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CONTENTS

REVIEW ARTICLE

UNROOFING TECHNIQUE AS AN OPTION FOR THE ENDOSCOPIC TREATMENT OF GIANT GASTROINTESTINAL LIPOMAS

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Summary: Gastrointestinal lipomas are usually asymptomatic, detected incidentally. However, they can cause severe symptoms such as obstruction, invagination, and bleeding. The transsection of an infarcted or large lipoma by needle sphincterotome (needle knife) and/or snare polypectomy of the upper part of the tumour is an option for the endoscopy treatment of giant infarcted lipomas. Cutting a top of lipoma (unroofing technique) allowed flow out of adipose tissue from the lipoma.

Keywords: *Endoscopy; Giant gastrointestinal lipoma; Needle sphicterotome; Unroofing technique*

Introduction

Gastrointestinal lipomas are rare, benign, usually single, slowly growing mesenchymal tumours, mostly found in the colon (65%) and small bowel (20%) (1–6). Lipomas tend to occur in older population sections and they are usually asymptomatic, detected incidentally (7, 8). However, they can rarely cause severe symptoms such as abdominal pain, intestinal obstruction, invagination, life-threatening bleeding, diarrhoea or even perforation (9–23). Franc-Law et al. (15) reviewed 275 previously reported cases of colonic lipoma, 28 patients (10%) had a dramatic presentation with pain and/or rectal bleeding, being the most significant prodromal symptom. In this subset the lipomas tended to be larger, frequently had associated marked necrosis or ulceration, and were less likely to be located in the ascending colon and caecum. Such lipomas usually reveal marked ischaemic changes (15).

Diagnostics

There are no difficulties to diagnose gastrointestinal lipomas properly in vast majority of cases. Endoscopic appearance of a lipoma is quite characteristic, with its bright yellow colour. The lesions are soft and compressible (a cushion sign), the overlying mucosa is normal (1). Recognition at endoscopic ultrasound, computed tomography or magnetic resonance imaging is unequivocal and definite, too.

Colour of infarcted lipomas is dark purple and brown-reddish (with tiny islands of yellowish adipose tissue). Their surface is smooth, glossy and tight (24). Quite seldom, it might be difficult to distinguish other mesenchymal tumours (like liposarcoma), especially in symptomatic elderly people. Surgical resection with subsequent histology may be the solution in such a case (25).

Therapeutic options

Asymptomatic lipomas do not require any treatment. Symptomatic gastrointestinal lipomas could be removed endoscopically by means of snare polypectomy (5, 6, 17, 18, 23, 26–30) or by endoscopic submucosal dissection (31–33). Preventive submucosal injection (saline or epinephrine), clipping of a lipoma base and the use of detachable nylon or polyglactin loop could reduce the risk of complications such as bleeding or perforation (28, 34–39). Some authors recommend the use of a double-channel endoscope with placing a ligating loop device around the lipoma base with the assistance of a grasping forceps (40) or grasping-forceps-assisted endoscopic resection (41–44). Endoscopic polypectomy is considered to be possible in smaller size (less than 3 cm) and pedunculated lipomas (13, 45). Larger lipomas are suggested by some authors for surgery because of the risk of complications after endoscopic polypectomy of submucosal tumours (perforation, bleeding) (8, 13, 15, 45–48). Use of SB knife (a scissor type device for submucosal dissection) with double-balloon endoscopy has been reported as a safe option to avoid surgical resection of small intestinal lipoma (49). Large transmural lipoma should be always referred for surgery (22). Self-amputation of colonic lipoma is exceptional (50–52). Some authors recommend looping and ligating lipoma with the detachable snare without endoscopic resection ("Loop-and-let-go" or "Ligate-and-let-go" technique) (32, 33, 37, 40, 53, 54). This ligation produces an asymptomatic, slow mechanical transsection of the lipoma (54). The endoscopic ligation should not be attempted in the treatment of broad based or sessile colonic lipomas (55). In these circumstances, endoscopic or surgical resection may be appropriate (54).

Large colonic lipomas occlude the intestinal lumen thus making it difficult to snare the lesion. In such a case, another

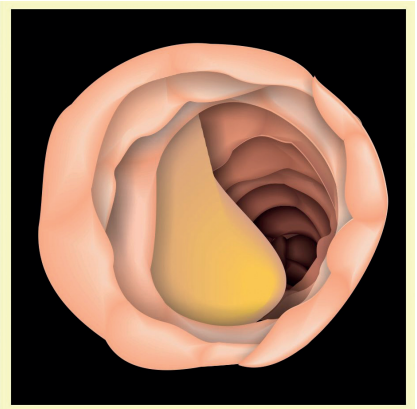

Fig. 1: Pedunculated lipoma of the large bowel. Bright yellow colour is characteristic.

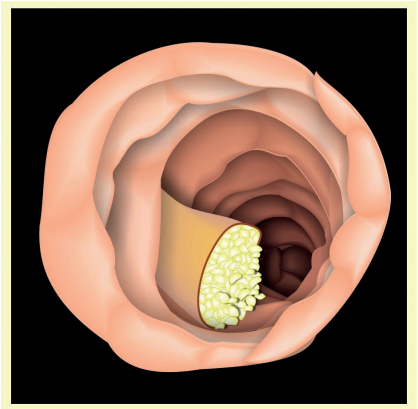

Fig. 2: Upper third of the lipoma body was cut off using a needle sphicterotome (a needle knife).

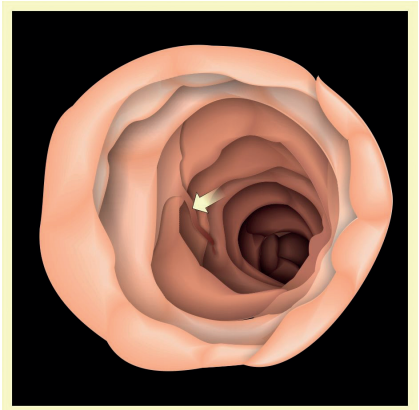

Fig. 3: Cutting off the polyp body allowed flow out of adipose tissue from the lipoma subsequently within couple of days or a few weeks. The remnant of lipoma stalk is marked with an arrow

option for giant lipomas is endoscopic treatment by means of unroofing technique (24) to avoid surgery.

Unroofing technique

Using the unroofing technique we cut off only the upper half or one third of the lipoma body using electrocautery snare. The remaining adipose tissue is subsequently extruded from the open surface. Therefore, this is a simple technique that allows both histological confirmation and complete treatment with minimal risk of perforation (see Figures No. 1–3). Using duodenoscope and grasp-and-snare technique in the management of a large, duodenal lipoma or combine this technique with a double-channel endoscope is also possible. Another possibility is consecutive dissecting the overlying mucosa on the lipoma body by means of a needle-knife in order to completely extrude the mass of the fat tissue (56–63).

We recommend this unroofing technique especially for giant and/or infarcted lipomas (54). We start with an initial cutting with an incision of the visible part of the polyp by means of a needle sphincterotome (needle knife). This transsection made it possible to subsequently grasp the lipoma by a snare and to cut off upper third of the tumour (24). Cut covering of lipoma should be extracted for histology.

Mimura et al. (56) were probably the first who reported this method by for endoscopic resection of colonic lymphangioma. Hizawa et al. (57) as the first used unroofing technique for the endoscopic resection of a large lipoma. They cut the upper third of large duodenal lipoma. This revealed a hole in the overlying mucosa and adipose material rapidly exuded from the cut surface through this opening (57). This technique only cuts off the upper half of the submucosal tumour, thus reducing the risk of complications. Since this initial experience, successful endoscopic treatment using unroofing technique has been reported by several authors (29, 31, 34, 58–60).

Binmoeller et al. (61) and Lee et al. (62) recommended endoscopic partial resection with the unroofing technique also for diagnostics of subepithelial tumours originating from the muscularis propria, such as gastrointestinal stromal tumours, leiomyoma or neuroendocrine carcinoma. Unlike unroofing technique of lipomas, procedural blood oozing was relatively common (9/16 cases; 56%) but easily controlled by argon plasma coagulation (62). There are no reports on local recurrence of lipomas after their endoscopic treatment, no data on follow-up of these patients are given in available literature.

Complications

Complications of the method are very rare. Adipose tissue contains not enough water to facilitate conduction of electric current, which is why endoscopists apply higher electrical output for snare during procedure, causing thermal injury on the colon wall adjacent to the mass and increasing the likelihood of perforation (58). The unroofing technique prevents this complication. In cases of polypectomy, polyps larger than 1 cm in the right colon or larger than 2 cm in the left colon and multiple polyps carried an increased risk of bleeding and/or perforation (63–65). Generally, lipoma with a broad base or a large diameter has the risk for complication after endoscopic resection (58).

Conclusions

In conclusion, transsection by means of electrocautery snare and/or needle sphicterotome is an optional and effective technique for endoscopic treatment of giant symptomatic gastrointestinal lipomas. The cut cover of the lipoma is possible to remove for histopathology. Although the transformation to liposarcoma is extremely rare (described only as sporadic case reports in the literature), biopsy from large

lipomas is recommended. Cutting the lipoma body (unroofing technique) allowed flow out of adipose tissue from the lipoma. This technique is quite safe as the risk of perforation and/or bleeding is unlikely.

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HISTOLOGICAL CHANGES OF THE MIDDLE EAR OSSICLES HARVESTED DURING CHOLESTEATOMA SURGERY

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Summary: Background: In the cholesteatoma surgery ossicles can be replaced to reconstruct middle ear function. It is important that these ossicles are free of squamous epithelium, to prevent residual disease. This study focuses on the histological findings of the malleus and incus harvested during cholesteatoma surgery. Materials and Methods: Eighty middle ears ossicles were examined in vivo and histologically to consider the relationship of cholesteatoma to ossicles, grade of bone destruction and invasion of cholesteatoma to deeper layers of bone. Results: Serious ossicular destruction was observed more frequently in incus compared to malleus ($p = 0.0065$). Difference of ossicles destruction between children and adults was not significant ($p = 0.3032$). Deep invasion of cholesteatoma into the vascular spaces or inner core of the bone was not observed. Conclusions: Autograft ossicles from cholesteatomatous ears should not necessarily be rejected for reconstruction of the ossicular chain. Regarding the histological finding, the authors suggest mechanical cleaning of the ossicle surface to eliminate residual disease.

Keywords: *Cholesteatoma; Middle ear ossicles; Incus; Malleus; Surgery*

Introduction

Autograft ossiculoplasty has been well known for more than fifty years. The first report was published by Hall and Rytzner in 1957 (1). The malleus and the incus have been used in middle ear surgery due to biocompatibility, low cost and long-term stability. However, the risk of cholesteatoma transmission limits autograft ossiculoplasty in cholesteatomatous ears. Cholesteatoma attacks middle ear ossicles in most patients. It depends on location and spreading of the cholesteatoma (Fig. 1). In these patients autologous ossiculoplasty could lead to reimplantation of cholesteatoma due to microscopic residue of squamous cell epithelium in the ossicles (2–10). Could one remove the cholesteatoma from ossicles and utilize malleus and incus for reconstruction without risk of residual disease? We studied the cholesteatoma relationship to ossicles in order to answer whether the cholesteatoma is present only on a superficial layer of the middle ear ossicle or invades deeper into the vascular spaces and inner core.

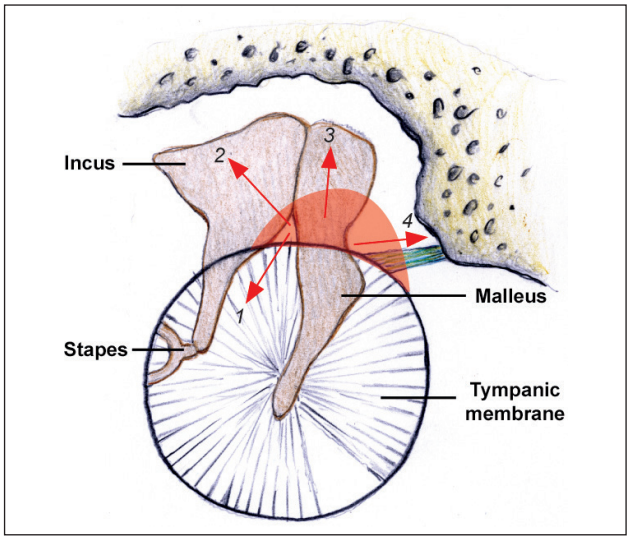

Fig. 1: Scheme of the attic cholesteatoma spreading – arrow 1 to the mesotympanum behind long process of incus, arrow 2 to the medial attic behind the body of incus, arrow 3 to the superior attic and tegmen tympani above the head of malleus, arrow 4 to the anterior attic and protympanum. (Adapted from Chrobok V et al. (20))

Materials and methods

Eighty middle ear ossicles were used for this study. As specimens, we examined mallei and incudes harvested during middle ear cholesteatoma surgery. Inclusion criteria were: chronic otitis media with cholesteatoma, primary cholesteatoma surgery, evidence of cholesteatoma on the ossicle surface. The surgeries were carried out between 2006 and 2011. The ossicles were examined and measured under microscopy. The ossicles were grouped as follows (consistent with malleus and incus erosion classification) (11):

- Ossicle destruction grade I: size of the malleus head > 2 mm, size of the incus body >3 mm in diameter.
- Ossicle destruction grade II: size of the malleus head < 2 mm, size of the incus body < 3 mm in diameter.

All specimens were fixed in 10% formaldehyde. Decalcification was performed by electrolysis in decalcifier system (SAKURA TDE™ 30 Decalcifier System, Sakura Finetek Europe B.V., Alphen aan den Rijn, The Netherlands). The tissue blocks were serially sectioned and stained with standard hematoxylin and eosin and examined under light microscopy.

Subjects gave written informed consent. The study was approved by our institutional Ethical Committee.

