Does neuraxial anesthesia damage DNA as general anesthesia?

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Background

DNA damage during general anesthesia has been well documented and it yields a significant burden to the patients, as it is iatrogenic damage. Data on neuraxial anesthesia are missing so far. Our aim was to assess the amount of DNA damage in patients under general anesthesia (GA) and neuraxial anesthesia (NA).

Methods

43 patients undergoing elective traumatology or orthopedic surgery on limbs and/or big joints were allocated to the GA or NA group. Blood samples were obtained from patients after informed consent with the study before and right after the surgery.

The blood was then subjected to DNA damage analysis using the DNA Comet method. Samples were centrifuged with LSM (Biotech, Austria) to obtain lymphocytes. PBS buffer was used to re-suspend the lymphocytes and adjust the concentration to 1 million cells /ml. 35 µl of the suspension was spread onto pre-coated microscopy slides on 85 µl of solidified high melting point agarose and mixed with 85 µl of low melting point agarose. Cells were lysed for 1 hour at 4°C in high salt and detergent solution to obtain the nuclear DNA on the agarose gel. The nuclear DNA was incubated for 45 minutes with the specific enzymes ENDO III for detection of oxidized pyrimidines and FPG to detect damaged purines. Slides were then exposed to alkali for 40 minutes for DNA unwinding and cleavage of alkali-labile sites. Then the electrophoresis was applied for 30 minutes at 4°C and DNA migrated to the anode and created comets. After neutralization and staining with ethidium bromide, the gels were analyzed by a specialized semiautomatic software Lucia (Laboratory Imaging, Czech Republic) by fluorescence microscopy.

The software measured the ratio of DNA intensity in the tail relative to the head of the comet and provided the ratio of single-stranded DNA damage (SSD), pyrimidine damage (ENDO) and purine damage (FPG).

For comparison of results paired the Wilcoxon test was used. The statistical significance was at $p = 0,05$. Data are presented as a median and interquartile range.

Results

Complete data were obtained from 24 patients in GA group and 19 patients in NA group. In GA group there was a significantly higher amount of SSB, ENDO, and FPG after the surgery as shown in table 1. In NA group the elevation of these parameters was not significant as described in table 2.

Conclusion
Our results declare that GA significantly damages nuclear DNA, unlike NA. Due to the lower load of genotoxic agents whenever possible patients should be undergoing traumatology and orthopedic surgery in NA.

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	Baseline	GA	p value
SSD	7, 58 (5,13 - 10,1)	10, 53 (7,41 - 11,67)	< 0,0001
ENDO	8,66 (6,85 - 10,3)	12,57 (9,33 - 13,72)	< 0,0001
FPG	8,09 (5,9 - 12,6)	11,75 (8,38 - 15,32)	< 0,0001

Table 1: Results of the general anesthesia group.

	Baseline	NA	p value
SSD	2,65 (0,93 - 6,55)	2,17 (0,41 - 6,79)	0,63
ENDO	6 (3,69 - 16,6)	5,73 (3,74 - 16,91)	0,46
FPG	6,72 (4,38 - 19,7)	8,33 (4,9 - 19,05)	0,81

Table 2: Results of the neuraxial anesthesia group.

DETECTION OF PANTON-VALENTINE LEUCOCIDIN IN METHICILN-SUSCEPTIBLE AND METHICILIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS COLLECTED FROM PATIENTS IN FACULTY HOSPITAL HRADEC KRALOVE

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Introduction

Staphylococcus aureus is common pathogen causing suppurative skin and soft tissue infections or purulent complications of wounds and burns, osteomyelitis, arthritis and acute endocarditis. It harmlessly colonises skin and mucosa of healthy population, persistent nasal carriage is present in 30%, transient colonization is described in up to 70% of people [1]. Colonizing strains may become in eligible conditions source of the infection. Serious therapeutic problem in hospitals worldwide are methicillin-resistant *S. aureus* strains (MRSA). Panton-Valentine leucocidin (PVL) is a staphylococcal cytotoxin that causes lysis of neutrophils and macrophages by forming pores in their membrane and prevents effective cell immune response. It consists of two components named S (slow) and F (fast), according to their electrophoretic mobility. Separated components themselves have no biological activity, but work synergistically when combined. PVL-producing strains mostly cause recurrent abscesses, boils and necrotic skin lesions, invasive infections are rare. Serious necrotizing pneumonia is not common in our conditions, but often end up lethally. In literature, less than 2% of *S. aureus* isolates are PVL producers, but the production of PVL toxin is characteristic for MRSA of community origin (CA-MRSA) where the prevalence could be higher [2].

