent muscarinic receptor antagonist on the ileum with selectivity for M<sub>3</sub> receptor in absence of cardiovascular effects and with selectivity over inhibition of salivary secretion (20).

However, the musculotropic action of dicyclomine in bladder muscle has been attributed to its local anesthetic activity. This „local anesthetic” property is not sufficient to explain the action, as showed Downie and McGuire (21). They found that contractions elicited by replacement of calcium during depolarization with 80 mM K<sup>+</sup> were only slightly affected by atropine or scopolamine, but were antagonized in a noncompetitive manner by dicyclomine (21).

Maggi and Meli (5) showed that intravenously administered dicyclomine produced a dose-dependent decrease of eserine-induced muscle tone and suppressed phasic contractions in rabbit detrusor (5).

In our in vitro experiments using organ baths we found that dicyclomine causes dose dependent decrease of acetylcholine-induced contractions in guinea pigs urinary bladder smooth muscle. This finding is in consistence with literary sources regarding the effect of dicyclomine on gastrointestinal smooth muscle.

One of the most effective treatments for overactive bladder disease represents the anticholinergic therapy (oxybutynin) (22). Oxybutynin causes depression of detrusor hyperactivity, which is reached by the blockade of muscarinic receptors, direct relaxation of detrusor and by a local anesthetic effect. However, the local anesthetic effect is present only by
intravesical administration of oxybutynin. Oxybutynin possesses higher affinity to muscarinic receptors M1 and M3 than to M2 subtype. The clinical importance of this affinity is still unclear, as oxybutynin acts through its active metabolites. Oxybutynin solidly inhibits the urinary bladder hyperreactivity and is therefore recommended as first line therapy of the overactive bladder (22).

However, the dryness in mouth during a standard dosage regimen was reported in a relatively high number of patients – 80 % (22). Therefore, other agents with potential effect on the urinary bladder smooth muscle are tested, including calcium channel blockers verapamil and nifedipine (23), highly selective antagonist of M₃ receptor subtype darifenacine (24), or imipramine with anticholinergic action and 5-hydroxytryptamine blocking effect (22). Local effect and desensitization of the sensory receptors in urinary bladder participates in effect of intravesically-administered capsaicin (25).

Dicyclomine, used in the therapy of irritant bowel disease, showed in our in vitro experiments the ability to suppress the contractility of urinary bladder smooth muscle, which was, however, significantly lower than that of oxybutynin in the same concentration. Therefore, its usage in urinary bladder disorders is questionable and needs further studies.

In conclusion, according to our results, we can confirm that dicyclomine significantly influenced the reactivity of urinary bladder smooth muscle in guinea pigs to acetylcholine. By comparing the influence of oxybutynin (6) we can conclude that oxybutynin caused significantly stronger decrease of reactivity to acetylcholine than dicyclomine. These findings are still objects of further research.

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STUDY OF LESIONAL T–LYMPHOCYTE SUBTYPES AND SYSTEMIC PSORIASIS TREATMENT
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Abstract
The ethiopathogenesis of psoriasis vulgaris (PV) has been studied for many years and in spite of many achievements there are still problems waiting to be resolved. The aim of our work was to follow changes in T-cell subpopulations in inflammatory psoriatic skin lesions after topical cyclosporin A treatment. Nineteen patients suffering from severe PV were examined. Material for immunohistopathological examination was taken from the margins of chronic psoriatic lesions of 19 patients before and 4-week after the treatment with cyclosporin A. Numeric value of CD4 T-lymphocytes, CD8 T-lymphocytes, their ratios and the values of Psoriasis area and severity index (PASI) score were followed. Significant decrease in CD4 T-lymphocytes and slight decrease in CD8 T-lymphocytes were observed after treatment. CD4:CD8 ratio dropped from the values of 2.10 to 1.68. Dramatic decrease in PASI score was observed – from the average value of 23.34 to the average value of 11.97. The authors compare and discuss the results of a few similar cytophotometric studies found in the available literature. The obtained results are in agreement with those reported in the literature and indicate that cyclosporin A treatment of PV patients is well-founded.

Key words: psoriasis, PASI, lesional CD4,CD8 lymphocytes, cyclosporin A treatment

INTRODUCTION
Pathogenesis of psoriasis is still not quite clear. Attention has been focused on pathogenetic spectrum of this disease mainly from the point of view of abnormalities in keratinocyte proliferation and their differentiation (1). Complex nature of the disease is underlined by heavy presence of inflammatory cells in infiltrate and multiple action of various cytokines and adhesion molecules. The latest knowledge in the field of immunology of psoriasis and interaction processes between the cells at the site of inflammation suggest possible autoimmune background of this disease (2).

Healthy skin contains a certain number of T-lymphocytes, that may significantly increase in the course of many inflammatory skin diseases (5). In PV, major pathological abnormalities occur in epidermis due to increased turnover of keratinocytes. Hyper- and parakeratosis in the psoriatic sites are accompanied by inflammation in the dermis. Cytomorphological and pharmacological studies of psoriatic plaque confirmed the pivotal role of T-lymphocytes and their subpopulations like helper CD4+ cells and suppressor/cytotoxic CD8+ cells (21). Following this paradigm, we have studied the effect of psoriasis treatment with cyclosporin A (CyA) (Sandimmun – Neoral, Novartis) on the local levels of CD4+ and CD8+ lymphocytes. Cyclosporin A (CyA) is a lipophilic undecapeptide isolated from the fungus Tolypocladium inflatum. It is an effective immunosuppressant with selective and reversible inhibitory effect on T-lymphocytes. In animal studies, as well as in clinical practice, CyA has been proved as effective in prophylaxis and treatment of autoimmune dermatoses including psoriasis (6). It is used as a drug of choice for the treatment of moderate to severe forms of psoriasis vulgaris including arthropatic form.