Statistical analyses

All statistical analyses were performed with SAS 9.2 (Statistical Analysis Software release 9.2, SAS Institute Inc., Cary, North Carolina, USA). The results were statistically evaluated by means of the Fisher Exact Test. A *P*-values less than 0.05 were considered to be statistically significant in all statistical analyses.

Results

Middle ear ossicles were harvested from 46 patients. There were 27 male and 19 females. Their ages ranged from 5 to 73 years with an average age of 37 years and median 43 years.

In total, 80 middle ear ossicles were histologically examined. Harvested ossicles included 43 mallei and 37 incudes. All ossicles showed evidence of cholesteatoma. Serious erosion, grade II, was observed in 24 ossicles, mild erosion grade I in 56 ossicles.

Difference of destruction between malleus and incus

Ossicular destruction grade II was observed more frequently in the incus. We found destruction grade II in 46% of incudes and only in 16% of mallei. The difference is statistically significant (p = 0.0065, Table 1). Malleus with destruction grade II accompanied incus with the same grade of destruction or complete destruction of incus.

Tab. 1: Difference of destruction between malleus and incus.

Ossicle	N	Destruction				*P*-value*
		Grade I		Grade II		
		N	%	N	%	
Incus	37	20	54.1	17	45.9	0.0065[a]
Malleus	43	36	83.7	7	16.3	
Total	80	56	70.0	24	30.0	

[a] Fisher exact test
* Difference is significant at the significance level p < 0.05.

Difference of ossicle destruction grade II between children and adults

Ossicular destruction grade II was observed in 38% of children (under 18 years of age) and 39% of adults. The difference is not statistically significant (p = 0.3032, Table 2).

Tab. 2: Difference of ossicles destruction between children and adults.

Patients	N	Destruction				*P*-value*
		Grade I		Grade II		
		N	%	N	%	
Children[1]	16	10	62.5	6	37.5	0.3032[a]
Adults[2]	28	17	60.7	11	39.3	
Total	44	27	61.4	17	38.6	

[1] ≤18 years of age
[2] >18 years of age
[a] Fisher exact test
* Difference is significant at the significance level p < 0.05.

Lymphocyte infiltration

Lymphocyte infiltration of the inner core of the ossicle was found in 5 cases, 3 malleus and 2 incudes. Statistical significance of this difference was not tested due to the small number of infiltrated ossicles.

Cholesteatoma invasion to the deeper bone layers

Cholesteatoma appeared on the surface of ossicles. In one malleus, a plug of squamous cell epithelium was also found underneath a thin bone lamella (Fig. 2). No deeper in-

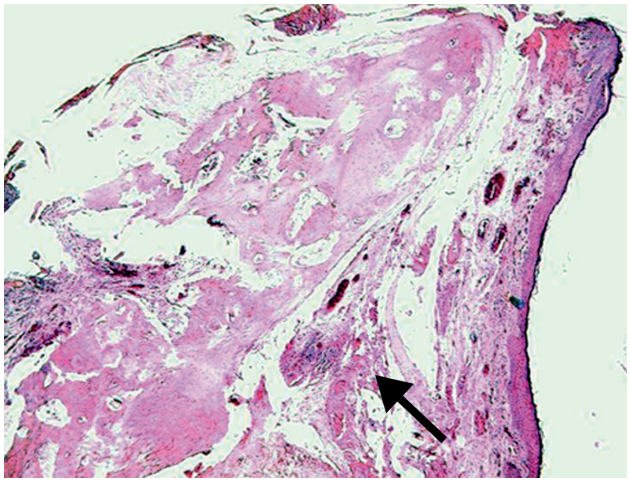

Fig. 2: Histological cross-section of incus with cholesteatoma (decalcification, haematoxylin and eosin staining, magnification 100×). The black arrowhead points to the cholesteatoma plug under thin bone lamella.

vasion of cholesteatoma into the vascular spaces or marrow spaces of the bone was observed.

Discussion

Autograft ossicles have been the choice for otologist for their biocompatibility, good hearing results and low cost. However, in patients with cholesteatoma the ossicles have been rejected because of a risk of residual disease. If the cholesteatoma is only superficial on the bone surface, mechanical cleaning of the ossicular surface should eliminate residual disease from the ossicles. Deeper invasion into the bone would exclude the possibility of mechanical cleaning of ossicles.

In this study, the residue of squamous cell epithelium was only superficially located. According to the literature, no sign of deeper invasion into the ossicle marrow was seen (2–10). One of our cases showed a plug of squamous cell epithelium underneath thin bone lamella. This finding explains the risk of residual cholesteatoma after ossicular stripping. If the surgeon eliminates the superficial soft tissue only by cold instruments (stripping), without drilling of the ossicular surface, the plug of squamous epithelium can persist underneath bone lamella. Ng et al. (6) found residual disease in 6 of 104 cleaned ossicles. Dornhoffer et al. (2) found residues in 7 of 11 specimens treated only by stripping without drilling. Vartiainen and Karjalainen (13) reported a low cholesteatoma recurrence rate of only 4%. The risk of residual disease is lowered by drilling of all ossicular surfaces under microscopic control and increased in cases of badly eroded ossicles (2, 6, 11, 12). Because badly eroded ossicles are deformed and flimsy, mechanical cleaning is technically more difficult and limited in efficacy. These severely eroded ossicles could be treated by autoclaving but badly deformed ossicles are usually not suitable for reconstruction of the ossicular chain (1, 11, 12).

We grouped the destruction of the malleus and incus into two grades. Our grading system is consistent with malleus and incus erosion classification (11, 12). Ossicle destruction grade I is mild erosion and ossicle is available for autograft ossiculoplasty. Ossicles with destruction grade II are badly eroded ossicles useless for ossiculoplasty.

Destruction grade II of incus is significantly more frequent compared to malleus. A badly eroded malleus was observed only in cases with badly eroded incus or completely destroyed incus. These findings could be explained by lower resistance of the incus against cholesteatoma. However, histological findings did not reveal important morphological differences between malleus and incus. In incus, large marrow spaces can persist this would not influence superficial erosion of the incus body. The persistence of large marrow spaces could be important for resorption of the long process of the incus in chronic otitis media. In the long process, there is only thin bone lamella protecting the bone marrow.

The second explanation for frequent destruction of the incus is the position of the cholesteatoma. Spread of cholesteatoma is consistent with the way the middle ear is ventilated. Preferential growth of cholesteatoma on the medial surface of the incus can explain its more frequent destruction as compared to the malleus.

Controversy exists as to whether cholesteatomas in childhood are more aggressive than cholesteatoma in adults. Multiple studies have shown that the rate of residual cholesteatoma is 2–3 times higher in children (14–16). Reasons for this difference are still quite unclear. Some have accented better-aerated mastoids in children in comparison with the usually sclerotic temporal bone in adults. A well-aerated mastoid provides an access of cholesteatoma to deeper aerated cells and more difficult elimination for surgeons. Current studies test levels of growth factors in cholesteatomas (17–19). Bujia et al. (18) haveproved a higher proliferation rate in pediatric cholesteatoma with increased levels of MIB-1; a nuclear antigen expressed by cells active in the cell cycle. De Carvalho Dornelles et al. (19) have demonstrated thicker epithelial matrices in pediatric cholesteatoma, higher levels of matrix metalloproteinases and exaggerated inflammatory profile. These findings suggest biologically more aggressive phenotype of pediatric cholesteatoma compared to adults. However, in our study, cholesteatoma in children was not found to be more aggressive to the middle ear ossicles. The ossicular destruction grade II in children was not significantly higher in comparison with adult cholesteatoma.

Conclusions

Cholesteatoma affects only superficial part of middle ear ossicles. A plug of squamous epithelium could spread underneath a thin bone lamella, but no deep invasion was observed. Autograft ossicles from cholesteatomatous ears should not necessarily be rejected for reconstruction of the ossicular chain. Regarding the histological finding, the authors suggest mechanical cleaning of the ossicle surface to eliminate residual disease.

Ethics committe approval

Ethics committee approval was received for this study from the ethics committee of University Hospital Hradec Králové (case number 200605 S07P).

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Conflict of interests

None declared.

Financial disclosure

None declared.

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DENTAL AND ORAL LESIONS IN HIV-POSITIVE INDIVIDUALS IN EAST BOHEMIA – CZECH REPUBLIC, SINGLE CENTRE EXPERIENCE

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Summary: Background: Human immunodeficiency virus (HIV) continues to be a serious health issue and one of the world most devastating epidemics. An estimated 1.5 million people died from AIDS-related illnesses in 2013, and an estimated 37 million people with AIDS have died worldwide since the epidemic has begun. HIV infection is known for its oral manifestations which causes discomfort and pain for infected individuals. The objective of this study was to document oral conditions of HIV positive patients and the pattern and frequency of oral and dental lesions. Methods: All patients with confirmed HIV infection who were treated at the Department of Dentistry, University Hospital in Hradec Králové, were examined. Results: During the study period, 29 HIV positive patients were examined and treated – 19 men, 10 women, with mean age of 32.9 years (range 22–58 years). 72.41% patients received ART. In total, all patients underwent 186 visits. The most frequent treatments were associated with teeth and periodontal lesions (71.80%), oral mucosal lesions were diagnosed and treated only in 3.96% cases. Conclusion: Since the introduction of ART, the frequency of oral mucosal lesions is minimal in patients with HIV infection.

Keywords: *HIV; Dental lesions; Oral mucosal lesions*

Introduction

Human immunodeficiency virus (HIV) which causes acquired immunodeficiency syndrome (AIDS) continues to be a very serious health issue and one of the world's most devastating epidemics. According to the WHO documents (1, 2) there were about 2.1 million new cases of HIV in 2013 worldwide. About 35 million people are living with HIV all around the world. An estimated 1.5 million people died from AIDS-related illnesses in 2013 and an estimated 37 million people suffering from AIDS have died since the epidemic has begun. Sub-Saharan Africa bears the biggest burden of HIV/AIDS, with nearly one in 20 adults living with HIV. Other regions significantly affected by HIV/AIDS include Latin America, the Caribbean, Eastern Europe, Central Asia, South and Southeast Asia.

The history of AIDS in medicine started in 1981 in USA where the disease was firstly described including oral signs. Discovery of HIV as the cause of AIDS was done later from blood samples originated from Central Africa. The virus was initially identified by Luc Montanier in 1983 (3) and then was fully characterized in 1984 by Robert Gallo (4–7). Later, two different types of the virus, HIV-1 and HIV-2, were recognized (8).

The objective of this study was to document oral conditions in a cohort of HIV-positive individuals and the pattern and frequency of oral mucosal and dental lesions including the treatment need found during their appointments at the Department of Dentistry, Faculty of Medicine and University Hospital in Hradec Králové, Czech Republic. This information was expected to give us a fair estimation of an impact of the HIV infection on their oral health.

Material and methods

A cohort of 29 adult HIV-positive individuals was examined at the Department of Dentistry, Faculty of Medicine and University Hospital in Hradec Králové from January 2000 to September 2013 (according to cumulative data from 1 October 1985 to 31 January 2014 of National Laboratory of references for AIDS, there were 39 HIV positive individuals diagnosed in East Bohemia (9)). All these patients were simultaneously treated at the Department of Infectious Diseases, University Hospital in Hradec Králové. Both departments serve as parts of the AIDS Centre in Hradec Králové, one of seven AIDS Centres in Czech Republic.

Each patient received a comprehensive oral and perioral examination focused on current dental, periodontal and mucosal status including radiographic examination, as well. Smears and biopsies were taken to verify the diagnosis, if necessary. All examinations were conducted by certified clinical specialists.

Following dental and oral lesions were noted: Dental caries and its complications, mostly pulpitis, pulp necrosis and apical periodontitis; plaque-related periodontal disease, e. g., gingivitis and periodontitis; salivary glands disease; mucosal disorders, e. g., herpes simplex infection, oral lichen planus, candidiasis, Kaposi sarcoma, non-specified oral ulcerations. Appropriate treatment and advice were administered. Following characteristics were also noted: Gender, age, stage of the HIV-related disease according to the WHO (10), treatment mode of the HIV infection, other systemic infectious diseases. The study has been performed according to the Declaration of Helsinki, and the procedures have been approved by the local ethics committee (reference number 201504 S16P, date of submission 26 March 2015). No statistical analysis was performed due to the small count of patients.

Results

Within the study time period, 29 HIV-positive individuals were referred to the Department of Dentistry. 19 patients (65.3%) were males, and 10 patients (34.8%) were females. The age of patients was from 22 to 58 years, with the mean age of 32.9 (+/−8.9 years). Gender, age, clinical staging of the HIV infection, treatment mode and comorbidities are summarized in Table 1.

Tab. 1: Distribution of demographic and clinical data of HIV positive patients.

Gender	n
Male	19
Female	10
Age (in years)	
20–24	4
25–29	8
30–34	9
35–39	6
40–49	1
50–59	1
Clinical staging (WHO)	
Category A (stage II)	14
Caregory B (stage III)	5
Category C (stage IV)	10
HIV treatment	
Non	7
Prophylaxis	1
ART	10
ART + prophylaxis	11
Comorbidities (systemic disease)*	
Tuberculosis	3
HIV encephalophaty	2
Lues	2
Herpes zoster	3
CMV	2
Hepatitis	3
Bacterial pneumonia	1
Wasting syndrome	1
Toxoplasmosis	1
Klebsiela	1
Arbovirosis	1

* Some patients had more than one systemic disease.

According to the WHO clinical staging of the HIV/AIDS, 14 patients (48.3%) were of stage II, 5 patients (17.2%) of stage III and 10 patients (34.5%) of stage IV. 21 patients (72.4%) received active antiretroviral treatment (ART) or active antiretroviral treatment combined with antibiotic prophylaxis. 8 patients (37.6%) recieved no therapy.