Aims

The aim of this study was to detect PVL in selected methicilin-susceptible *S. aureus* (MSSA) strains and MRSA strains by multiplex polymerase chain reaction (mPCR). Another goal was to identify CA-MRSA strains based on the result of antimicrobial susceptibility testing.

Methods

Twelve MSSA strains from patients presented in 2010 – 2016 were chosen, twelve MRSA strains were recovered during January - June 2017. Most of strains originated from patients with skin infections, there were no strains from patients with clinical symptoms of necrotizing pneumonia tested. Isolates were cultivated 18 hours on Blood agar plates (Oxoid). Disc diffusion test was performed in all strains according to EUCAST v.7. clinical breakpoints. PVL production was determined by presence of the gene for fast component (luk-F gene, 83 bp) as recommended by The National Food Institute (EURL-AR). Simultaneously, presence of methicilin resistance genes (*mecA* 138 bp and *mecC* 162 bp) was tested. DNA was isolated by QIAamp DNA Mini Kit (Qiagen). Amplification proceeded in 30 cycles: 30 seconds denaturation at 94 °C, 1 minute annealing at 59 °C and 1 minute extension at 72 °C. After amplification, the resulting product was analyzed by electrophoresis in a 2% agarose gel with addition of ethidium bromide. The electrophoresis was carried out for 70 minutes at 110 V. The visualization was performed by UV illumination of the gel.

Results

PVL production was proved in two of twelve tested MSSA strains. These strains were susceptible to all tested antibiotics. Both patients suffered from recurrent abscess recently, so the clinical manifestation is corresponding with laboratory results.

Of the twelve tested MRSA strains, the gene encoding PVL was detected in three isolates. Antibiotic resistance profile indicates, these are community strains. Two of the PVL-producing MRSA strains originated from children with skin abscess. Third strain was obtained from young foreiner patient with soft tissue skin infection. All tested isolates carried *mecA* gene, which prevails in most human MRSA strains.

Discussion

PVL producing strains are usually associated with community-acquired skin infections, generally affect healthy children and young adults. Mostly have moderate course, but recurrence is typical and infection often affects more household members. Surgical treatment followed by antibiotic therapy is recommended. Besides culture and susceptibility testing of the strain, PVL production should be considered in patients with typical history of complaints. Toxin detection is available in National Reference Laboratory for Staphylococci and it is completed within few days. When PVL production in such a case is proven, it is recommended to treat staphylococcal carriage in all household members to prevent recurrence. In Europe, PVL positive strains are mostly MSSA, prevalence of MRSA strains is low, 1-3% [3]. On the other hand in some states in the USA is prevalence up to 50% in community patients suffering from skin infections [4].

All tested MRSA strains were *mecA* positive, as expected. *MecC* gene is typical for animal strains and prevalence of MRSA harbouring *mecC* gene is less than 0,5 % [5].

Conclusion / Summary

In the present study were tested twelve MSSA strains from patients mostly suffering from recurrent skin infections, PVL-producing strains were confirmed in two cases. Majority of tested MRSA were hospital-associated MRSA strains. Community origin is assumed in three isolates. Most of cases of CA-MRSA infections worldwide are caused by a few specific clones, which are defined by molecular criteria that distinguish sequence type (ST). The main European clone is ST80, in the USA is ST8 (USA300) the most prevalent, while ST30 is prevalent in Asia and Oceania [6]. Further molecular characteristic of detected PVL-producing CA-MRSA stains would be interestig.

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The role of the methylation status in therapy of high grade non-muscle invasive bladder tumour