PATIENTS AND METHODS

Nineteen patients (11 males, 8 females) suffering from severe form of psoriasis vulgaris were examined and treated with cyclosporin A microemulsion (Sandimmun – Neoral, Novartis). The age

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of patients ranged from 32 to 58 yrs, 42 years on average. Extent and severity of PV were measured using PASI (psoriasis area and severity index) score. Before starting the treatment and 4 weeks after, skin excision was taken from the lesion margins using 5 mm punch biopsy needle. Specimen was processed by the method of frozen cuts, and monoclonal antibodies against CD4 and CD8 markers (DAKO) were applied. Ten fields of vision were examined under magnification of 480x (semiquantitative method). Following thorough morphological evaluation, T-lymphocytes with positive reaction were counted. Absolute numbers of CD4+ and CD8+-cells, their ratios, as well as their changes in the course of treatment were statistically evaluated using two-tailed paired t-test.

Treatment of psoriatic patients with cyclosporin A was conducted according to approved guidelines with the average dosage of 3mg/kg/day. Evaluation of the extent and severity of the disease was made using PASI score.

The study was approved by institutional Ethical Committee assembled ad hoc in Martin University Hospital.

RESULTS

The results obtained in our group of patients after the first and second skin excisions are summarized in Table 1. Before the treatment, CD4+ number was within the range of 22 - 178, with the average of 74.8 of CD4+ per 10 fields of vision. CD8+ were less numerous, their values ranged from 8 to 83, with the average of 38.5 of CD8+ per 10 fields of vision. CD4+/CD8+ ratio during the first excision was about 2.1. PASI score in the patients was 23.3 on average, ranging from 6.3 up to 42.8.

After 4-weeks of CyA treatment, the numbers of activated lymphocytes were decreased. The CD4+ cells decreased to the average value of 52.8, the difference was statistically significant (p-level < 0.05). The CD8+ cells decreased to the average value of 31.9, the difference was not statistically significant.

Tab.1. CD4+ and CD8+ counts measured before (1st excision) and after (2nd excision) the tretment with cyclosporin A in 19 patients suffering from severe form of psoriasis. The ratio CD4/CD8 was calculated and the clinical findings were expressed as PASI score.
The decrease of CD4+/CD8+ ratio from the value of 2.10 to 1.68 was slightly exceeding the limit of significance (p = 0.055) but complying to the trend. Decrease in PASI score from the average value of 23.34 to the average value of 11.97 was the most remarkable. Its statistical significance reached the value of 2.92 \cdot 10^{-6}.

**DISCUSSION**

Psoriasis is an autoimmune disease mediated by clones of activated T-lymphocytes (7). There is a consensus in literature that CD4+ and CD8+ subpopulations of lymphocytes and the cytokine profile typical for T_{h1} route of activation are pivotal in the pathogenesis of the disease. Dermal infiltrate consists mostly of CD4+ cells, while T-lymphocytes scattered or infiltrating the epidermis belong mostly to CD8+-cells (8).

Intercellular adhesion molecules like ICAM-1 contribute significantly to retention of T-lymphocytes in the epidermis and their firm bond with keratinocytes (7). In our material, epidermal lymphocytes were found only occasionally and in insignificant quantity, therefore we did not pay any special attention to evaluate this finding. This condition may be the result of preceding local therapy with corticoid externals used by psoriatic patients. T-lymphocyte populations in the peripheral blood of patients with PV were examined using flow-cytometry. In patients with psoriasis, the activated lymphocytes presented were recruited mainly from the CD4+-cells (64% - 85%), and less frequently from the CD8+-cells (10% - 32%) (8).

In the peripheral blood of healthy people, Petrisková (17) found referential values of CD4+-cells in 33%-53% and CD8+-cells in 24%-50% of activated T-lymphocytes. It has been shown that in the peripheral blood of psoriatic patients, CD4+ were increased while CD8+ are decreased. Thus, in the blood of psoriatic patients not only changes in absolute number of the T-lymphocyte subpopulations has been observed but also shifts in their proportion. The reversal in the proportion of CD4+ and CD8+-cells is considered significant during psoriasis remissions and relapses (5).

Literature data on increased presence of activated T-lymphocytes in the peripheral blood are not uniform. This is attributed to the fact that their final numbers are influenced by other processes in the body (9). Only few authors have studied CD4 and CD8 expression on T-lymphocytes in the skin of psoriatic patients. Baker et al. (1) have counted 204(+24) CD4+-cells, and 119(+18) CD8+-cells with their ratio of 2.05(+0.26) in 50 fields of vision of frozen slices taken from the margins of psoriatic lesions. CD4+/CD8+ ratio was higher than 1.28 found in healthy population (9). There is a lack of literary data regarding the effect of CyA on lymphocyte subpopulations in inflammatory psoriatic lesions. Our study showed that before treatment, the CD4+/CD8+ ratio was similar to that found in psoriatic patients by Baker et al.(10). Decrease of absolute values in T-lymphocyte subgroups can be explained by specific immunosuppressive effect of CyA influencing predominantly IL-2–dependent pathways of activation (11).

Despite effective CyA treatment and dramatic drop in PASI score, the numbers of CD8+-cells did not increase. Decrease in CD4+/CD8+ ratio was due to more rapid decrease in CD4+ than CD8+. Effective treatment was reflected in steady return of measured values of CD4+-cells to 52.8% on average, that is close to values observed in healthy persons, that range between 33%-53% (12, 13). Similarly, the return of CD8+-cells to the average values of 31.9% was close to normal range of 24%-50% (13). Analogous results were published in dermatological literature after various types of systemic treatment of inflammatory skin diseases (14,15). Our results complement in detail the bulk of data corroborating clinical efficacy of CyA in the treatment of skin diseases (16, 17, 18).