Infectious systemic diseases included tubercolosis and viral hepatitis, both in 3 cases (10.3%), herpes zoster, lues, CMV disease and HIV encephalopathy, all in 2 cases (6.9%). We have also observed one case of a bacterial pneumonia, toxoplasmosis, *Klebsiella* infection and arbovirosis (each 3.5%). Oral pain and discomfort was the most frequent complaints of evaluated individuals occuring 131 times during 186 visits (70.4%). Oral and dental lesions are summarized in Table 2.

Tab. 2: Patients dental and oral diagnose.*

Dental leasion	n
Dental caries	16
Pulpitis	4
Tooth pulp necrosis	16
Periodontal disease	
Gingivitis	23
Periodontitis	2
Oral mucosal leasion	
Candidosis	3
Herpes simplex virus	1
Non-recurrent oral ulceration	1
Haemangiom	1
Kaposi sarcoma	1
Erosive oral lichen planus	1
Sialoadenitis	1

*Some patients had more than one disease/lession.

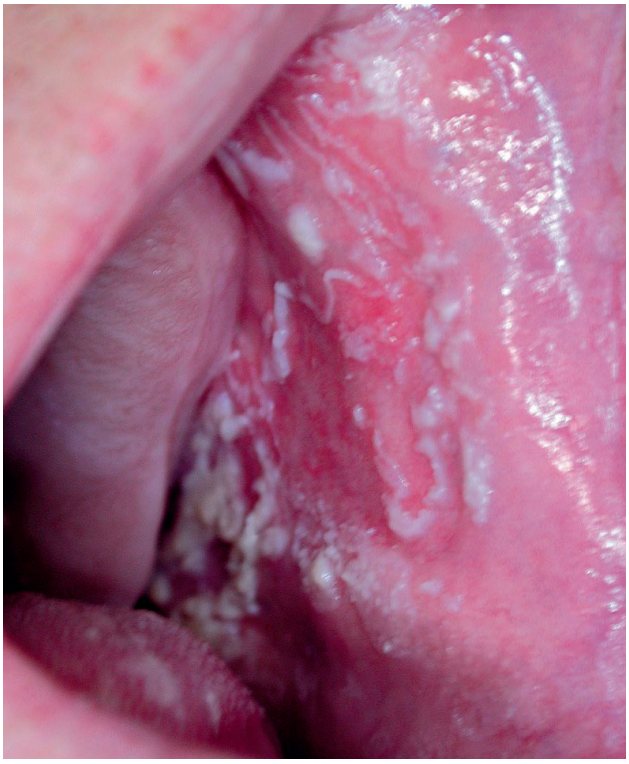


Fig. 1: Acute pseudomembranous form of the oral candidasis of the bucal mucosa. Typical white lesions combined with redness in surroundings.

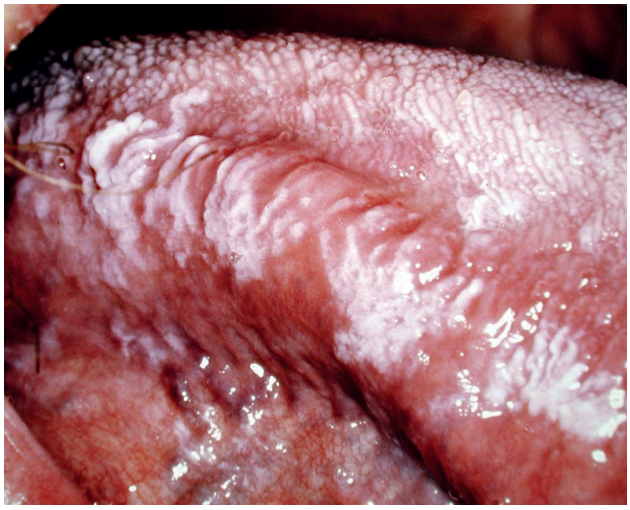
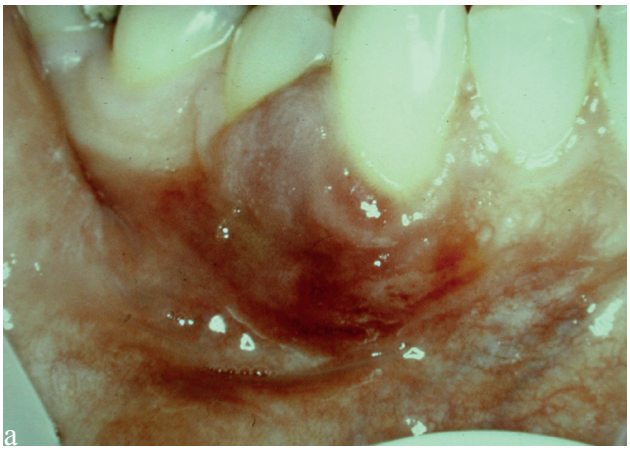


Fig. 3: Hairy leukoplakia, a chronic viral infection caused by Epstein-Barr virus, known for 40 years in the association with HIV.



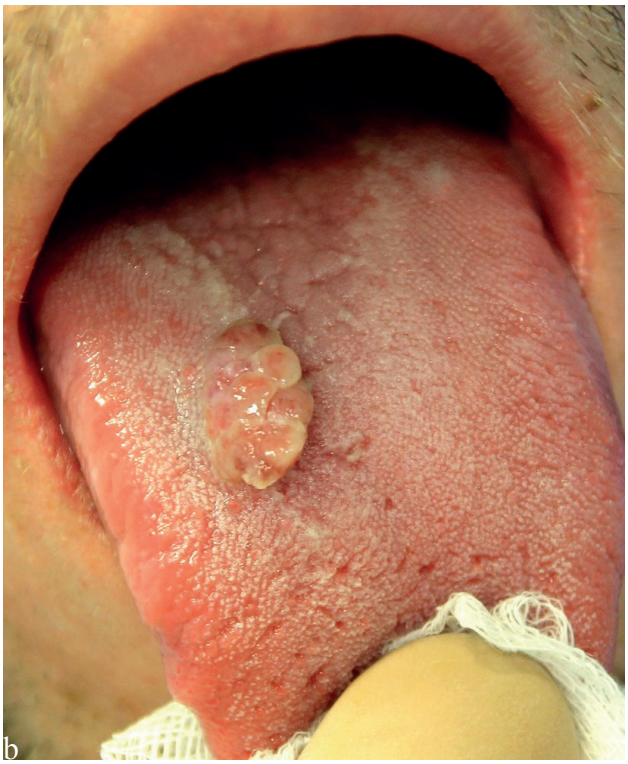


Fig. 2: Typical form of the Kaposi sarcoma of the gingiva (a) and an atypical exophytic form of the tongue tumor (b).

The most common diagnoses were chronic form of the plaque-induced gingivitis in 23 patients (79.3%), pulp necrosis in 16 patients (55.2%), tooth decay in 16 patients (55.2%), pulpitis in four patients (13.8%), and apical periodontitis in two patients (6.9%). Only 7 patients (24.1%) revealed one type of oral mucosal lesions, one of them (3.45%) with two types of various mucosal lesions simultaneously. The most frequent oral lesion was oral candidosis detected in three patients (10.3%) (Fig. 1). Other oral mucosal diagnoses such as intraoral herpes simplex, non-specified oral ulcerations, erosive form of the oral lichen planus, haemangioma, Kaposi sarcoma, and sialoadenitis of the parotid gland, were seen in each one individual (Fig. 2, 3).

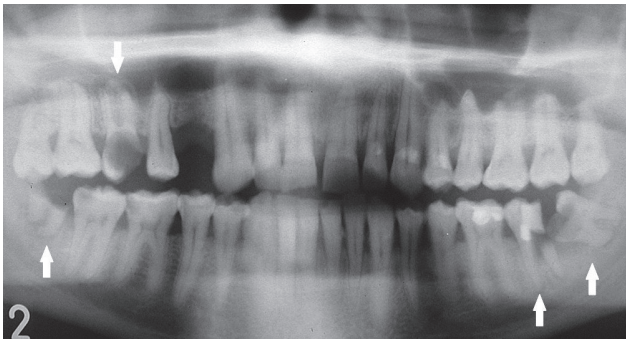


Fig. 4: Panoramic radiograph of the dentition of an HIV-positive younger individual. Note posterior teeth 16, 37, 38, 48 (arrows) fully destroyed by dental decay and indicated for extractions.

In total, the cohort of patients underwent 186 dental visits. The most frequent treatment procedures were associated with teeth and periodontal lesions (71.8%), and oral mucosal lesions were treated in 9 visits (4%). 79 restorative treatment of dental caries (34.8%) were done. Due to the extensive destruction of the crown, 38 teeth (16.7%) needed extractions (Fig. 4). Periodontal diseases were treated in 25 (11%) cases. The patients also underwent 13 endodontic treatment procedures (5.7%), and 7 prostethics restorative procedures (3.08%). A surgical procedure was needed in one patient to treat persistant oroantral comunication after the extraction of an upper molar (0.4%). All therapeutic procedures are summarized in Table 3.

Tab. 3: Distribution of dental and oral lesion treatment.

	n
Preventive treatment	55
Restorative treatment (filling)	79
Endodontic treatment	13
Extraction	38
Wassmund plastic	1
Crown	7
Periodontal disease treatment	25
Oral lesion treatment	9

Discussion

The majority of patients in our study were male (65.3%). A similar findings were reported by Ranganathanet (77.4%) (11), Nittayananta (72.5%) (12), Sharma (72.2%) (13), and Bendick (60.3%) (14). Different gender distribution in a similar study was described by Adedigba with 36.4% of female patients (15). The median age for both genders was 32.9 years.

That means practically no differences in comparizon with other clinical studies (Bendick 32 years (14), Nittayananta 28 years (12)).

The most frequent patient's oral complaint was acute pain. If untreated, in HIV positive individuals can result in odynophagia and/or dysphagia which may lead to serious problems with chewing and swallowing. This fact could be followed by a dehydratation, rapid weight loss and malnutrition resulting to the HIV-wasting syndrome. It interferes with already damaged immune system response (16, 17). In general, 70.4% of dental examinations were conditioned by the presence of an acute oral pain, comparable to the study of Agbelusi (18), who recorded pain in 80%. There is only one study by Pinheiro (19) concerning prevalence of dental caries treatment need (see Table 4 for comparison). Our results of the dental treatment need and preventive treatment need were similar. Restorative treatment need was twice higher as in the study mentioned above (78.9%), compared to our results (34.8%). In 6.2% cases in Pinheiro's study, a tooth had to be extracted (19), on the other hand, in our study tooth extraction was indicated in 16.7% only.

The most common mucosal lesion associated with HIV infection in our cohort was oral candidiasis as a very frequent fungal infection found in immunocompromised individuals. Necrotizing periodontal disease, inraoral herpes, herpes zoster, non-specified oral ulcers and Kaposi sarcoma in the oral cavity also caused pain (20, 21, 22). Interestingly, our findings showed that mucosal manifestations of the HIV infection were present in 27.6% of our HIV-positive patients only, which coresponds with results of Pinheiro (33.5%) in 2004 (19). On the other hand, different studies showed much higher prevalence of mucosal lesions, e. g., 60.4% in a study published by Arendorf (23), 79.2% by Sharma (13), 86% by Ranganathan (11), and 90% by Bendick (14). Oral candidiasis was the most frequent mucosal lesion among HIV-positive patients in this study in 3 of 29 patients (10.3%). This was far less frequent than reported by Sharma (13) 44.5%, Agbelusi (18) 43%, and Arendorf (23) where oral candidiasis was evident in 37.8 %. Lower prevalence rate (13.7%) was also reported by Schuman (24). Since the patients with oral candidosis had already been given antimycotics prophylacticaly, therapeutical dosage of antimycotics was prescribed.

Tab. 4: Percentage prevalence of dental lesion treatment needs comparison (in percentages).

Study	**Dental treatment need**	**Preventive treatment**	**Restoreative treatment**	**Endodontic treatment**	**Extraction**
A. Pinheiro et. al	78.9	26.1	77.6	0	6.2
Our study	85	24.2	34.8	5.7	16.7

Since there are seven AIDS Centres in the Czech Republic, it would be very informative to compare our findings with others. This would give us a fair idea of the problem across our country. Unfortunately, there are no similar retrospective analyses done in the Czech Republic.

Conclusion

Since the introduction of ART in clinical practice and early testing for the infection, in 2013, about 12.9 million people living with HIV had access to antiretroviral therapy (9), and the frequency of oral mucosal lesion had become significantly less frequent (25). If this trend continues, HIV/AIDS will become more manageable and such patients will be able to live without major problems and limitations related to their relatively good oral health. Nonetheless even with good care and available medication for HIV infection, people shouldn't stop taking precautions again this infection.

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NATURAL DETOXIFICATION CAPACITY TO INACTIVATE NERVE AGENTS SARIN AND VX IN THE RAT BLOOD

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Summary: Background: The method of continual determination of the rat blood cholinesterase activity was developed to study the changes of the blood cholinesterases following different intervetions. Aims: The aim of this study is registration of cholinesterase activity in the rat blood and its changes to demonstrate detoxification capacity of rats to inactivate sarin or VX in vivo. Methods: The groups of female rats were premedicated (ketamine and xylazine) and cannulated to a. femoralis. Continual blood sampling (0.02 ml/min) and monitoring of the circulating blood cholinesterase activity were performed. Normal activity was monitored 1–2 min and then the nerve agent was administered i.m. (2× LD_{50}). Using different time intervals of the leg compression and relaxation following the agent injection, cholinesterase activity was monitored and according to the inhibition obtained, detoxification capacity was assessed. Results: Administration of sarin to the leg, then 1 and 5 min compression and 20 min later relaxation showed that further inhibition in the blood was not observed. On the other hand, VX was able to inhibit blood cholinesterases after this intervention. Conclusions: The results demonstrated that sarin can be naturally detoxified on the contrary to VX. Described method can be used as model for other studies dealing with changes of cholinesterases in the blood following different factors.

