Impaired nucleotide excision repair pathway as a possible factor in pathogenesis of head and neck cancer


A R T I C L E   I N F O

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A B S T R A C T

Tobacco smoking is one of the major risk factors in pathogenesis of head and neck squamous cell carci-
nomas (HNSCC). Many of the chemical compounds present in tobacco are well-known carcinogens
which form adducts with DNA. Cells remove these adducts mainly by the nucleotide excision repair
pathway (NER). NER also eliminates a broad spectrum of pyrimidine dimers (CPD) and photo-products
(6-4PP) induced by UV-radiation or DNA cross-links after cisplatin anti-cancer treatment. In this study DNA
damage and repair was examined in peripheral blood lymphocytes obtained from 20 HNSCC patients
and 20 healthy controls as well as HTB-43 larynx and SSC-25 tongue cancer cell lines. DNA repair kinetics
in the examined cells after cisplatin or UV-radiation treatment were investigated using alkaline comet
assay during 240 min of post-treatment incubation. MTT assay was used to analyse cell viability and the
Annexin V-FITC kit specific for kinase-3 was employed to determine apoptosis after treating the cells with
UV-radiation at dose range from 0.5 to 60 J/m². NER capability was assessed in vitro with cell extracts by
the use of a bacterial plasmid irradiated with UV-light as a substrate for the repair. The results show that
lymphocytes from HNSCC patients and HTB-43 or SSC-25 cancer cells were more sensitive to genotoxic
 treatment with UV-radiation and displayed impaired DNA repair. Also evidenced was a higher rate of
apoptosis induction after UV-radiation treatment of lymphocytes from the HNSCC patients and the HTB-
43 cancer cells than after treatment of those from healthy donors. Finally, our results showed that there
was a significant decrease in NER capacity in HTB-43 or SSC-25 cancer cells as well as in peripheral blood
lymphocytes of HNSCC patients compared to controls. In conclusion, we suggest that the impaired NER
pathway might be a critical factor in pathogenesis of head and neck cancer.

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1. Introduction

Head and neck squamous cell carcinomas (HNSCC) comprise about 6% of all malignant neoplasms. Head and neck carcinogen-
esis is associated with abnormalities in DNA repair, apoptosis and carcinogen metabolism. One of the major risk factors of HNSCC is
tobacco smoking. There are approximately 4 thousand chemical compounds identified in tobacco smoke. Many of them, like poly-
cyclic aromatic hydrocarbons (PAHs) and aromatic amines (AA) are well-known carcinogens forming adducts with DNA, which are
then eliminated by nucleotide excision repair (NER) [1]. Cancer response to genotoxic treatment may depend on its repair capacity,
especially in respect of DNA damage processing by NER might be a critical factor in pathogenesis of head and neck cancer.
The most important task of molecular oncology is identifying the mechanisms responsible for cancer development. Genome
integrity is maintained by an intricate network of DNA repair proteins [1,2]. Organisms have developed several DNA-repair path-
ways as well as DNA-damage checkpoints. Although each pathway is addressed individually, cross-talk exists between repair path-
ways and there are instances in which a DNA-repair protein is involved in more than one pathway. Abnormalities in this com-
plex machinery are associated with genotoxic susceptibility and
familial predisposition to cancer [3]. Increasing evidence links environmental exposure, subtle modifications in DNA repair efficiency, and cancer risk [4]. Establishing this connection has been challenging due to the complexity of interactions that affect the repair pathways [5,6].

NER removes a wide range of DNA damage through dual incisions on both sides of the lesion followed by DNA re-synthesis, using the complementary strand as a template, and ligation. NER is a versatile DNA repair system that eliminates a broad spectrum of base lesions generated on one strand, including UV-induced cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone photoproducts (6-4PP), as well as other bulky lesions that can be induced by numerous chemical compounds such as benzo(a)pyrene (BP) [7]. Benzo(a)pyrene is a highly carcinogenic polycyclic aromatic hydrocarbon (PAH) present in cigarette smoke, typically 10 ng per cigarette [8]. BP is one of the carcinogens in cigarette smoke that may be involved in the etiology of HNSCC cancer [9]. It is metabolically activated into benzo(a)pyrene-7,8-diol-9,10-epoxide (BPDE) which reacts with DNA predominantly at the N2-position of guanine to produce primarily N2-guanine lesions, e.g. benzo(a)pyrene-7,8-diol-9,10-epoxide-N2-deoxyguanosine (BPDE-dG) adduct [10]. The nucleotide excision repair pathway is also common for removing cisplatin induced adducts from the DNA structure [11-14]. Cisplatin (cis-diaminedichloroplatinum(II); CDDP) is broadly used in the treatment of many kinds of cancer including HNSCC. It was found that the high efficiency of the NER pathway contributed to lower sensitivity of cancer cells to cisplatin [13,14].