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Introduction

Bladder Cancer (BC) is the second most common malignancy of the urinary tract. BC has two categories. Approximately 75% of patients with BC present with disease confined to the mucosa or submucosa (non muscle invasive bladder tumor – NMIBC). Carcinoma in situ (CIS) is a very specific subgroup of NMIBC, because it is not a papillary lesion but a flat tumor, which is why CIS can be missed in cystoscopy[3]. CIS is always a high grade tumor. Without any treatment, approximately 54% of patients with CIS progressed to muscle-invasive or metastatic disease. The second category covers patients with muscle-invasive BC (MIBC). These patients have a higher prevalence of progression rates and higher cancer-specific mortality. Patients with NMIBC are indicated to transurethral tumor resection (TUR) alone or with adjuvant treatment (intravesical chemotherapy or intravesical Bacillus Calmette-Guérin - *BCG* immunotherapy). BCG is an attenuated mycobacterium developed as a vaccine for tuberculosis that has demonstrated antitumor activity in BC intravesical instillation, and significantly reduces the progression risk of high grade papillary lesion and CIS. The therapy of MIBC is radical cystectomy – hard mutilating surgery with urinary diversion. This procedure has significant impact on the quality of the patient's life. Patients with superficial (NMIBC) high grade tumor and CIS are the focus of our interest. It is a borderline subgroup, where radical and non-radical treatments are both possible. Non-radical therapy means complete tumor resection with adjuvant intravesical immunotherapy (BCG vaccine). Radical therapy means radical cystectomy. At present we have no markers which would be helpful in deciding optimal therapy. Weighing the risk of failure of non-radical treatment against overtreatment by radical therapy constantly presents a dilemma. The etiology of BC is multifactorial, driven by the multistep accumulation of environmental, genetic and epigenetic factors. Methylation status differences were evaluated in our study subgroups. A new model for the mechanism of carcinogenesis has been proposed in which hypermethylation of unmethylated cytosine-phosphate-guanine (CpG) islands in the promoter regions of tumour suppressor genes (TSG) in normal cells silence these genes and this leads to the cells becoming cancerous.

Aims

Genetic and epigenetic alterations play an important role in urothelial cancer pathogenesis. Deeper understanding of these processes could help us achieve better diagnosis and management of this life-threatening disease. The aim of this research was to evaluate the methylation status of selected tumor suppressor genes for predicting BCG response in patients with high grade non-muscle-invasive bladder tumour.

Methods

We evaluated retrospectively 82 patients with high grade non-muscle-invasive bladder tumor (stage Ta, T1, CIS) who had undergone BCG instillation therapy. We compared epigenetic methylation status in BCG-responsive and BCG-failure groups. We used the MS-MLPA (Methylation-Specific

Multiplex Ligation-Dependent Probe Amplification probe sets ME001 and ME004) (1, 2). The control group was 13 specimens of normal urotel (bladder tissue).

Discussion

Meta-analyses have confirmed that BCG instillation after TUR is superior to TUR alone or TUR and chemotherapy. BCG therapy reduces relative progression risk by about 27%. Without any treatment, approximately 54% of patients with CIS progressed to muscle- invasive disease[4]. Radical cystectomy is the sole radical procedure. Although BCG has good clinical outcomes in general, we have many patients with BCG failure. This problematic group is the focus of our interest. Clinical markers for the prediction of BCG response or failure are missing. BCG failure patients spend a lot of time in conservative procedures and radical therapy is delayed. This could be fatal in terms of the patient's oncology prognosis. The studies have shown that methylation status plays an important role in carcinogenesis in various organs, including NMIBC (3). Methylation status is a potent indicator for distinguishing patients responding to BCG from those who are failing to do so and who need the radical therapy approach. Hypermethylation of unmethylated CpG islands in the promoter regions of specific TSGs can be associated with both good and poor prognoses. Hypomethylation (unmethylation, silent methylation) of specific TSGs also showed association with clinical outcome. CDKN2B is cyclin-dependent kinase inhibitor 2B. This gene lies adjacent to the tumour suppressor gene CDKN2A in a region that is frequently mutated and deleted in a wide variety of tumours. This gene encodes a cyclin-dependent kinase inhibitor, which forms a complex with CDK4 or CDK6, and prevents the activation of the CDK kinases, and thus the encoded protein functions as a cell growth regulator that controls cell cycle G1 progression. The expression of this gene was found to be dramatically induced by TGF beta, which suggested its role in TGF beta-induced growth inhibition.MUS81 (Structure-Specific Endonuclease Subunit) is a protein coding gene. The MUS81 protein belongs to a conserved family of DNA structure-specific nucleases that play important roles in DNA replication and repair. Unmethylation of CpG islands in CDKN2B especially and MUS81a TSGs is connected with BCG failure in our study. The mechanism by which unmethylation of CDKN2b and MUS81a favor the BCG failure is unknown.

Results

Newly identified methylations in high grade NMIBC were found in MUS81a**,** NTRK1 and PCCA**.** The methylation status of CDKN2B ($p=0.00312^{**}$) and MUS81a ($p=0.0191^{*}$) is associated with clinical outcomes of BCG instillation therapy response. CDKN2B and MUS81a unmethylation was found in BCG failure patients.