Keywords: *Sarin; VX; Detoxification; Rat; Blood; Cholinesterases*

Introduction

The toxicodynamics of nerve agents is based on irreversible acetylcholinesterase (AChE, EC 3.1.1.7) inhibition at the cholinergic synapses (4). The resulting accumulation of neuromediator acetylcholine at the cholinergic synapses overstimulates the cholinergic pathways and subsequently desensitizes the cholinergic receptor sites. Before AChE inhibition in the central and peripheral nervous system, the enzyme is inhibited in the transport system, in the blood according to the principle "first come, first serve" (6). Two enzymes in the blood are present, AChE in the erythrocytes and butyrylcholinesterase (BuChE, EC 3.1.1.8) in the plasma/serum. However, in the blood, the binding of the agent to cholinesterases is leading to the decrease of its concentration and toxic effect. Other detoxification reactions have occured, too. These characteristics are important for diagnosis, mechanism of action of nerve agents, and, especially, for prophylaxis.

These prophylactic countermeasures include i.a. AChE protection against inhibition, decrease of nerve agent level using stoichiometric, catalytic and pseudocatalytic scavengers (2, 5, 11). For review see e.g. (4, 5, 10, 12, 14, 19).

Enzymes capable of nerve agents decomposition (catalytic scavengers) are known many years. They are extensively studied with the aim to develop new ways of decontamination or to prevent nerve agent toxicity (2, 10, 12, 14, 19).

Among enzymes participating in metabolism of nerve agents, A-esterases, serum cholinesterase and carboxylesterases are involved. Their role and mechanism of action in detoxification process is different and was extensively discussed by Jokanovic (10).

Detoxification capacity was studied in rats with the aim to elucidate the action of nerve agents and, to improve prophylaxis against nerve agents and organophosphorus compounds. We developed the technique for continual monitoring of the blood cholinesterase activity (7). It allows to study the changes in the activity in the real time. The method was originally developed for the study of the blood cholinesterase changes following acute exposure with nerve agents. However, the technique can be used for modelling of effects of different factors influencing the enzyme and, in the present study, it was evaluated for the demonstration of detoxification of two nerve agents, sarin and VX.

Methodical approach

Animals: Female Wistar rats (VELAZ Prague), weighing 250–270 g, were used in this study. The animals were divid-

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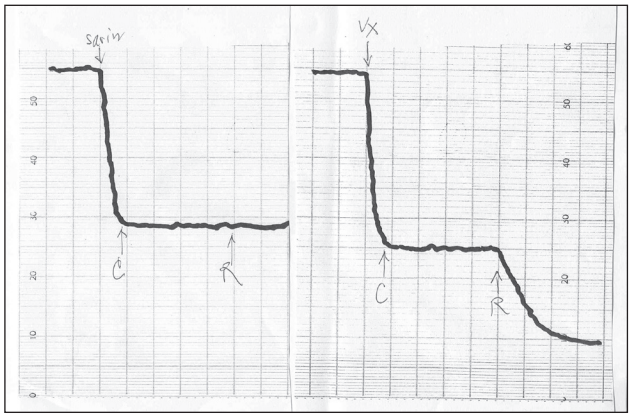

Fig. 1: Copies of original records of monitoring the rat blood cholinesterase activity. C – compression of the leg, R – relaxation of the leg.

ed into groups of 3–7 animals in each. Housing of rats was realized in the Central Vivarium of the Faculty of Military Health Sciences under veterinary control. All the experiments were performed under permission and supervision of the Ethics Committee of the Faculty of Military Health Sciences, Hradec Králové.

Chemicals: Nerve agents (sarin, VX) were obtained from Military Technical Institute of Protection (Brno, Czech Republic). They were of minimally 99% of purity and stored in glass ampullas (1 ml). The solutions in saline for experiments were prepared before use.

Continual monitoring of the rat blood cholinesterase activity: The method described by Cabal et al. (7) based on Ellman's method (8) with acetylthiocholine as substrate was used. Generally, the groups of female Wistar rats weighing 250–270 g (n = 3–7) were premedicated (ketamine and xylazine) and cannulated to a. femoralis. Continual blood sampling (0.02 ml/min) and monitoring of the circulating blood cholinesterase activity were performed (the activity represents all enzymes hydrolyzing acetylthiocholine). Normal activity was monitored 1–2 min and then the agent (sarin or VX) was administered i.m. into one leg in dose of 2× LD_{50}. Using different combinations of the leg compression and relaxation (different time intervals before/following agent injection), cholinesterase activity was monitored and according to the inhibition obtained, detoxification capacity was derived.

Results

Intoxication with sarin and VX caused strong decrease of the blood cholinesterase activity. Copies of original records of the blood cholinesterase activity is shown in Fig. 1. When administration of the agent was performed and the leg was compressed immediately (1 min) after the intoxication, the activity was decreased to 40–60% of normal activity. After relaxation 20 min later, two different changes were observed: following sarin intoxication, the inhibition was remained on the same level while following VX administration, the inhibition was continuing after relaxation (Fig. 2). When the compression was performed 5 min after the intoxication (and relaxation 20 min later), the inhibition was more expressed and continued after relaxation following VX administration while following sarin intoxication, further inhibition was not demonstrated (Fig. 3). If the compression was done later (10 min after the intoxication, then relaxation 20 min), the inhibitions by sarin and VX were not very different after relaxation in both cases (following sarin and VX administration).

Discussion

The results suggested that resorption of the agent (both sarin and VX) is very fast and 1 min after the intoxication, approximately 50% of the blood cholinesterases are inhibited. The role of compression is not significant for haemodynamic changes at the time intervals studied as it

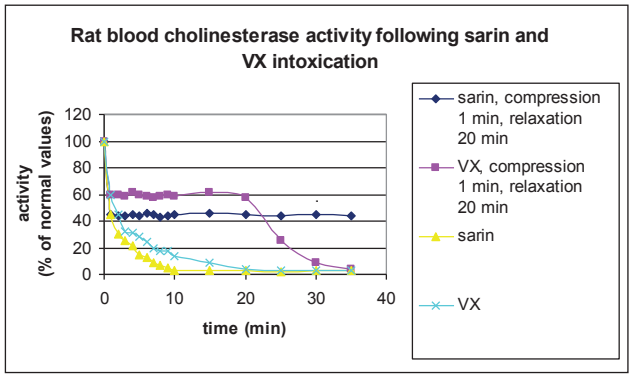

Fig. 2: Changes in the rat blood cholinesterase activity following intoxication with sarin and VX without compression and with compression 1 min after the intoxication and relaxation 20 min later [sarin (6), VX (7)]. The number in brackettes indicate the number of animals in group. The results are means only, SD were not higher than ±10%.

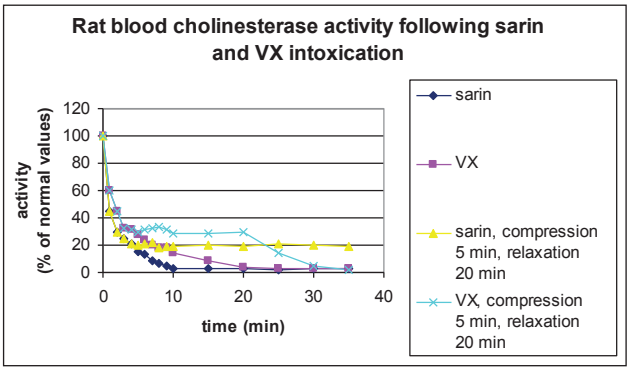

Fig. 3: Changes in the rat blood cholinesterase activity following intoxication with sarin and VX without compression [sarin (3), VX (4)] and with compression 5 min after the intoxication and relaxation 20 min later. The number in brackettes indicate the number of animals in group. The results are means only, SD were not higher than ±10%.

was described earlier: there were observed haemodynamic changes following femoral vein ligation but the changes were demonstrated after relatively long time (6 hours) after the ligation (20).

Comparison of these results is difficult because a lack of similar data in literature. The half-lives of inhibition of the blood ChE following intoxication with VX and sarin (1) can be compared with our results and the half-times of inhibition are similar. The blood cholinesterase activity on swine was monitored following percutaneous intoxication with VX (17); the inhibition was lower in comparison with our results. Toxicokinetic data with direct determination of the agents published by Schans et al. (21) show similar results as demonstrated in our experiments with inhibition efficacy in the blood.

Quite different cholinesterase changes obtained following sarin and VX intoxication (after compression and relaxation of the leg) suggested that the loss of inhibition capacity can not be scavenger effect (in that case, similar results for both agents sarin and VX would be expected). The loss of inhibition capacity is probably caused by detoxification indicating that sarin is able to be detoxified. Similar results (detoxification of nerve agents including sarin except VX) were demonstrated by Ohmori et al. (18) in decontamination study. Degradation of G-type of nerve agents was also described (15); it was caused by carboxylesterases: their amount in mice and rats was higher than in rabbits or guinea pigs leading to higher LD_{50}.

Since VX does not react with carboxylesterases, VX can not be detoxified by these enzymes (16). It could be also a reason for different cholinesterase inhibition by G- and V-nerve agents in the brain structures as demonstrated earlier (9, 3). Similar problem was solved by Wills et al. (22) using another approach: fresh frozen plasma (FFP) was used to demonstrate detoxification of G- and V-agents in vitro; the authors concluded suitability of FFP for the treatment of intoxication with G-agents but not for V-agents. Using the method of continual determination of the blood cholinesterase activity, it is possible to monitor cholinesterase activity not only for irreversible but also for reversible inhibitors used for prophylaxis against nerve agent intoxication, e.g. physostigmine, tacrine, huperzine etc. (4, 13).

Conclusions

Described methodical approach allows in real time and real conditions in vivo to monitor the changes of the blood cholinesterases following administration of different experimental interventions, e.g. agents administration, and the effect of detoxification.

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OESOPHAGEAL MANOMETRY IN EXPERIMENTAL PIGS: METHODS AND INITIAL EXPERIENCE

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Summary: The aim of this study project was to prepare our own method of porcine oesophageal manometry. Ten mature experimental pigs entered the study. Conventional water-perfused system was decided for manometry. Porcine resting and relaxed pressures of the lower oesophageal sphincter are fully comparable with healthy human subjects. Evocable swallowing is doable and oesophageal peristalsis is quantifiable. Basic manometric parameters were different in male and female animals. Oesophageal manometry in experimental pigs is feasible. Porcine oesophageal manometry will be usable for preclinical studies in future.

Keywords: *Experimental pigs; General Anaesthesia; Oesophageal Manometry*

Introduction

Pigs, as an omnivorous representative, can be used in various preclinical experiments due to their relatively very similar gastrointestinal functions compared to humans (1, 2). Our group demonstrated that gastric myoelectrical studies (using electrogastrography) are reliable and feasible in experimental pigs (3–7). The aim of this current project was to work out our own method of oesophageal manometry in experimental pigs.

Material and methods

Animals

Five mature male and five mature female experimental pigs entered the study (*Sus scrofa f. domestica*, hybrids of Czech White and Landrace breeds; 3–4 month old; weighing 27.5–41.5 kg, mean 32.0 ± 4.6, median 30.5 kg). Animals were fed twice a day (standard assorted food A1) and were allowed free access to water. All manometry investigations were performed under general anaesthesia in the morning after 24 hours of fasting. Intramuscular injections of ketamine (20 mg per kg; Narkamon, Spofa, Praha, Czech Republic) and azaperone (2.2 mg per kg; Stresnil, Janssen Animal Health, Saunderton, UK) were used as an introduction. General anaesthesia was carried out by propofol (2.2 mg/kg; Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany).

Oesophageal manometry

Water-perfused disposable catheters were used (MMS G-88402, conventional 12 French, 8 channels with central lumen; MMS – Medical Measurement Systems B.V., Enschede, the Netherlands). Catheters were introduced into the oesophagus through mouth (using a dedicated mouthpiece). Their correct position was verified endoscopically. We used video-gastroscope Olympus GIF160 dedicated for animal use only (Olympus Optical Co, Tokyo, Japan). All animals were lying in supine position. Oesophageal manometry was performed for 10 minutes by means of the Polygraf UPS 2020 (UPS-2020 manometry system from MMS – Medical Measurement Systems B.V., Enschede, the Netherlands). Dry swallowing was induced by massage of lower part of the neck. All evaluated parameters were assessed as an average measure of four consecutive values.

Statistics

The data were analysed using SigmaStat software (Version 3.1, Jandel Corp., Erkrath, Germany). Descriptive statistics, Fisher's exact test and un-paired t-test were used.

Ethics

The Project was approved by the Institutional Review Board of the Animal Care Committee of the University of Defence, Faculty of Military Health Services, Hradec Králové, Czech Republic (Protocol Number 14/2012). Ani-

mals were held and treated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (8).

Results

Oesophageal manometry was successfully accomplished in all animals. Lower oesophageal sphincter was easily identified in all animals (Fig. 1). Its middle part is 50 to 55 cm far from incisors. It was possible to evaluate all recordings in all 10 animals. In the absence of oesophageal contractions, artefacts can be readily identified, caused by respiration (18 cycles per minute in average) (Fig. 2). Oesophageal peristalsis during dry swallowing was evocable in all animals, with substantial relaxation of the lower oesophageal sphincter (Fig. 3). Values of basic parameters are given in Table 1. Male and female pigs were comparable in age and weight. Baseline pressure of the lower oesophageal sphincter and peristaltic wave pressure were different in male and female experimental pigs (see Figs. 4 and 5).