In conclusion, this study confirmed that the number of CD4+ and CD8+ –cells is increased in the margins of chronic psoriatic lesions. The positive therapeutic effect of CyA is in correlation with the decrease of activated T-lymphocytes in the site of inflammation.
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THE IMPACT OF ORAL ESTRADIOL AND PROGESTERONE SUBSTITUTION ON ESTROGEN AND PROGESTERONE RECEPTORS EXPRESSION AND APOPTOSIS IN HUMAN ENDOMETRIUM

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Abstract

Objective: Exogenous hormonal manipulation may alter physiological cyclic pattern of endometrium. This article evaluates the effect of oral estrogen-progesterone substitution on the expression of estrogen and progesterone receptors and the level of apoptosis in the secretory endometrium of substituted cycles by comparison with natural cycles in the same patient.

Methods: Serum levels of estradiol (E₂), sex hormone-binding globulin (SHBG) and progesterone (P) were determined and biopptic samples of endometrium were taken on the 5th and 7th day of luteal phase in 26 infertile women who entered the assisted reproduction program first in a spontaneous and later in an estrogen-progesterone substituted cycle. E and P receptors (ER and PR) were estimated by immunostaining and image analysis in stroma and glandular epithelium. The level of apoptosis was evaluated by the TUNEL assay. Wilcoxon matched-pairs test was used for the statistical analysis of data.

Results: The mean serum levels of E₂, SHBG and P were higher in substituted cycles (p<0.05, p<0.001, p<0.01, resp.). The expression of ER and PR in stroma was lower than in glandular epithelium in both types of cycle and did not change between the 5th and the 7th day after ovulation or P addition. The expression of ER and PR in glandular epithelium in substituted cycles was higher than in spontaneous cycles on the day 5th (p=0.01, p=0.02, resp.). A decline between the 5th and the 7th day was seen in both types of cycle, but was much more prominent in substituted cycles (p<0.01, p<0.001, resp.). The mean level of apoptosis was always higher in glands than in stroma. Higher levels of apoptosis were more often found in compact layer than in spongy layer of glands, in substituted cycles than in spontaneous cycles and in the second biopsy samples than in the first ones.

Conclusions: Our findings are in accordance with the supposed effect of oral ovarian steroid cycle substitution which may often elicit supraphysiological peripheral P levels. The higher expression of ER and PR observed in substituted cycles seemed to have no negative effect on endometrial receptivity.

Key words: estradiol, progesterone, hormonal substitution, apoptosis, steroid receptors

INTRODUCTION

Embryo implantation is a complex process initiated by an interaction of the trophoblast with endometrial epithelial cells. This interaction can only happen when endometrium has reached a short phase of receptivity called „implantation window“. Endometrium is a target organ of ovarian steroids. Cyclical variations in peripheral blood levels of these hormones are promptly reflected in histological and morphological changes of the endometrium.

The action of ovarian steroid hormones is mediated by specific nuclear receptor proteins localized in glandular, stromal and vascular cells. The expression of steroid receptors in different endometrial cells vary during the different stages of menstrual cycles (1). Estradiol induces the expression of estrogen receptors (ER) and progesterone receptors (PR) which in natural cycles were found to be maximally expressed during the periovulatory and early luteal phase. On the other hand, certain level of progesterone (P) induces steroid receptor down-regulation. The timing of down-regulation of steroid receptors by P in epithelium has been shown to coincide with the establishment of endometrial receptivity (2, 3).

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In some assisted reproduction procedures it is necessary to prepare endometrium to be receptive by the controlled estrogen-progestagen substitution. The effects of such administration may vary with the dosage and duration of supplementation as well as with the activity of steroid receptors in the individual patient (4).

The success rate of transfer of cryopreserved-thawed embryos is still lower than that of embryos. This can be possibly caused by freezing and thawing procedures (5) or by the non-synchronous endometrial maturation in substituted cycles. It has been suggested (6) that exogenous hormonal manipulation can affect endometrial receptivity. Accelerated or delayed appearance of „implantation window” has been reported (7, 8). These studies on limited numbers of patients have shown high inter-individual variations. More studies are therefore needed to better understand how these processes are reflected by different markers of endometrial receptivity. The ultimate goal is to show if the pregnancy rate of transfer of cryopreserved-thawed embryos can be improved by selecting the individually optimal time of embryo transfer.

METHODS

Study group: Twenty six infertile women that had cryopreserved embryos in pronuclear (PN) stage from previous IVF cycle were recruited in a single infertility centre over three years interval 2001-2003. All women gave their informed written consent to the study, which was approved by the Institutional Review Board of the Palacký University.

Women had a history of infertility of more than 12 months, were less than 40 years old, had regular menstrual cycle with normal concentration of serum progesterone in the mid-luteal phase, physiological basal serum levels of follicle-stimulating hormone, FSH (<10 IU/l) and prolactin on the 3rd day of the cycle previously. Infertility evaluation revealed tubal (40%), idiopathic (16%), immunological (4%), endometriosis (4%) and male factors (36%) of infertility. Patients were examined in the course of two menstrual cycles.

1. Spontaneous cycle: All subjects monitored by themselves urinary LH excretion daily from cycle day 10 using (Simtech Biore Inc, USA). From cycle day 11, repeated vaginal ultrasound examination (Hewlett Packard, probe 7,5 MHz) was performed till the ovulation (day 0). Endometrial biopsies and blood sampling for E2 and P determination were performed on luteal days +5 and +7.

2. Substituted cycle: Hormonal substitution was started on the first day of another cycle using progressively increasing doses of estradiol-valerate (2 mg/d from day 1 to 6,4 mg/d from day 7 to 10 and 6 mg/d from day 11 to 15). From the 11th day of substitution serum levels of E2 and P were measured as in the spontaneous cycle. If on day 15 the endometrial thickness reached 8 mm or more micronised progesterone (P) was added in a dose 600 mg/d and the dose of estradiol-valerate was decreased to 4 mg/d. Endometrial biopsies as well as blood samples were taken on days 5 and 7 of P addition.