Conclusion

The results show the methylation status of selected tumor suppressor genes (TSGs) has the potential for predicting BCG response in patients with NMIBC high grade tumours. Tumour suppressor genes such as CDKN2b, MUS81a are very promising for future research.

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IN SEARCH FOR POTENTIAL BIOMARKERS: DEREGULATION OF SELECTED MICRORNAS IN SQUAMOUS CELL SINONASAL CARCINOMA

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Introduction

Malignant tumors arising from nasal cavity and paranasal sinuses make up 3 % of all cancers of head and neck area with squamous cell carcinoma (SCC) being the most common subtype. Sinonasal carcinomas (SNC) are characterized by unfavorable outcome due to difficult diagnosis, treatment and prognosis of the disease corresponding with the anatomic complexity of the region. Risk factors for developing SNC include cigarette smoking professional exposure to various cancerogenous substances (wood-dust, leather dust) and HPV infection [1]. MicroRNAs (miRNAs) are short (18 – 25 nt) non-coding RNA molecules that are part of gene expression and their primary role is negative regulation of translation as part of the RNA-induced silencing complex (RISC) [2]. The aim of this study was to investigate relative expression levels of selected miRNAs in squamous cell sinonsal carcinoma samples and to compare the results with recorded clinicopathological data.

Methods

A total of 63 formalin fixed, paraffin embedded samples of squamous cell sinonasal carcinoma and normal sinonasal tissue were analyzed (46 sinonasal cancer samples and 17 samples of control tissue). Relative expression of miR-21, miR-9, miR-145, miR-99a, miR-137, miR-484 and let-7d were measured by real-time PCR with specific TaqMan® Advanced miRNA Assays (Applied Biosystems) on Rotor-Gene Q and calculated using the $2^{-\Delta\Delta Ct}$ method [3]. One-way analysis of variance and regression analysis were used to analyze the correlation between relative expression levels of miRNA and recorded clinicopathological characteristics such as gender, age at the time of diagnosis, smoking history, occupation, tumor localization, TNM classification, tumor subtype, invasion, recurrence, metastasis or HPV infection status. The Kaplan-Maier method and Logrank test were used to determine overall survival rate and corresponding statistical significance.

Results

Real-time PCR data show statistically significant upregulation of three miRNA miR-21 ($p < 0.001$), let-7d ($p < 0.001$), and miR-9 ($p < 0.001$) in SCC samples in comparison to control tissue. On the other hand miR-145 was significantly downregulated ($p < 0.001$). Kaplan-Maier survival analysis showed, that survival of patients with high upregulation of miR-21 ($p = 0.063$) and high expression of miR-137 (0.0278) was significantly impaired. Patients with higher upregulation of miR-9 (0.026) and high expression of miR-99a (0.068) survived longer. After comparing relative expression levels of the miRNAs with clinicopathological data, we identified correlation between expression of miR-21 and tumor localization (nasal cavity × maxillary sinus, $p = 0.026$). Higher expression of miR-145 was in patients with angioinvasion ($p = 0.037$), lower expression of miR-99a had tumors with

perineural spread (p = 0.0055). Expression of miR-137 and let-7d correlated with local recurrence (p = 0.045, p = 0.025), miR-9 expression with regional recurrence (p = 0.045) and miR-145 and miR-484 expression with HPV infection status (p = 0.019 and p = 0.016).

Discussion

Although miRNA research is currently on the rise and many authors investigated expression of miRNAs in many cancerous tissues including head and neck cancer subtypes, there are not many information about miRNA expression and regulation in sinonasal cancer. We observed statistically significant upregulation of miR-9, let-7d and miR-21, which corresponded with other head and neck cancer studies [4] and downregulation of miR-145 also observed by Karatas et al. [5] in head and neck cancer. Kaplan Maier survival analysis showed significant differences between groups of patients with different miRNA expression levels. Higher expression of miR-21 and miR-137 resulted in impaired survival. On the other hand high upregulation of miR-9 and miR-99a corresponded with longer survival time of the patients. This confirms that selected miRNAs might be used as prognostic biomarkers of the disease [6]. Correlation analysis with clinicopathological data shows that levels of expression of some miRNAs correlate with localization of the tumor, angioinvasion, perineural spread, local recurrence and HPV infection status.

Conclusion

Our data show that our selected miRNAs may represent important regulatory molecules involved in development and progression of the disease and survival time of sinonasal carcinoma patients. On top of that, they could be potentially used as valuable prognostic biomarkers of the disease.

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