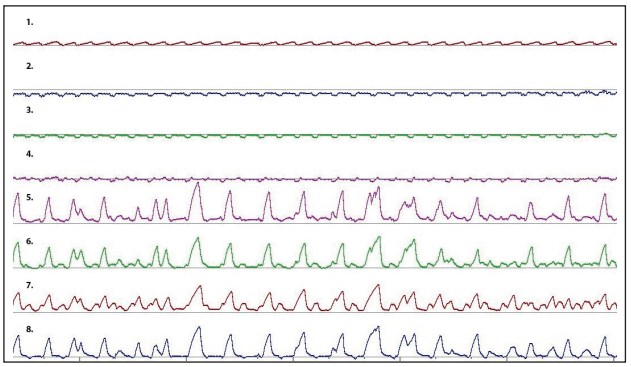

Fig. 1: Porcine oesophageal manometry using a water-perfused system.
Four proximal pressure sensors are localised in the oesophageal body (1.–4.) and four distal sensors are placed in the area of the lower oesophageal sphincter (LOS; 5.–8.). Manometry without swallowing shows a high-pressure LOS zone in distal four sensors (5.–8.). Pressure values displayed on the Y-axis; time course on the X-axis.

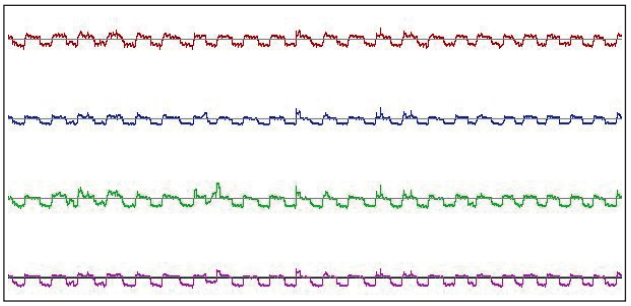

Fig. 2: Respiratory artefacts.
Four pressure sensors localized in the oesophagus show respiratory artefacts in the absence of oesophageal contractions.

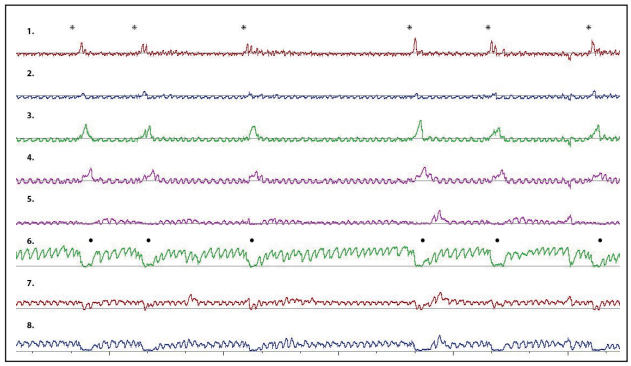

Fig. 3: Propulsive peristaltic contraction of the porcine oesophagus. Zone of high pressure is produced by the lower oesophageal sphincter and diaphragm (5.–8.). Several peristaltic sequences are recorded, with a propulsive increase of oesophageal pressure in the oesophageal body (sensors 1.–4.). Asterisks indicate dry swallows. Relaxation of the lower oesophageal sphincter is marked with black closed circles.

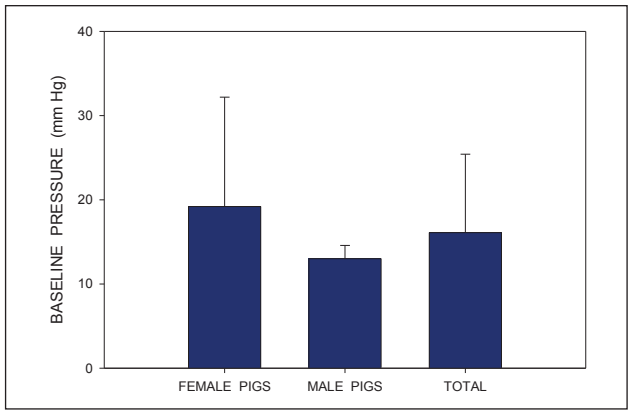

Fig. 4: Baseline pressure of the lower oesophageal sphincter in total and separately in female and male experimental pigs. The difference is not statistically significant (with power below desired value of 0.8).

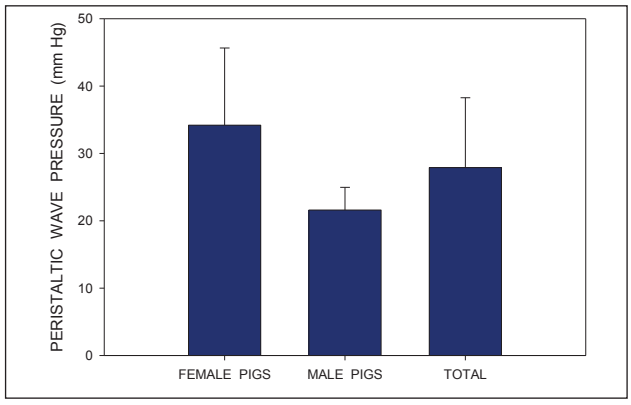

Fig. 5: Peristaltic wave pressure in total and separately in female and male experimental pigs. The difference between female and male pigs is statistically significant ($p = 0.046$).

Tab. 1: Oesophageal manometry in experimental pigs.

Animal No. / sex	**Baseline pressure of LES** (mm Hg)	**Relaxation of LES** (%)	**Duration of relaxation** (s)	**Peristaltic wave pressure** (mm Hg)	**Duration of peristaltic wave** (s)
1 / F	12	85	5.7	46	3.3
2 / F	23	100	23.0	20	4.5
3 / F	40	89	8.4	46	2.8
4 / F	7	100	2.4	29	2.3
5 / F	14	100	3.5	30	1.5
6 / M	15	95	5.5	24	1.7
7 / M	13	98	7.5	19	1.4
8 / M	11	100	1.5	26	1.5
9 / M	14	100	5.4	21	2.0
10 / M	12	100	4.4	18	2.0

F – female; M – male
LES – lower oesophageal sphincter
s – seconds

Mean relaxation of the lower oesophageal sphincter was 96.7 ± 5.4%, duration of relaxation was 6.7 ± 6.1 s. Mean duration of peristaltic wave was 2.3 ± 1.0 s. These two time intervals were longer in female pigs (Table 1), however, the difference did not reach a statistical significance. Propulsive peristalsis was found in 86%, there were simultaneous (3%), interrupted (4%) or non-transmitted ones (7%) in remaining cases.

Discussion

Our group elaborated our own method of oesophageal manometry in experimental pigs. It was a feasibility study in fact. Porcine hiatal and gastro-oesophageal anatomy and physiology are similar to human ones. The muscle is thicker at the point where the clasp (on the right side) and sling fibres (on the left) concentrate. The pressure profiles are axially and radially asymmetric in coincidence with the thickness variations of the corresponding muscle layers. Sphincteric pressure is recorded as a plateau, whereas diaphragmatic crural pressure appears as phasic oscillations in synchrony with respiration. The sphincter relaxed upon deglutition (9).

We were able to find out only two papers published so far that can be partly compared with our study. Ciotola et al. (10) performed peroral endoscopic myotomy (POEM) in five pigs. Mean pre-myotomy pressure of the lower oesophageal sphincter was 36 ± 8 mm Hg. After myotomy, the pressure significantly dropped to 10.6 ± 3.2 mm Hg (10). Perretta et al. (11) found mean preoperative pressure 22.2 ± 3.3 mm Hg and mean pressure 11.3 ± 2.7 mm Hg after POEM in four experimental pigs.

In humans, various authors reported different normal range of basic manometric parameters in healthy subjects, beside other things influenced by ethnicity and/or age (12–18). There are also significant pressure differences between solid-state and water-perfused systems in lower oesophageal sphincter measurement (16). Common values of baseline pressure of the lower oesophageal sphincter are about 5–25 mm Hg and relaxation 90–100% (with duration 5–10 s). Common peristaltic wave pressure is about 30–160 mm Hg in humans (with duration of contraction 2–6 s) (18). Based on our study, the crucial parameters of porcine oesophageal manometry are comparable with those in healthy humans.

Surprisingly, basic manometric parameters were different in male and female experimental pigs in our current study. Peristaltic wave pressure was significantly higher in female pigs. There was also a clearly distinct trend in other parameters in favour of female gender (higher baseline pressure of the lower oesophageal sphincter, longer duration of relaxation and longer duration of peristaltic wave), although they did not reach a statistically significant difference, mostly because of a small number of subjects. Gender-related difference of the oesophageal motility has not been reported in porcine manometry yet, but it was already described in healthy humans. Differences have been observed in water ingestion, oropharyngeal transit, duration of opening of the upper oesophageal sphincter, and pressure duration in the oropharynx with swallows (for review see ref. 19). Women also had longer duration of oesophageal contraction in the distal oesophageal body (19). The explanation for the results observed may be anatomic and/or hormonal differences between genders (20).

We are fully aware of possible limits of our current study. Primarily, this is our very initial experience with porcine oesophageal manometry. Number of subjects was sufficient for a usual animal setting but not for detailed statistics, especially correlation analysis. We decided a conventional water-perfused system, not a high-resolution manometry. All measurements were accomplished under general anaesthesia that could also influence the acquired results. Nevertheless all the obtained data are consistent.

Conclusions

Oesophageal manometry in experimental pigs is feasible. Porcine resting and relaxed pressures of the lower oesophageal sphincter are fully comparable with healthy human subjects. Evocable swallowing is doable and oesophageal peristalsis is quantifiable. Porcine oesophageal manometry will be usable for preclinical studies in future.

Conflicts of interest

The authors declare no conflict of interest.

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THE EVALUATION OF THE POTENCY OF NEWLY DEVELOPED OXIMES (K727, K733) AND TRIMEDOXIME TO COUNTERACT ACUTE NEUROTOXIC EFFECTS OF TABUN IN RATS

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Summary: Aim: The ability of two newly developed oximes (K727, K733) to reduce tabun-induced acute neurotoxic signs and symptoms was evaluated and compared with currently available trimedoxime in rats. Methods: The neuroprotective effects of the oximes studied combined with atropine on Wistar rats poisoned with tabun at a lethal dose (380 μg/kg i.m.; 90% of LD_{50} value) were evaluated. Tabun-induced neurotoxicity was monitored by the functional observational battery consisting of 38 measurements of sensory, motor and autonomic nervous functions at 2 hours following tabun challenge. Results: All tested oximes combined with atropine enable tabun-poisoned rats to survive till the end of experiment. Both newly developed oximes (K727, K733) combined with atropine were able to decrease tabun-induced neurotoxicity in the case of lethal poisoning although they did not eliminate all tabun-induced acute neurotoxic signs and symptoms. Conclusion: The ability of both novel bispyridinium oximes to decrease tabun-induced acute neurotoxicity was slightly lower than that of trimedoxime. Therefore, the newly developed oximes are not suitable for the replacement of commonly used oximes such as trimedoxime in the treatment of acute tabun poisonings.

Keywords: *Tabun; Neurotoxicity; Functional observational battery; Oximes; Rats*

Introduction

Highly toxic organophosphorus compounds called nerve agents have been developed as chemical warfare agents. They exert their toxic effects primarily via irreversible inhibition of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) by phosphylation (phosphorylation or phosphonylation) of its active site serine in the central and peripheral nervous system. The inhibition of AChE after exposure to nerve agents leads to the accumulation of the neurotransmitter acetylcholine in the synaptic cleft and to subsequent overstimulation of both muscarinic and nicotinic cholinergic receptors that results in excitotoxicity, seizures, brain damage, long-term behavioral aberrations including cognitive deficits and other signs and symptoms of acute cholinergic crisis. The death is usually caused by central and peripheral respiratory failure resulting from bronchospasm, excessive bronchial secretion, paralysis of respiratory muscles, and depression of brain respiratory centers (1–3).

A current standard treatment of poisoning with nerve agents usually consists of a combined administration of an anticholinergic drug (preferably atropine) and an oxime (preferably pralidoxime or obidoxime). Generally, anticholinergics are used for relieving muscarinic signs and symptoms whereas oximes are used for reactivation of nerve agent-inhibited AChE (2, 4, 5). Unfortunately, currently available antidotal treatment is not able to sufficiently counteract acute toxic effects of nerve agents because of limited ability of oximes to reactivate nerve agent-inhibited AChE, especially in the case of acute poisoning with tabun, soman and cyclosarin (6–8). Tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate) is one of the most resistant nerve agents. Its deleterious effects are extraordinarily difficult to antagonize due to the changes in hydrogen bonding and conformational changes of AChE-tabun complex in the AChE active site that make the nucleophilic attack of oximes very difficult (9, 10).

In the case of severe intoxication, some nerve agents including tabun can cause centrally mediated seizure activity that can rapidly progress to status epilepticus and contribute to profound brain damage that is associated with long-lasting neurological and psychological injuries (11, 12). Therefore, the ability of antidotes to counteract acute neurotoxic effects of nerve agents and prevent nerve agent-poisoned organisms from irreversible lesions in the central nervous system (CNS) is very important for the successful antidotal treatment of acute nerve agent poisonings. Generally, the oximes exert more potent effects in the peripheral nervous system compared to CNS due to their low penetration across the blood-brain barrier (BBB). Although the percentage of reactivation of nerve agent-inhibited AChE in the brain is lower compared to the peripheral nervous system, the role

of reactivation of nerve agent-inhibited AChE in the brain is important for survival from nerve agent exposure (2).