Tissue collection: Uterine endometrial sequential biopsies were taken within the interval of 48 hours using Novak curette by single investigator while patient was under sedation. The first biopsy was taken from the right side, second, two days later, from the left side of anterio-fundal area of the uterus to ensure that the second sample was taken from a different area. Biopic samples were fixed in methacarn for 24 hours and embedded in parafin. Sections (7 µm) were pre-treated by exposing to microwave or Proteinase K.

Serum levels of E2, P and SHBG were determined using commercial RIA kits supplied by Immunotech as described previously (9).

Steroid receptors positivity was detected separately in glandular and stromal cells and examined by a standard 3-step immunohistochemical method using commercially available primary monoclonal antibodies ER1D5 (Immunotech) specific for ERα and PR1A6 (Immunotech) detecting both isoforms of PR. Primary antibodies were detected by the Biotin-Streptavidin AP conjugate and NBT/BCIP as a substrate (ROCHE). Computer image analysis (ACC 4.0 software) was used to quantify the expression as labelling index (LI) in % (count of immunostained cells to all cell present). The mean LI of 3 different areas was calculated. The expression of ERα and PR were estimated separately in stroma and glandular epithelium.
Fig. 1. Expression of ER in stromal and glandular cells of the endometrium on the luteal day 7th of the substituted cycle.

Fig. 2. Expression of PR in stromal and glandular cells of the endometrium on the luteal day 7th of the substituted cycle.
Apoptosis was detected using the TUNEL kit (ROCHE) in stromal cells and glands. In glands, apoptosis was evaluated separately in spongy and compact layers. Apoptosis was quantified by dividing samples into 7 categories (0%, 0-10 %, 10-20%, 20-35%, 35-50 %, 50-75 %, >75%).

Statistical evaluation was performed by Wilcoxon matched-pairs test using STATISTICA software, p < 0.05 was considered statistically significant.

RESULTS

Serum hormone levels: The mean serum levels of E₂, SHBG and P in substituted cycles were higher than in spontaneous cycles (p<0.05, p<0.001, p<0.01, resp.). The increase of E₂ was only moderate, while the levels of SHBG and P were markedly increased, in several instances up to
ER and PR expression: The typical example of ER and PR staining is shown in Fig.1 and 2. The expression of both steroid receptors in endometrial biopsies was extremely variable and also focal positivity was often found. Therefore the Box-Whiskers graphs, medians and 25 to 75 percentiles were used to illustrate the differences between spontaneous and substituted cycles. (Fig.3-6). The expression of ER was generally higher in glandular epithelium than in stroma. The expression of ER in glandular epithelium on the 5th day in substituted cycles was higher than in spontaneous cycles (p=0.01) and also the decline from the 5th to the 7th day was significant (p<0.01) only after hormonal substitution. The mean expression of ER in stroma did not differ significantly between the cycles or days of biopsy, though in individuals a tendency to increase from the 5th to the 7th day prevailed.

The expression of PR was also lower in stroma than in glandular epithelium in both types of cycle and in stroma did not differ between the types of cycle or in the subsequent biopsies. The expression of PR in glandular epithelium on the 5th day was significantly higher in substituted cycles (p=0.02) and was more dramatically declined in the subsequent biopsies in the natural (p=0.02) as well as in the substituted cycle (p<0.001).

Apoptosis: The staining of apoptosis is shown in Fig.7. The expression was often focal. In general, apoptosis in stroma was lower than in glands, mostly only up to 20% of cells stained and has a tendency to increase in natural cycle between the days 5th to 7th. The staining in spongy layer of glands was usually between 20 to 50% with slight increase in both cycles. The highest staining was found in compact layer (50 to 75%).

supraphysiological levels. There was no significant change of mean steroid levels between the 5th and the 7th day after ovulation or P addition.
DISCUSSION

In IVF programs the endometrial preparation is used in women receiving donated oocytes as well as frozen-thawed embryos. The endometrium is prepared with exogenous hormones by either oral, vaginal or intramuscular administration. Although the bioavailability of oral steroid is lower than in other routes of administration the oral steroids may be still preferred for practical reasons. There are several protocols for uterine preparation either with incremental doses of \( E_2 \) (10, 11) or fixed doses of \( E_2 \) (12) during the proliferative phase of the cycle. Micronised P as well as didrogesterone are used for oral supplementation of luteal phase.

We have shown that oral substitution of ovarian hormones induced increased serum P and SHBG concentrations (often supraphysiological) in mid-luteal phase. It can be deduced that grossly increased SHBG in substituted cycles decreased the availability of free, biologically active \( E_2 \) in spite of moderately increased total \( E_2 \) and further contributed to the already higher \( P/E_2 \) ratio in substituted cycles.

Studies in animals suggest that the impact of P on different uterine cell types is partly determined by the receptor availability. In canine uterus nuclear staining for PR was observed in epithelial cells of the surface epithelium, glandular ducts and basal glands of the endometrium, in endometrial stroma cells and in myometrial smooth muscle cells. This staining was positively correlated with the \( E_2/P \) ration, and reflected the positive effect of \( E_2 \) and the negative influence of P on the receptors (13). Similar pattern was found in human endometrium (14).

Higher expression of ER and PR receptors in human glandular epithelium in our study may be induced by higher late proliferative phase peripheral \( E_2 \) in substituted cycles where there is steady \( E_2 \) level in contrary to the abrupt preovulatory decrease in spontaneous cycles. The more pronounced decline of the ER and PR expression between the day 5th and 7th in substituted cycles could be a result of accelerated down-regulation by high P levels (15).

Apoptosis, programmed cell death, is a basic biological phenomenon with widespread implications in tissue kinetics. Already in 1975, Hopwood (16) studied apoptosis in human endometrium. Nowadays it is supposed that apoptosis can represent another important regulatory mechanism of endometrial function (17), though the knowledge about the hormonal control of apoptosis in various cell types is still limited. Estrogen has been shown to upregulate the protective, anti-apoptotic protein of the Bcl gene family, Bcl-2 (18). Its expression is the highest in the late proliferative phase (19) and decreases soon after the onset of P production (20) which coincides with the increased secretion of the pro-apoptotic protein Bax.

The peripheral serum hormone levels, the expression of ERs and PRs and the level of apoptosis in mid-luteal phase of spontaneous and estrogen-progestin substituted cycles found in this study was individually variable. Nevertheless, in general, the presented results obtained on a more representative sample are in accordance with our preliminary observations (21, 22). The reasons for this variability may be multiple: individual variability in bioavailability of orally administered ovarian steroids due to polymorphism of isoforms of cytochrome P 450 in intestinal wall (23, 24), short halftime of P elimination after oral administration which necessitates the exact time intervals for blood sampling after taking the pill. In some cases the focal positivity of staining for receptors as well as for apoptosis seen more often in stroma could lead to bias in the results.

We can conclude that oral hormonal substitution used in this design induced similar dynamic changes in the midsecretory endometrium as were observed in the natural cycle. The decrease of ER and PR in the midsecretory phases in both cycles has been related to the occurrence of other morphological markers of endometrial receptivity (25, 26). The higher expression of ER and PR observed in substituted cycles seemed to have no negative effect on endometrial receptivity according to the obtained pregnancy rate after transfer of cryopreserved-thawed embryos in patients included in this study.
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The main purpose of care of patients with chronic diseases is to improve their quality of life (QoL). Patients with peripheral arterial occlusive disease (PAOD) suffer from pain, intermittent claudication and threat of the loss of lower limb, their QoL is therefore seriously impaired.

130 patients with PAOD were hospitalized at the Clinic of Surgery in Martin in 2003. The authors assessed QoL in a series of 40 patients. To measure health-related QoL, a standardized, non-disease-specific EuroQol questionnaire was used. The aim of the work was to use QoL scores in order to express the effect of reconstructive vascular surgeries and amputations of lower extremities.

The best overall health-related QoL was assessed in the patients after reconstructive surgeries. On the other hand, the mean QoL of the amputees was the worst, worse than QoL in the patients who did not undergo any surgery for PAOD. Reconstructive surgeries improved mobility of the patients and their ability of self-care and reduced anxiety and depression. The amputees had problems with mobility, suffered from pain or discomfort, had problems with self-care and usual activities. One third of them were extremely anxious or depressed, one third was not anxious at all.

QoL instruments provide information which increase physician's understanding of the patient's disease. The QoL assessment is irreplaceable. The reconstructive vascular surgeries evidently improve QoL. Although amputation means a drastic impact on a patient's life, it still remains one of the basic surgical procedures because it is a life-saving operation.

Key words: quality of life, EuroQol, peripheral arterial occlusive disease

INTRODUCTION

The quality of life (QoL) of a patient is an important criterion of medical treatment’s direct benefit to the patient. The growing interest in QoL of surgical patients is one of the important aspects of humanization of medicine (1). It is a modern approach to assessment of the outcome of surgery using evidence-based ideas, because surgery has been trying to catch up with evidence-based medicine (2).

Patients with peripheral arterial occlusive disease (PAOD) suffer from pain and intermittent claudication. Pain and restricted mobility considerably reduce their QoL not only in the physical, but also in the psychological and social spheres (3). They are even threatened with the loss of lower limb. Although amputation is a life-saving procedure, it seriously affects patient’s life. We recognize QoL as an important decision criterion for medical treatment of surgical patients. The main purpose of care of patients with chronic diseases is to improve their QoL (4).

The aim of this work was to find an optimal and valid method of assessment of surgical patients’ QoL (5), to gain patients’ cooperation and understanding, and to use QoL in expressing the effect of surgical procedures, such as reconstructive vascular surgeries and amputations.

METHODS

Our study sample consisted of 40 patients, treated at the Clinic of Surgery in Martin. Inclusion criteria were intermittent claudication (stage II of PAOD according to Fontaine) or presence of rest pain (Fontaine’s stage III) and symptoms of gangrene and ulceration (stage IV). We recorded each patient’s history and physical examination, to assess the claudication distance and to determine localization and severity of the PAOD.

In order to use QoL as a criterion of surgical treatment’s benefit to patients, we identified...
three groups of patients. The first group (group A) consisted of 13 patients who did not undergo any operation for PAOD, the second (group B) of 18 patients after reconstructive vascular surgeries, and the third (group C) of 9 amputees. 8 out of 9 amputees were diabetics.

We measured health-related QoL with a standardized, non-disease-specific instrument EuroQol, which consists of a questionnaire and a visual analogue scale (VAS) (6). The questionnaire is anonymous. It takes only few minutes to complete, is relevant to respondents of all ages and of different degree of functional disability, and is capable of producing a single index value (7). EuroQol-5D defines health in terms of mobility, self-care, performing usual activities, pain/discomfort and anxiety/depression. Each dimension is divided into three levels: no problem, some or moderate problems and extreme problems, which are coded as 1, 2 and 3, respectively. There are defined 243 potential health states. Thus the EQ-5D self-classifier is a descriptive system of health related QoL. The derived information can be converted into a single summary index. On EQ VAS rating scale individual respondents rated their own health state between 0/the worst imaginable health state and 100/the best imaginable health state. We expressed the global values as mean +/- standard deviation (SD) for the study sample.

RESULTS

There were 130 patients with PAOD hospitalized at the Clinic of Surgery in Martin in 2003. It means that 6% out of all hospitalized were patients with PAOD. 68% of them were men with mean age 63 years, 32% were women with mean age 68 years. Considering the percentage of males and females hospitalized at our clinic yearly, our series of 40 patients is a representative sample. In 2003 there were 3067 outpatients treated at the surgical ward specialized in the treatment of vascular diseases.