As currently available antidotal treatment consisting of atropine and commonly used reactivator of inhibited AChE (pralidoxime, obidoxime, trimedoxime) is not able to sufficiently counteract acute toxic effects of tabun because of low ability of oximes to reactivate tabun-inhibited AChE (8), the replacement of commonly used oximes with a more effective oxime has been a long-standing goal for the treatment of tabun poisoning. Therefore, we are still searching for a more efficacious oxime able to sufficiently reactivate tabun--inhibited AChE. For this purpose, two novel oximes, K727 [naphtylene-2,7-diyl-bis(2-hydroxyiminomethylpyridinium) dibromide] and K733 [4-(ethylcarboxyl)-2′-(hydroxyiminomethyl)-1,1′-(phenylene-1,3-diyl)-bispyridinium dibromide] (Figure 1), were synthesized at our Department of Toxicology and Military Pharmacy to improve the efficacy of antidotal treatment of tabun poisoning. They were developed based on the structure-activity relationship study and they were chosen based on the data obtained from molecular docking and *in vitro* evaluation of their ability to reactivate acetylcholinesterase inhibited by organophosphorus compounds. The evaluation of their potency to reactivate tabun-inhibited hAChE using *in vitro* methods showed that the reactivating efficacy of both newly developed oximes is comparable with trimedoxime and obidoxime.

The aim of this study was to compare the potential neuroprotective effects of two newly developed oximes (K727, K733) with trimedoxime in combination with an anticholinergic drug atropine in tabun-poisoned rats. The tabun-induced neurotoxic signs were determined using a functional observational battery, a non-invasive and relatively sensitive type of neurological examination for a wide range of neurobiological functions including measurements of sensory, motor and autonomic nervous functions (13).

Materials and methods

Animals

Male albino Wistar rats weighing 220–250 g were purchased from VELAZ, Czech Republic. They were kept in climate- and access-controlled rooms (22 ± 2 °C and 50 ± 10% relative humidity) with the light from 07:00 hr to 19:00 hr and were allowed access to standard food and tap water *ad libitum*. The rats were acclimatized in the laboratory vivarium for 14 days before starting the experiments, and they were divided into groups of 8 animals. Handling of the experimental animals was done under the supervision of the Ethics Committee of the Faculty of Military Health Sciences, Czech Republic.

Chemicals

Tabun was obtained from the Military Technical Institute in Brno (Czech Republic) and was 90% pure as assayed by acidometric titration. The basic solution of tabun (1 mg/1 mL) was prepared in propyleneglycol three days before starting the experiments. Actual solution of tabun was prepared from its basic solution with the help of saline immediately before administration. All oximes studied (Figure 1) were of 98.5% purity and were synthesized at the Department of Toxicology and Military Pharmacy of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic). Their purities were analyzed using HPLC (14). All other drugs and chemicals of analytical grade were obtained commercially (Sigma Aldrich, Prague, Czech Republic) and used without further purification. The saline solution (0.9% NaCl) was used as a vehicle. All substances were administered intramuscularly (i.m.) at a volume of 1 mL/kg body weight (b.w.).

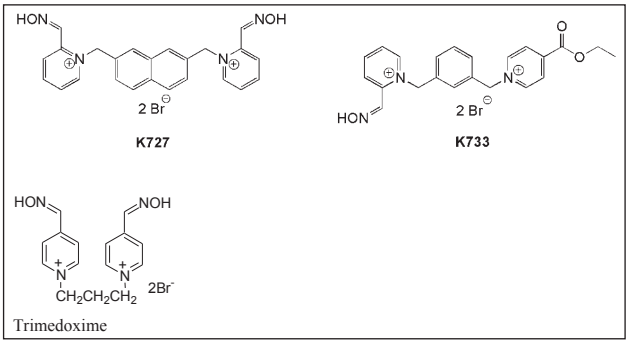

Fig. 1: Chemical structure of oximes studied.

In vivo experiments

Tabun was administered i.m. at a lethal dose (380 μg/kg b.w. – 90% LD_{50}). The relatively high dose of tabun was used to achieve maximum number of tabun-induced neurotoxic signs and symptoms at the time when the neurotoxicity of tabun was monitored by the functional observational battery. One minute following tabun poisoning, the rats were treated i.m. with atropine sulfate (10 mg/kg b.w. corresponding to 1% of its LD_{50} value) in combination with the oxime K727, K733 or trimedoxime at equitoxic doses corresponding to 5% of their LD_{50} values (15). The neurotoxicity of tabun was monitored using the functional observational battery at 2 hours following tabun poisoning. This time interval was chosen because we wanted to assess the maximal ability of antidotal treatment to avoid or at least diminish all tabun-induced neurotoxic signs and symptoms during acute cholinergic crisis when the full clinical picture of acute poisoning with tabun is developed and visible. The evaluated markers of tabun-induced neurotoxicity in experimental animals were compared with the parameters obtained from control rats given saline instead of tabun and antidotes at the same volume (1 mL/kg b.w.). In addition, the markers of tabun-induced neurotoxicity in treated tabun-poisoned rats were compared with the parameters obtained from non-treated tabun-poisoned rats.

Tab. 1: Functional observational battery.

MARKER	Scored values only									
	−2	**−1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
POSTURE				*sitting or standing*	*rearing*	*asleep*	flattened	lying on side	crouched over	head bobbing
CATCH DIFFICULTY				passive	*normal*	defense	flight	escape	aggrression	
EASE OF HANDLING				very easy	*easy*	moderately difficult	difficult			
MUSCULAR TONUS	atonia	hypotonia	*normal*	hypertonia	rigidity	fasciculations				
LACRIMATION			*none*	slight	severe	crusta	coloured crusta			
PALPEBRAL CLOSURE				*open*	slightly drooping	half-way drooping	completely shut	ptosis		
ENDO-EXOPHTHALMUS		endo	*normal*	exo						
PILOERECTION			*no*	yes						
SKIN ABNORMALITIES			*normal*	pale	erythema	cyanosis	pigmented	cold	injury	
SALIVATION			*none*	sllight	severe					
NOSE SECRETION			*none*	slight	severe	coloured				
CLONIC MOVEMENTS			*normal*	repetitive movements of mouth and jaws	nonrhythmic quivers	mild tremors	severe tremors	myoclonic jerks	clonic convulsions	
TONIC MOVEMENTS			*normal*	contraction of extensors	opisthotonus	emprostho-tonus	explosive jumps	tonic convulsions		
GAIT			*normal*	ataxia	overcompen-sation of hindlimbs movements	feet point outwards from body	forelimbs are extended	walks on tiptoes	hunched body	body is flattened against surface
GAIT SCORE				*normal*	slightly impaired	somewhat impaired	totally impaired			
MOBILITY SCORE				*normal*	slightly impaired	somewhat impaired	totally impaired			
ACTIVITY				very low	sporadic	reduced	*normal*	enhanced	permanent	

AROUSAL (level of unprovoked activity)				very low	sporadic	reduced	*normal*	enhanced	permanent	
TENSION			*none*	partial (ears)	stupor					
STEREOTYPY			*none*	head weaving	body weaving	grooming	circling	others		
BIZARRE BEHAVIOR			*none*	head	body	self–mutilation	abnormal movements	others		
APPROACH RESPONSE				no reaction	*normal*	slow reaction	energetic reaction	exaggerated reaction		
TOUCH RESPONSE				no reaction	*normal*	slow reaction	energetic reaction	exaggerated reaction		
CLICK RESPONSE				no reaction	*normal*	slow reaction	energetic reaction	exaggerated reaction		
TAIL-PINCH RESPONSE				no reaction	*normal*	slow reaction	energetic reaction	exaggerated reaction		
PUPIL SIZE		miosis	*normal*	mydriasis						
PUPIL RESPONSE			no reaction	*normal reaction*						
RIGHTING REFLEX				*normal*	slightly uncoordin.	lands on side	lands on back			

Tab. 2a: The values of tabun-induced activity and neuromuscular neurotoxic markers measured at 2 hours following tabun challenge by the functional observational battery (No. 1–2, 4–10: scored values; No. 3, 11–17: values in absolute units). Statistical significance: * $p < 0.05$ (comparison with the control values).

2 hours		**Controls**		**Tabun + A + trimedoxime**		**Tabun + A + K733**		**Tabun + A + K727**		**Tabun**	
No.	***Marker***	***x/M***	***−/+s***	***x/M***	***−/+s***	***x/M***	−/+s	x/M	−/+s	x/M	−/+s
1	posture	1.00		**3.00***		**3.00***		**3.00***		**7.00***	
2	muscular tonus	0.00		**−2.00***		**−2.00***		**−2.00***		**−2.00***	
3	rearing	5.00	3.00	**1.00***	1.00	**0.00***	0.00	**1.00***	2.00	**0.13***	0.35
4	clonic movements	0.00		0.00		0.00		0.00		**2.00***	
5	tonic movements	0.00		0.00		0.00		0.00		**5.00***	
6	gait	0.00		**1.00***		**1.00***		**1.00***		**7.00***	
7	gait score	0.00		**3.00***		**3.00***		**3.00***		**4.00***	
8	mobility score	1.00		1.00		1.00		**2.00***		**4.00***	
9	activity	4.00		4.00		3.00		3.00		**1.00***	
10	righting reflex	1.00		1.00		1.00		1.00		**4.00***	
11	landing foot splay (mm)	79.67	20.58	78.33	12.00	74.17	13.42	82.00	16.61	103.00	0.39
12	forelimb grip strength (kg)	5.31	0.32	**3.81***	1.00	**3.49***	2.00	**3.67***	0.89	**2.85***	2.00
13	hindlimb grip strength (kg)	1.09	0.21	**0.49***	0.21	**0.63***	0.35	**0.37***	0.08	**0.45***	0.42
14	grip strength of all limbs (kg)	16.22	2.87	**9.28***	4.00	**5.82***	3.00	**7.30***	1.28	**4.97***	5.20
15	vertical activity	258.67	149.90	97.33	98.2	**71.33***	90.53	**87.83***	78.19	**8.00***	17.66
16	horizontal activity	45.33	36.21	**1.80***	4.00	**0.33***	0.82	**8.00***	19.00	**0.00***	0.00
17	total motor activity	304.00	172.32	**98.23***	99.47	**71.67***	90.32	**95.83***	82.01	**8.00***	17.66
		n = 8		n = 8		n = 8		n = 8		n=3	

Tab. 2b: The values of tabun-induced sensorimotor and excitability neurotoxic markers measured at 2 hours following tabun challenge by the functional observational battery (scored values). Statistical significance: * $p < 0.05$ (comparison with the control values).

2 hours		Controls		Tabun + A + trimedoxime		Tabun + A + K733		Tabun + A + K727		Tabun	
No	*Marker*	*x/M*	*−/+s*	*x/M*	*−/+s*	*x/M*	*−/+s*	*x/M*	*−/+s*	*x/M*	*−/+s*
1	catch difficulty	2.00		**1.00***		**1.00***		**1.00***		**1.00***	
2	ease of handling	2.00		**1.00***		**1.00***		2.00		**1.00***	
3	arousal	1.00		1.00		1.00		1.00		**4.00***	
4	tension	0.00		0.00		0.00		0.00		0.00	
5	stereotypy	0.00		0.00		0.00		0.00		0.00	
6	bizarre behavior	0.00		0.00		0.00		0.00		0.00	
7	approach response	2.00		2.00		2.00		2.00		**1.00***	
8	touch response	2.00		2.00		2.00		**3.00***		**1.00***	
9	click response	2.00		2.00		**1.00***		2.00		**1.00***	
10	tail−pinch response	2.00		2.00		2.00		2.00		**1.00***	
		n = 8		n = 8		n = 8		n = 8		n = 3	

Tab. 2c: The values of tabun-induced autonomic neurotoxic markers measured at 2 hours following tabun challenge by the functional observational battery (No 1–6, 9–10: scored values; No 7–8, 11: values in absolute units). Statistical significance: * $p < 0.05$ (comparison with the control values).

2 hours		Controls		Tabun + A + trimedoxime		Tabun + A + K733		Tabun + A + K727		Tabun	
No	*Marker*	*x/M*	*−/+s*	*x/M*	*−/+s*	*x/M*	−/+s	x/M	−/+s	x/M	−/+s
1	lacrimation	0.00		0.00		0.00		0.00		**4.00***	
2	palpebral closure	1.00		1.00		1.00		1.00		**4.00***	
3	endo/exophtalmus	0.00		0.00		0.00		0.00		**−1.00***	
4	skin abnormalities	0.00		0.00		0.00		0.00		**3.00***	
5	salivation	0.00		0.00		0.00		0.00		**2.00***	
6	nose secretion	0.00		0.00		0.00		0.00		**3.00***	
7	urination	5.00	5.00	0.17	0.41	2.33	4.00	1.50	2.00	**0.00***	0.00
8	defecation	0.00		0.00		0.00		0.00		0.00	
9	pupil size	0.00		**−1.00***		**−1.00***		**−1.00***		**−1.00***	
10	pupil response	1.00		**0.00***		**0.00***		**0.00***		**0.00***	
11	body temperature (°C)	37.68	0.34	**36.33***	1.00	**35.50***	0.28	**35.38***	0.38	**36.18***	0.80
		n = 8		n = 8		n = 8		n = 8		n = 3	

The functional observational battery (FOB) consists of 38 measurements of sensory, motor and autonomic nervous functions. Some of them are scored (Table 1), the others are measured in absolute units (13, 16). The first evaluation was obtained when tabun-poisoned rats were in the home cage. The observer evaluated each animal's posture, palpebral closure and involuntary motor movements. Then, each rat was removed from the home cage and briefly hand-held. The exploratory activity, piloerection and other skin abnormalities were noted. Salivation and nose secretion were also registered and scored. Then, the rats were placed on a flat surface which served as an open field. A timer was started for 3 min during which the frequency of rearing responses was recorded. At the same time, gait characteristics were noted and ranked, and stereotypy and bizarre behaviors and abnormal posture were evaluated. At the end of the third minute, the number of fecal boluses and urine pools on the adsorbent pad was registered. Reflex testing comprising recording each rat's response to a frontal approach of the blunt end of a pen, a touch of the pen to the posterior flank and to an auditory clic stimulus was also used. The response to a pinch on the tail and the ability of pupils to constrict in response to light were then assessed. These measures were followed by a test for the aerial righting reflex and by the measurements of forelimb and hindlimb grip strength, body temperature and finally hindlimb landing foot splay. The whole battery of tests required approximately 6–8 min per rat. Motor activity data were collected shortly after finishing the functional observational battery, using an apparatus for testing of a spontaneous motor activity of laboratory animals (constructed at the Faculty of Military Health Sciences, Hradec Králové, Czech Republic). The animals were placed for a short period (10 min) in the measuring cage and their movements (total, horizontal and vertical activity) were recorded. The observer was blind to treatment condition.