Out of the series of 40 subjects there were 27 (68%) men and 13 (32%) women. Their mean age was 63 years, the highest age was 84 and the lowest 31 years. 50% were smokers, 50% were treated for hypertension, 45% for diabetes mellitus, 24% for hyperlipidaemia. Body mass index higher than 30 was found in 17% of the patients. 40% of the subjects had diseased both legs. Claudication distance of 30% of the patients was more than 50 meters (stage IIa of PAOD), of 36% it was less than 50 meters (stage IIb). 34% of patients suffered from rest pain and ulceration or gangrene (stage III or IV).

We created an EQ-5D profile as a frequency distribution of the EQ-5D descriptive system for comparison of three groups (Table 1) (8). Mobility was improved in some patients after reconstructive surgery. A part of patients after surgery had no problems with mobility. Some amputees were confined to bed or wheelchair. There was improved self-care in patients after reconstructive surgery. Only one amputee was unable to wash or dress himself. More patients after any surgery for PAOD were able to perform their usual activities. There was almost no difference among three groups in amount of patients who had no or some problems with performing their usual activities. Almost the same part of patients in each group suffered from extreme pain or discomfort. Pain and discomfort ment problems especially for the patients after operations. Two patients (an amputee and a patient after reconstructive surgery) had neither pain nor discomfort. Among the patients who did not undergo any surgery, there was nobody without pain. Extremely anxious or depressed were only some patients after surgery, but nobody without surgery. Almost a half of the patients after reconstructive surgery were not anxious or depressed at all. Almost two thirds of amputees were anxious or depressed.

The scores of EQ-5D index express overall health-related QoL of the study groups and are presented as mean scores with standard deviations (Fig. 1). The scores of EQ VAS indicating mean self-rated health index are presented in the same manner (Fig. 2) (8). The best overall health-related QoL had the patients after reconstructive operations. The amputees had much worse QoL than the patients who did not undergo any surgery for the disease. On the other hand, the best self-rated health was found in the patients without previous operation. The worst subjective health state was rated by patients after amputation of lower limb.
Table 1. Frequency distribution of the EQ-5D descriptive system for the comparison of patients after no surgery for PAOD, after reconstructive vascular surgeries and after amputations.

<table>
<thead>
<tr>
<th>Q-5D profile</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(No surgery for PAOD)</td>
<td>(After reconstructive vascular surgeries)</td>
<td>(After amputation of lower limbs)</td>
</tr>
<tr>
<td>Mobility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no problems (%)</td>
<td>0</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>some problems (%)</td>
<td>92</td>
<td>88</td>
<td>44.5</td>
</tr>
<tr>
<td>confined to bed (%)</td>
<td>8</td>
<td>6</td>
<td>44.5</td>
</tr>
<tr>
<td>Self-care</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no problems (%)</td>
<td>15</td>
<td>44.5</td>
<td>11</td>
</tr>
<tr>
<td>some problems (%)</td>
<td>77</td>
<td>44.5</td>
<td>78</td>
</tr>
<tr>
<td>Unable to (%)</td>
<td>8</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Usual Activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no problems (%)</td>
<td>23</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>some problems (%)</td>
<td>46</td>
<td>61</td>
<td>56</td>
</tr>
<tr>
<td>Unable to (%)</td>
<td>31</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Pain / Discomfort</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none (%)</td>
<td>0</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>moderate (%)</td>
<td>77</td>
<td>61</td>
<td>56</td>
</tr>
<tr>
<td>extreme (%)</td>
<td>23</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Anxiety / Depression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none (%)</td>
<td>31</td>
<td>44</td>
<td>33</td>
</tr>
<tr>
<td>moderate (%)</td>
<td>69</td>
<td>39</td>
<td>33</td>
</tr>
<tr>
<td>extreme (%)</td>
<td>0</td>
<td>17</td>
<td>33</td>
</tr>
</tbody>
</table>

Fig. 1. EQ-5D index values are presented as mean scores with standard deviations.
DISCUSSION

The QoL in patients with PAOD depends mainly on the level of functional disability. It has been emphasized that the assessment of QoL is important because objective disease indicators, such as the peripheral Doppler pressure and the angiographic severity of the disease, do not reflect the subjective state of the disease adequately (9, 10). We were interested in how the specific surgical procedures affect life of these patients, how they influence functional disability and psychosocial distress. In general, improved QoL is expected as a result of surgery or medical treatment.

Only one third of the patients were women, and they were 5 years older than men in average. Each patient with PAOD who did not undergo an operation had problems with mobility and suffered from pain. Most of them had also problems with self-care and performing usual activities. But there was nobody among these patients who would feel extremely anxious. Reconstructive surgeries improved mobility of the patients, and often also their ability of self-care. But most of the patients still had problems with performing usual activities, and only 6% of them felt no pain or discomfort. Reconstructive surgeries improved QoL in the emotional sphere, reduced anxiety and depression. Amputation is a serious procedure. The impaired mobility of amputees is even more important factor for their QoL than the psychosocial distress (11). In our series of patients, there was only one amputee who had no problems with walking and one who had no pain or discomfort. The amputees had problems with self-care and performing usual activities. It should not be neglected that one third of the amputees were extremely anxious or depressed. On the other hand, one third of them felt no anxiety, perhaps because they had realized that amputation was a life-saving procedure.

The best overall health-related QoL was assessed in the patients after reconstructive surgeries. We would expect even better QoL, but most of these patients come to visit the doctor only if they have troubles or new problems. However, we have to admit that a surgery itself can be a source of anxiety and fear. Maybe therefore the subjective health state of these patients was worse than of the patients who underwent no surgery. On the other hand, the mean health-related QoL of the amputees was the worst.

Some patients had problems to realize the importance of physician’s interest in their QoL. Especially some amputees refused to rate their health state. These patients were still hospitalized after the surgery, and we assessed the stage of negativism as a part of the individual process.