Data collected with the FOB include categorial, ordinal and continuous values. Their statistical analyses were performed on a PC with a special interactive programme NTX (13). The categorial and ordinal values were formulated as contingency tables and judged consecutively by Chi-squared test of homogeneity, Concordance-Discordance test and Kruskal-Wallis test, respectively. The continual data were assessed by successive statistical tests: CI for Delta, Barlett test for Equality of Variance, Williams test and Test for Distribution Functions. The differences were evaluated at the significant level $p < 0.05$.

Results

The results of the experiments related to the measurement of tabun-induced neurotoxicity at 2 hours following tabun poisoning are divided into three parts (activity and neuromuscular measures, sensorimotor and excitability measures and autonomic measures) (16) and summarized in Table 2a–c. While five non-treated tabun-poisoned rats died before the evaluation of tabun-induced neurotoxicity by FOB, all tabun-poisoned rats treated with atropine in combination with an oxime survived till the end of experiment.

The evaluation of tabun-induced neurotoxic signs at 2 hours following intoxication proved significant alteration of 32 observed parameters. Tabun produced passive behavior of rats during handling and retention, severe miosis, lacrimation, salivation, nose secretion and a decrease in muscular tone. The posture of tabun-poisoned rats was seriously altered and skin abnormalities were observed. Exploratory and rearing activities were significantly reduced, righting reflex was altered, clonic and tonic convulsions were observed, gait and mobility were impaired and ataxia was found. In addition, no reaction during a reflex testing consisting of recording each rat's response to the frontal approach of the blunt end of a pen, to the touch of the pen to the posterior flank, to an auditory click stimulus and to a pinch on the tail was found. The pupils of the tabun-poisoned rats did not constrict in response to light because of tabun-induced miosis. A significant decrease in forelimb and hindlimb grip strength and body temperature was also observed at 2 hours following tabun challenge. In addition, vertical and horizontal motor activity was markedly reduced (Table 2a–c).

Both newly developed oximes (K727, K733) in combination with atropine were able to prevent some tabun-induced signs of neurotoxicity observed at 2 hours following tabun challenge with the exception of alteration of posture, passive behavior of rats during retention, a decrease in muscular tone and rearing activity, the absence of touch and click response and pupil response to light, a decrease in forelimb and hindlimb grip strength, body temperature and vertical as well as horizontal motor activity (Table 2a–c). On the other hand, the ability of trimedoxime to eliminate tabun-induced signs of acute neurotoxicity was slightly higher compared to the novel oximes studied. It was not be able to eliminate or reduce alteration of posture, passive behavior of rats during retention, a decrease in muscular tone and rearing activity, the absence of pupil response to light, and a decrease in forelimb and hindlimb grip strength, body temperature and vertical as well as horizontal motor activity (Table 2a–c).

Discussion

The severe poisoning with nerve agents including tabun brings centrally mediated seizures that can rapidly progress to status epilepticus and cause irreversible seizure-related brain damage if left untreated (17). It is known that atropine alone fails to prevent nerve agent-induced acute neurotoxic effects (18). As the potential benefit of atropine alone to counteract the acute neurotoxicity of nerve agents is negligible, atropine should be combined with a reactivator (oxime) of nerve agent-inhibited AChE for the antidotal treatment of nerve agent poisonings to improve its neuroprotective efficacy.

Generally, the ability of currently available oximes to eliminate tabun-induced acute neurotoxic effects is relative-

ly low (19). Among them, trimedoxime seems to be the most effective to counteract tabun-induced acute neurotoxicity in rats although it is not able to completely eliminate or at least reduce all tabun-induced signs of neurotoxicity in the case of lethal tabun poisoning, either (18). Therefore, new oximes with higher potency to counteract tabun-induced acute neurotoxicity are still searched to increase the efficacy of antidotal treatment of acute tabun poisonings.

The ability of oximes to reactivate nerve agent-inhibited AChE in the brain is one of the most important factors influencing their potency to counteract acute neurotoxicity of nerve agents mostly caused by the irreversible inhibition of AChE in the brain (20). Bispyridinium oximes are generally more effective to reactivate nerve agent-inhibited AChE than monopyridinium oximes, however, their ability to penetrate across blood-brain barrier (BBB) is lower, in maximum of 6% (21, 22). Therefore, a design of newly developed oximes should respect not only the goal to increase their reactivating efficacy via higher affinity to AChE but also the goal to increase their BBB penetration as much as possible. It was demonstrated that proper length between covalently connected proper peripheral site ligand and a non-ionic part containing nucleophilic aldoxime in the structure of AChE resulted in higher reactivation potency (23). From this point of view, AChE reactivators consisting of AChE proper peripheral site, ensuring the affinity to AChE and highly nucleophilic moiety (e.g. aldoxime, ketoaldoxime) capable of BBB permeation with balanced chemical-physical properties represent a very promising area in drug development against nerve agent poisoning (24). As the limitation of BBB penetration of bispyridinium oximes is caused above all by the presence of two quaternary nitrogens in their structure, uncharged reactivators represent a new hope in a way of increased bioavailability in the central compartment and better therapeutic management of nerve agent poisoning (25).

The experience from long-term oxime development work and the data from structure-activity relationship studies realized at our Department of Toxicology and Military Pharmacy were used for the chemical structure prediction and synthesis of both novel oximes studied (26–29). Both newly developed bispyridinium oximes (K727, K733) were designed as reactivators with aromatic connecting linker that was formerly found to be beneficial for the reactivation of cyclosarin, tabun and organophosphorus pesticides *in vitro* and *in vivo* (30–32). In addition, the oxime K733 was designed with ethoxycarbonyl moiety as a representative of carboxylic derivatives that were previously found to decrease toxicity of the reactivator (33). Therefore, the oxime K733 showed markedly lower acute toxicity than the oxime K727 (15).

To compare the neuroprotective efficacy of newly developed oximes (K727, K733) with trimedoxime, the ability of both novel oximes to eliminate or reduce tabun-induced neurotoxic signs and symptoms was slightly lower. As the neuroprotective efficacy of both novel oximes is not possible to explain exclusively by their central reactivating efficacy that was low or negligible (15), their neuroprotective efficacy could be also caused by their direct pharmacological effects such as inhibition of acetylcholine release, interaction with presynaptic cholinergic nerve terminals and/or with postsynaptic receptors (34–36).

Conclusion

The results presenting in this paper bring a novel information about the ability of both newly developed oximes to counteract some acute neurotoxic signs and symptoms induced by the lethal dose of tabun in spite of negligible central reactivating activity. However, the benefit of both novel oximes for neuroprotective efficacy of antidotal treatment of acute tabun poisonings is not so high to make the decision about the replacement of commonly used oximes (especially trimedoxime) in the antidotal treatment of acute tabun poisonings.

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CASE REPORT

PNEUMOPERITONEUM IN IN-VITRO CONCEIVED QUADRUPLET NEONATE: RARE MANIFESTATION OF HIRSCHSPRUNG'S DISEASE – REPORT OF A CASE

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Summary: Introduction: Hirschsprung's disease is a congenital colonic aganglionosis, usually presented as inability or difficulty in passing of meconium, chronic and persistent obstipation, maleficent feeding, vomiting, distension and lethargy. Case presentation: We presented a case of an in-vitro conceived quadruplet premature neonate who presented with pneumoperitoneum caused by transverse colon spontaneous perforation and microcolon appearance of distal bowel, treated by resection and temporary colostomy turns to be a rare manifestation of Hirschsprung's disease. Conclusion: Assisted reproductive technologies increases chances for multiple pregnancies and may increase chance for major congenital anomalies. Rare manifestation of Hirschsprung's disease is spontaneous pneumoperitoneum which remains a surgical emergency. Delay in recognizing and treatment can significantly worsen prognosis. In neonate with intestinal perforation one should consider Hirschsprung's disease.

Keywords*: Hirschsprung disease; Pneumoperitoneum; quadruplets; In vitro fertilization*

Introduction

Hirschsprung's disease (HD), a congenital colonic aganglionosis, usually presents as inability or difficulty in passing meconium, chronic and persistent obstipation, poor feeding, vomiting, distension and lethargy. Rare manifestation of the disease is spontaneous perforation which usually takes place proximally to aganglionic colon segment (1, 2). Since introduction in 1978 in-vitro fertilization (IVF) has greatly increased incidence of multiple pregnancies, which raises chances of premature delivery and accompanying complications (3, 4). We present a case of in-vitro conceived premature neonate from quadruplet pregnancy presented with abdominal distension and pneumoperitoneum as a complication of HD.

Case report

Premature male born by caesarean section at 32 weeks + 2 days of gestation presented with spontaneous pneumoperitoneum. Birth weight was 1630 grams. Two fraternal sisters and identical brother were born from quadruplet pregnancy. Mother was healthy 35 years-old, underwent IVF procedure and gave uncomplicated vaginal birth 3 years earlier. Current pregnancy was result of triple transfer of the previously cryopreserved embryos. There were no congenital disorders in family anamnesis. The patient showed signs of respiratory distress syndrome after birth and was immediately intubated and given endotracheal surfactant. Total parenteral nutrition was introduced. During the first day of life patient was extubated because of satisfactory spontaneous breathing and placed in incubator with oxygen supplementation. Meconium passage was delayed and he had first stool 48 h after birth. Third day of life marked abdominal distension was noticed. Abdominal X-ray revealed massive pneumoperitoneum (Fig. 1).

The patient underwent emergency surgery. Transverse laparotomy was performed and perforation measuring 1 × 2 cm was found on antimesenteric border of distal transverse colon with minor local spillage. Distal to perforation colon was narrow – microcolon appearance (Fig. 2). Caecum and ascending colon appeared normal. Resection of affected microcolon was performed, followed by colostomy proximal to the site of perforation. Early postoperative course was uneventful.

Pathohistological analysis confirmed the diagnosis – short segment HD with aganglionic segment beginning on descending colon (Fig. 3).

The child had regular stools via colostomy and was thriving well. The patient made rapid recovery and was discharged from the hospital on seventh day after the surgery. Six months later colostomy was closed and standard transabdominal Soave procedure was performed.

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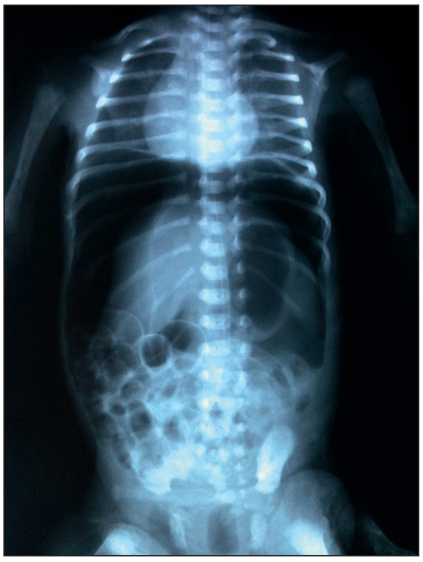

Fig. 1: Abdominal X-ray: massive pneumoperitoneum.

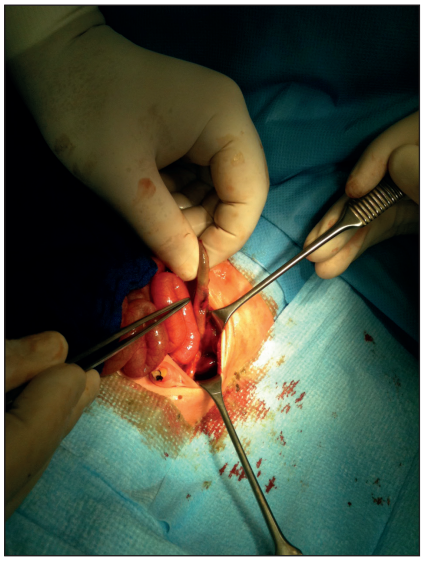

Fig. 2: Perforation on antimesenteric border of distal transverse colon with minor local spillage. Colon distal to perforation site was narrow – microcolon appearance.

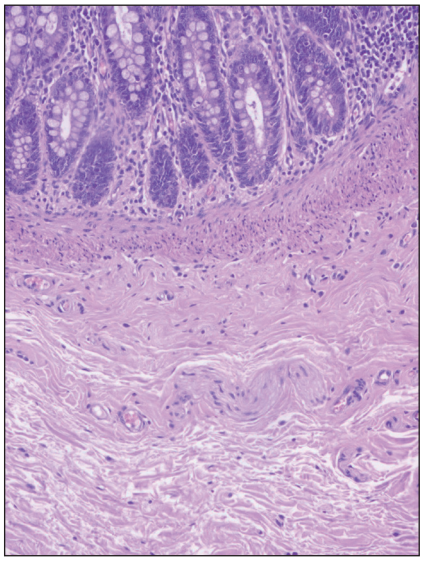

Fig. 3: Pathohistological findings – Submucosal (Meissner) plexus from the resected specimen of the colon: nerve fibers of the plexus are present, but ganglion cells are absent (HE, ×200).

Discussion

Assisted reproductive technologies (ART) is general term reffering to methods used to achive pregnancy by artificial means. Several studies showed increased risk of low-birth weight, cerebral palsy and major congenital malformations in newborns concieved in assisted reproductive technologies (4–6). The most important risk factor for children born after ART is high iatrogenic multiple pregnancy rate (4). This is directly related to the practice of transferring multiple embryos at embryo transfer procedure. Even after restriction of number of embryos transferred to 3, percentage of multiple births remains relatively high. Quadruplets delivery rate is between 0.04–0.4% (3, 7). Multiple pregnancy is linked to increased risk of miscarriage, obstetrical complications, prematurity and neonatal morbidity. Neonatal outcome after IVF is worse than in general population, mainly due to the large proportion of multifetal births after IVF. Most mortality rates were twice as high as figures in the general population (8). Hansen et al. have found that infants conceived with use of intracytoplasmic sperm injection or in vitro fertilization have twice as high a risk of a major birth defect as naturally conceived infants (9). Among mentioned congenital malformation are gastrointestinal anomalies, such as cleft lip with or without cleft palate, oesophageal atresia and anorectal atresia. There are no data for incidence of HD among children concieved with ART. In general population HD occurs in approximately 1 per 5000 live births, and is defined as congenital aganglionosis of the distal bowel due to arrest of migration of neural crest cells during embryonic development. HD should be considered in any newborn with delayed passage of meconium (more than 48 hours). Pneumoperitoneum is rarely the first symptom. It occurs in only 3.4–6% of cases (1, 2, 10, 11). Traditionally, diagnosis and management of intestinal perforation revolves around necrotizing enterocolitis (NEC) which has been used synonymously to the neonatal pneumoperitoneum in the past (12). Classically NEC affects preterm low-birth weight infants. On the other hand, the majority of children with HD are born at term with a normal birth weight. Reports from developing countries warn that NEC participates in almost 50% cases of neonatal pneumoperitoneum, but other causes of perforation should be kept on mind; meconium ileus in cystic fibrosis, intestinal atresia, appendicular perforation, ventilation related barotraumas and particularly HD (12). The mechanism of perforation appears to be directly related to increased intraluminal pressure from distal obstruction. Perforation as first symptom of HD is exclusively seen in neonatal period and is more likely associated with long-segment or total colonic aganglionosis (1, 2). HD in children with neonatal perforation should be on surgeon's mind to avoid delay in the treatment and to plan the resection level. The goal is to place stoma proximal to aganglionic segment, because colostomy at the site of perforation is not satisfactory enough and is related with more complications (2).

Surgical management for HD aims at removing the aganglionic bowel and reconstructing the intestinal tract by bringing the normally innervated bowel down to the anus while preserving normal sphincter function. The surgical approach changed gradually from three-stage procedures to one-stage pull-through without colostomy. This has turned out to be as favorable as the multistage procedures with ben-

efits for the patients and reduction in health care costs due to shorter and fewer hospital stays (12). The development of new techniques has changed the surgical management of HD considerably during the last decade and they are dominating the modern treatment of HD today.The main operative approaches used today are the total transanal endorectal pull-through and the laparoscopic assisted pull-through.

Conclusion

Assisted reproductive technologies increases chances for multiple pregnancy and may increase chance for major congenital anomalies. Multiple pregnancies are burdened with premature deliveries, lower birth weight, shorter gestation period and associated perinatal morbidity and mortality. HD is congenital aganglionosis and might be related to our patient's perinatal history. Rare manifestation of HD is spontaneous pneumoperitoneum which remains a surgical emergency. Delay in recognizing and treatment can significantly worsen prognosis. In neonate with intestinal perforation, a suspicion of HD should be raised, especially if other causes such as NEC, intestinal atresia or meconium ileus are excluded. Although an uncommon cause, HD has to be kept on mind when facing neonate with free air in abdominal cavity.

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CONCHA BULLOSA MUCOPYOCELE: A CASE REPORT

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Summary: Concha bullosa (CB) is among the most common anatomic variations of sinonasal anatomy. Although usually asymptomatic, CB can occasionally cause nasal obstruction or headache. Obstructions within the mucociliary transport system can develop into a mucocele or mucopyocele. A 48-year-old female, with a history of progressive headache and nasal obstruction, was referred to our department. Paranasal sinus tomography revealed a nasal mass in the left nasal cavity resembling a mucopyocele in the middle turbinate. Under general anesthesia, the purulent material was aspirated, and the lateral part of the left turbinate was resected. Mucopyoceles are common within the paranasal sinuses, but uncommon with CB; thus, they should be considered in patients with a large hyperemic nasal mass.

Keywords: *Concha Bullosa; Mucopyocele; Mucocele; Nasal obstruction*

Introduction

Concha bullosa (CB) is among the most common anatomic variations of paranasal sinuses. CB is defined as pneumatization of the middle, inferior, or superior turbinate (1). Although typically asymptomatic, CB can sometimes lead to nasal obstructions or headache due to contact with the lateral nasal wall. The CB mucociliary transport system drains into the frontal recess or the middle meatus over the sinus lateralis (2). Obstruction of a CB can result in a mucocele. Secondary infections that develop over a mucocele are referred to as mucopyoceles (3). We report herein a case of a CB-related mucopyocele with a large hyperemic nasal mass that caused nasal obstruction and headache, and was treated endoscopically.

Case report

A 48-year-old female with a history of progressive headache and nasal obstruction was referred to our ear nose and throat department. The patient that has not got any complaint previously reported increasingly severe and frequent nasal obstruction and headache attacks during the previous week. She did not mention postnasal drip and olfactory impairment. On nasal endoscopic examination, a hyperemic mass that invaded the left nasal cavity was observed. Paranasal sinus tomography (CT) revealed a lesion in the left nasal cavity resembling a mucopyocele in the middle turbinate (Figure 1). CT sections of the paranasal sinuses were normal (Figure 2). Based on the clinical and radiologic findings, we decided to perform endoscopic CB resection. The middle turbinate included a large amount of purulent material (Figure 3), which was subsequently aspirated; the lateral part of the left turbinate was resected. Antibiotic therapy was provided using intravenous ampicillin/sulbactam at a dose of 4 g per day. Cultures were taken during surgery. Meticilin-sensitive *Staphylococcus Aureus* was detected on the culture; 10-day antibiotic therapy was started promptly. During the postoperative period, the patient's complaints subsided and she experienced marked improvement. The excision material was reported as mucocele at pathological examination. During the following 9 months, the patient developed no additional problems, and there was no recurrent pathology on her nasal endoscopic examination (Figure 4). Informed consent was obtained from the patient before the report was made.

Discussion

CB, which is principally characterized by pneumatization of the middle turbinate, is among the most common anatomic variations of sinonasal anatomy. The incidence of CB ranges from 14% to 53% (4), and it is encountered both unilaterally and bilaterally. Pneumatization of the middle turbinate CB usually arises from anterior or posterior ethmoid cells. CB typically causes no symptoms and is usually diagnosed incidentally on CT sections. Bolger et al. (5) classifies CB pneumatization according to its location; when it involves the vertical lamella it is referred to as lamellar, whereas involvement of the inferior bulbous segment is referred to as bulbous. When pneumatization involves the entire concha it is referred to as extensive; if it obstructs the sinus ostia nasal obstruction or headache can occur (6). In our patient, nasal obstruction was the main symptom.

A mucocele is a true cyst lined with pseudostratified ciliated columnar epithelium (6); if it becomes infected, it is referred to as a mucopyocele. Mucoceles and mucopy-

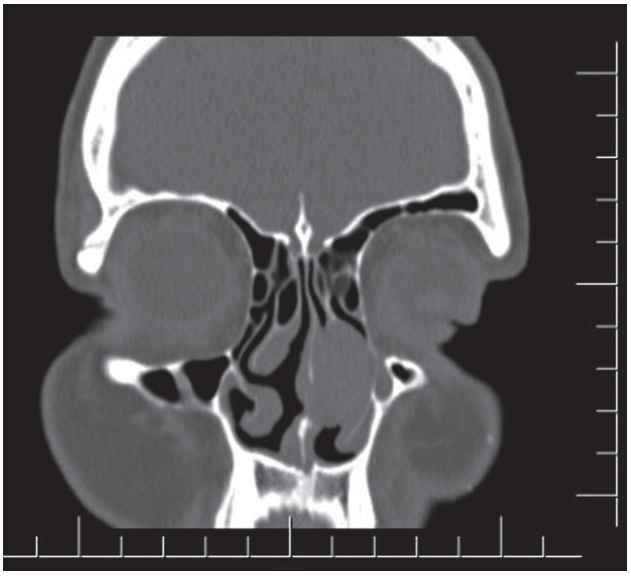

Fig. 1: The mucopyocele filled the left nasal cavity.

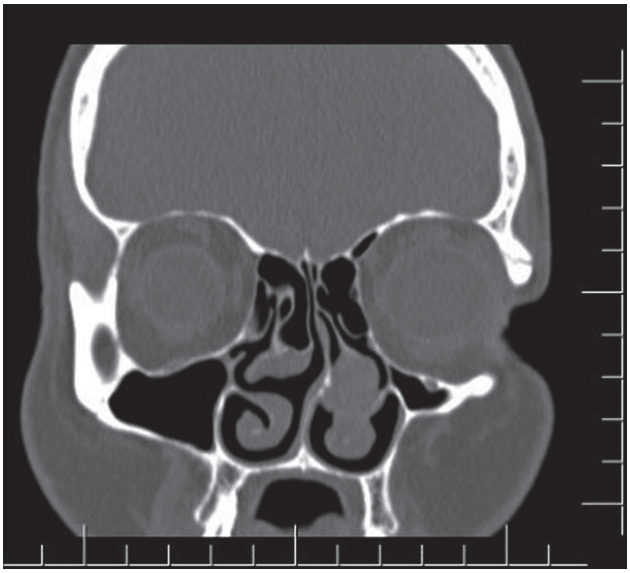

Fig. 2: There was no sign of sinusitis in the paranasal sinuses

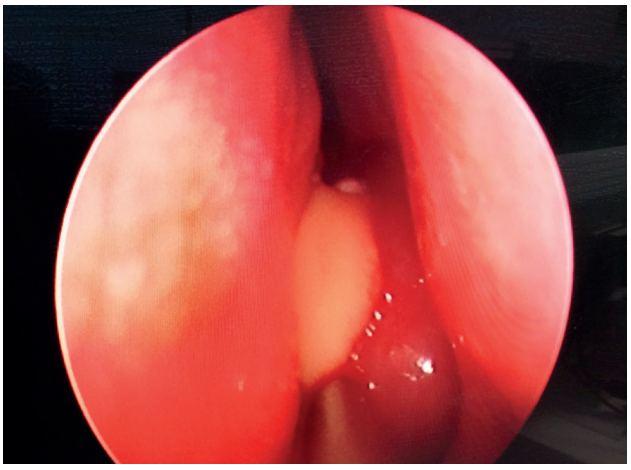

Fig. 3: Purulent secretion filled the middle turbinate.

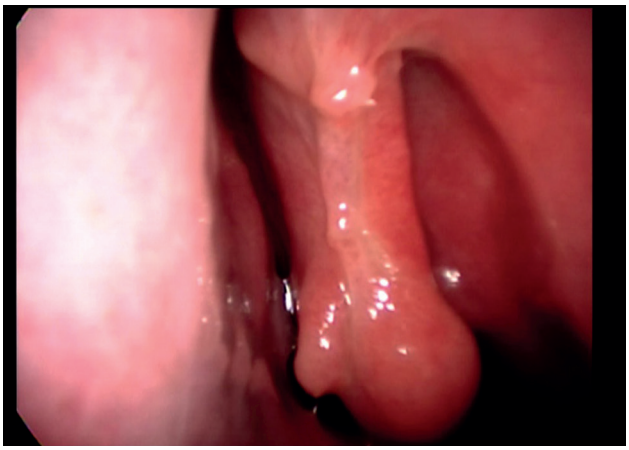

Fig. 4: There was no sign of infection in nasal cavity at endoscopic image in post operative 9 months.

oceles are uncommon in the CB, with only 10 previous reports of CB mucoceles or mucopyoceles (3, 6–8). Mucoceles of the paranasal sinuses or CB typically develop due to an obstruction in the mucociliary transport system. Nasal trauma or chronic rhinosinusitis may obstruct the sinus ostia. As a result of obstructed mucociliary flow, mucus secretion leads to an expansion of the middle turbinate, which can lead to bone erosion, diplopia, nasal obstruction, or headache. On endoscopic examination a conchal mass is usually seen in the nasal cavity. Axial and coronal CT images provide useful information concerning nasal and paranasal structures (3), which can appear as a mass of soft tissue surrounded by a thin plate of bone that may expand into surrounding structures (9). Magnetic resonance imaging can be used if intracranial or intraorbital complications are suspected.

The relationship between CB and chronic rhinosinusitis remains unclear. In several studies, there were no significant differences in chronic rhinosinusitis incidence between patients with and without CB (3, 10, 11). However, other studies report a significantly higher risk in CB patients (12–14). There have been 10 published cases of CB mucopyoceles, 7 of which describe patients with sinusitis (3). In our case, CT sections of the paranasal sinuses were normal.

In a study by Brook and Frazier (15), aerobic bacteria were identified in only 7 (19%) of 36 specimens; anaerobic bacteria alone were identified in 15 specimens (42%), and mixed aerobic and anaerobic bacteria were observed

in 14 specimens (39%). There was no correlation between clinical and medical history, including recent antimicrobial therapy and microbiologic results. We identified the aerobic bacteria *Staphylococcus Aureus*, and treated it using ampicilin/sulbactam.

The treatment of choice for mucopyoceles is endoscopic CB resection and drainage. We excised the lateral border of the CB and aspirated the mucopurulent secretion. Lateral marsupialization, medial marsupialization, crushing, and transverse excision represent other treatment options (16).

Conclusions

Mucopyoceles are common within the paranasal sinuses but are uncommon with CB, in which surrounding structures may be compressed potentially leading to nasal obstruction or headache. We suggest that CB mucopyoceles should be considered in patients with a large hyperemic nasal mass.

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This case report was presented as a poster at the 11th Turkish Rhinology Convention, 2015.

Conflict of interest

The authors have no conflicts of interest to declare.

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