**Fig. 2.** EQ VAS scores are presented as mean scores with standard deviations.

<table>
<thead>
<tr>
<th>Mean EQ VAS score</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(SD)</td>
<td>19</td>
<td>23</td>
<td>14</td>
</tr>
</tbody>
</table>
of getting used to live with their handicap. It would be ideal to measure QoL before and after, not during hospitalization, so that patients are able to assess how they live. The QoL measuring using EQ-5D is not very time-consuming. Sometimes, when a patient does not cooperate, it is optimal to ask intentional questions while recording patient’s history, instead of using questionnaires (5). Some patients think the questionnaires are impersonal. QoL instruments provide information about various areas in which a patients is affected, and thus increase the physician’s understanding of patient’s disease. The QoL assessment is irreplaceable. The information about QoL help to reach the unique agreement of goals of medical workers and needs of patient. The decisions about medical treatment always have to come out of patient’s perspective.

The reconstructive vascular surgeries evidently improve QoL. Especially important is their positive influence on the sphere of mobility and self-care by improving patient’s functional status. We emphasize importance of such procedures in reduction of the psychosocial distress of patients. Although amputation means a drastic impact on patient’s life, it still remains one of the basic and unique surgical procedures in order to save and prolong life.

REFERENCES


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PUBLIC HEALTH IMPORTANCE OF VACCINATION AGAINST SELECTED COMMUNICABLE DISEASES

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Abstract

Vaccination is a cost-effective preventive measure gaining priority in our primary prevention. The aim of this contribution was to analyse epidemiological situation in selected infectious vaccination-preventable diseases and the cumulative estimation of saved financial costs for their treatment in the period of 1951-2002. Preventive vaccination saved approximately 17.7 billion of Sk and prevented 1.39 million of diseases during the analysed period. Our analysis has confirmed that the used vaccination strategy had an important impact on the epidemiological and financial situation. Vaccination is a highly cost-saving preventive strategy with overall benefit for the whole society and individually for each and every vaccinated person.

Key words: vaccination, saved diseases, saved financial costs.

INTRODUCTION

Vaccination belongs in the world context to the primary public health interventions. This preventive strategy in struggle against infectious diseases is one of the most effective weapons and at the same time it is the cheapest way of health protection of the modern medicine. However it is still not possible to prepare vaccines against all infections. Evaluation of effectiveness and usefulness of vaccination strategy is possible only after a delay of certain period (1,2).

Our work focuses on the analysis of occurrence of the selected infectious vaccination-preventable diseases in the period of 1951-2002, and to the cumulative estimation of the saved financial costs for their treatment.

METHODS

Available epidemiological, demographic and economic data from official statistical annual reports (3) and from epidemiological information system EPIS were used in our work. Prices of remedies and special health materials were calculated from the Indication List of treatment order. Retrospectively, the analysis of epidemiological situation in communicable diseases - the vaccination against which started consecutively from 1950s - 1980s of last century - was performed. Diphtheria, tetanus, pertussis, poliomyelitis, measles, rubella and mumps belong to these selected diseases. Total (direct and indirect) financial costs for the year 2002 was calculated and compared with the model for treatment used before the introduction of vaccination strategy (4). Cumulative calculation of saved diseases and saved financial costs for years 1951–2002 was obtained as a sum of results of each year, calculated for the population in that year.

RESULTS

Table 1 includes the number of notified selected diseases before the introduction of compulsory vaccination (model situation) and after the introduction of vaccination strategy in compared
to the model year 2002. Positive changes were presented by the decrease of morbidity for pertussis, tetanus, mumps of 95.8-99.9% and for diphtheria, poliomyelitis and measles of 100.0%. Fifty-six notified cases in 2002 represent a decrease of 99.9% of the overall number of cases in pre-vaccination period.

Table 1. Comparison of morbidity of selected infectious diseases before the introduction of vaccination strategy and incidence of notified cases in Slovakia in 2002.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cases of disease</th>
<th>Changes in incidence of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model situation</td>
<td>Year 2002</td>
</tr>
<tr>
<td></td>
<td>Abs.</td>
<td>/100 000</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>2 656</td>
<td>66.5</td>
</tr>
<tr>
<td>Tetanus</td>
<td>48</td>
<td>1.2</td>
</tr>
<tr>
<td>Pertussis</td>
<td>2 265</td>
<td>56.7</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>283</td>
<td>7.4</td>
</tr>
<tr>
<td>Measles</td>
<td>17 672</td>
<td>381.9</td>
</tr>
<tr>
<td>Rubella</td>
<td>15 437</td>
<td>309.9</td>
</tr>
<tr>
<td>Mumps</td>
<td>21 465</td>
<td>417.8</td>
</tr>
<tr>
<td>All</td>
<td>59 826</td>
<td>x</td>
</tr>
</tbody>
</table>

Model costs for treatment of the analysed diseases represent 760 751 810 Sk. The highest part falls to the treatment of measles – 39.3% of total financial cost, the lowest was for the treatment of tetanus – 0.9%. The total financial cost of the treatment in 2002 was 1 167 841 Sk. The highest one was for the treatment of pertussis – 62.8%. Diphtheria, poliomyelitis and measles required no financial cost because of their zero incidence. The difference of costs of model treatment and treatment in the year 2002 was 759 583 969 Sk and it represents the decrease of 99.8% of the used financial costs – Table 2.

Table 2. Comparison of treatment costs of selected infectious diseases before the introduction of vaccination and costs used in Slovakia in 2002.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Financial costs in Sk</th>
<th>Changes in financial costs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model situation</td>
<td>Year 2002</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>89 671 133</td>
<td>0</td>
</tr>
<tr>
<td>Tetanus</td>
<td>6 543 456</td>
<td>282 392</td>
</tr>
<tr>
<td>Pertussis</td>
<td>60 178 889</td>
<td>733 609</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>22 624 212</td>
<td>0</td>
</tr>
<tr>
<td>Measles</td>
<td>299 335 200</td>
<td>0</td>
</tr>
<tr>
<td>Rubella</td>
<td>90 414 161</td>
<td>53 225</td>
</tr>
<tr>
<td>Mumps</td>
<td>191 984 759</td>
<td>98 615</td>
</tr>
<tr>
<td>All</td>
<td>760 751 810</td>
<td>1 167 841</td>
</tr>
</tbody>
</table>

Preventive vaccination saved approximately 17.7 billion of Sk and prevented 1.39 million of diseases during analysed period – Table 3. The highest rate of saved diseases and financial costs belonged to measles (39.5%), mumps (21.8%) and rubella (19.4%), the lowest one belonged to tetanus (0.2%).
Table 3. Overview of saved diseases and financial costs by preventive vaccination in the Slovak Republic in 1951-2002.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Period</th>
<th>No. of saved diseases</th>
<th>No. of saved financial costs (Sk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Abs.</td>
<td>%</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>1951-2002</td>
<td>155 225</td>
<td>11.1</td>
</tr>
<tr>
<td>Tetanus</td>
<td>1961-2002</td>
<td>2 058</td>
<td>0.2</td>
</tr>
<tr>
<td>Pertussis</td>
<td>1961-2002</td>
<td>106 801</td>
<td>7.7</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>1961-2002</td>
<td>15 352</td>
<td>1.1</td>
</tr>
<tr>
<td>Measles</td>
<td>1971-2002</td>
<td>551 466</td>
<td>39.5</td>
</tr>
<tr>
<td>Rubella</td>
<td>1986-2002</td>
<td>270 652</td>
<td>19.4</td>
</tr>
<tr>
<td>Mumps</td>
<td>1988-2002</td>
<td>293 281</td>
<td>21.0</td>
</tr>
<tr>
<td>All</td>
<td>1951-2002</td>
<td>1 394 835</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The aim of preventive vaccination measures is to decrease the morbidity, mortality and invalidation caused by vaccination-preventable communicable diseases. Elimination (and if reaching theoretically supposed conditions, also eradication) is an important point. Saving of direct and indirect financial costs given for the treatment of these diseases is an unneglectable reality (5,6,7).

The effect of vaccination strategy in population will not be immediate after introduction of preventive vaccination. First, epidemical appearance of diseases occurs but the importance and range is ever lower, usually targeting adults and no vaccinated children (8). Intra epidemical periods are growing longer, epidemics are decreasing and later sporadic occurrence is decreasing either (9,10).

Public health importance of immunisation programme can be divided to several periods. In pre-vaccination period materials supporting introduction of vaccination are prepared based on epidemiological analysis together with evaluation of other facts (social, ethical, etc). After the implementation of preventive strategy, and depending on the increased vaccinated population, the decrease of morbidity can be observed. In this period not favourable reactions after vaccination are being noted. After reaching and maintaining a certain level the lost credibility in vaccination can be noticed, this being reflected in the decrease of vaccination coverage, increase of morbidity and occurrence of smaller or greater epidemics. Regaining of credibility is followed by stabilisation of the level of the vaccination, by further important decrease of the number of diseases, and later by elimination and eradication (9,10). Not all diseases are eradicable. After stabilisation of epidemiological situation and its supporting by broad complex of health and non health rules, the vaccination can be stopped.

In recent 52 years more than 1.39 million diseases could be saved. Separate vaccination strategies as they were consecutively introduced, influenced epidemiological situation. The 1950s were influenced in an important way by vaccination against diphtheria, that was compulsory in Slovakia from 1946. In 1960 is the important decrease in morbidity from tetanus, pertussis and poliomyelitis occurs after introduction of vaccination in the second half of the 1950s. After the introduction of vaccination against measles in 1970s and mostly 1980s the extreme decrease of morbidity occurred, followed by successful vaccination against rubella (1985) and mumps (1987). Such a decrease happened because the three mentioned diseases represented the highest rate of notified diseases. In the last decade of the past century 99% decrease of selected notifiable infections was observed, representing 99.9% in 2002.

Together with the improvement of epidemiological situation, saving of total financial costs for treatment of selected communicable diseases occurred. Total costs for treatment consist of direct
and indirect costs. In direct costs the costs for home treatment and hospitalisation are involved. Indirect costs reflect absence and loss of productivity in employed persons due to family member attendance or work disability in adults and reimbursement of payments. The lost of gross domestic product and payed allowances represent lost for the whole society (11,12,13). High costs for treatment of selected infectious diseases were influenced by the number of notified cases (measles, rubella, mumps) need of hospitalisation (poliomyelitis, tetanus, diphtheria, perussis, measles), duration of home treatment and absence from work due to family member attendance or disability for work (pertussis, measles, rubella, mumps) and payment of social allowances and losses from non-production of the gross domestic product (4,14).

Modern medicine is oriented not only to the treatment but also to the primary prevention of diseases, which is considered as a natural requirement of modern society. Realisation of vaccination requests investments that can be seen first as too high, because their return can be seen only after rather a long time. Public health importance of such a preventive strategy depends on effective impact on individual health as well as on the society (15,16). Not neglectable is saving of financial costs of individuals, employers, health system and the whole society (17).

REFERENCES


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2. **Abstract** (separately) informative and structured in the extent of 1 standard page. Three to five key words are appended at the end of the abstract page.

3. **Introduction** should introduce into the problem and state the purpose of the article.

4. **Methods** should be complete to allow other workers to reproduce the results. Describe statistical methods. Indicate whether the procedure followed was in accordance with the ethical standards.

5. **Results**: Present your results in logical sequence in the text, tables and illustrations.

6. **Discussion**: Emphasize the new and important aspects of the study, link the conclusions with the presented goals of the study, relate the results to other relevant studies with a short summary of results at the end.

7. **References**: All publication cited in the text should be presented in references. References have to be numbered consecutively in the order in which they are first mentioned within the text. Identify references in the text by Arabic numerals in parantheses. Use abbreviations of the journals according to Index Medicus (List of Journal Indexed in Index Medicus, http://www.nlm.nih.gov).

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