The aim of this study was to estimate the NER capability in cells after treatment with cisplatin and UV-light. We examined cancer response to genotoxic treatment in peripheral blood lymphocytes from patients with HNSCC and HTB-43 larynx cancer as well as SCC-25 oral cancer cell lines. Lymphocytes and tissue cells from healthy donors were used as controls. Comet assay was used to examine DNA damage and repair effectiveness in cells after treatment with genotoxic agents. Repair effectiveness was also examined using in vitro functional NER assay. The cell viability and NER capability to apoptosis induction was also estimated after UV-radiation and cisplatin treatment.

2. Materials and methods

2.1. Patients

Twenty patients with head and neck squamous cell carcinomas were enrolled to the study (16 men and 4 women; mean age 56 ± 7). Most of the patients had been diagnosed with larynx cancer, among the patients 16 subjects had metastasis of HNSCC (neck, tongue, salivary glands, tonsils). The cells from 20 subjects without cancers (14 men and 6 women; mean age 54 ± 11) were used as controls.

The lymphocytes were collected from the patients before they had received any chemotherapy or radiotherapy for their primary disease. The diagnosis of healthy and tumor tissues was made after histopathological examination of the patients’ biopsies. Controls were selected based on family history, in order to exclude familial predisposition to cancer development. Prior to examination, the patients and control subjects did not receive other medication like antibiotics or steroids. An age difference of two years between the groups of patients and control subjects was statistically insignificant (P = 0.879). Patients and control subjects enrolled to the examination were non-smokers. Alcohol consumption was also an exclusion criterion. All patients and control subjects were recruited from the medical unit of the Department of Otolaryngology and Oncology, Medical University of Lodz. All subjects included into the study were unrelated Caucasians and lived in Lodz, Poland. The study was approved by the Local Ethic Committee and written consent was obtained from each patient or healthy blood donor before enrolling them into the study. Blinded replicates were used for quality control of examined samples. They were under the supervision of a clinician whose responsibility was also collecting the questionnaire of patients and control subjects. Due to the small amount of biological material obtained from healthy donors as well as from HNSCC patients, we pooled the samples taken from these groups.

2.2. Cells and cultures

Peripheral blood lymphocytes from the blood of healthy donors and HNSCC patients were isolated by centrifugation (15 min, 280 × g) in a density gradient of in Histopaque-1077 (Sigma, Poznan, Poland). The HTB-43 and SCC-25 cell lines were purchased from the American Type Culture Collection (ATCC, USA). HTB-43 larynx cancer cells were grown in an EMEM medium with 10% fetal bovine serum, 100 units/ml penicillin, and 50 μg/ml streptomycin at 37°C. SCC-25 tongue cancer cells were grown in DMEM Ham’s F-12 supplemented with 10% fetal bovine serum, 0.4 μg/ml hydrocortisone, 100 units/ml penicillin, and 50 μg/ml streptomycin in an atmosphere containing 5% CO2. Prior to examination the cells were trypsinized and suspended in a suitable medium to a final concentration of (1–2) × 106 cells/ml.

2.3. Cell viability

The cell viability was assessed using trypan blue staining method. Trypsinized cells were washed and then mixed 1:1 with trypan blue solution. They were then counted using the Bürker counting chamber (Carl Roth GmbH, Karlsruhe, Germany).

2.4. Cell extract preparation

The cell protein extract was prepared as described elsewhere [15]. Briefly, the cells were cultured in 175 cm2 flasks and incubated overnight to reach the mid-exponential growth phase. The cells were then washed three times with ice-cold phosphate-buffered saline and re-suspended at 106 cells/ml in buffer 1 (10 mM Tris–Cl, pH 7.8, and 200 mM KCl). After the addition of an equal volume of buffer II (10 mM Tris–Cl, pH 7.8, 200 mM KCl, 2 mM EDTA, 40% glycerol, 0.2% Nonidet P-40, 2 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 10 mg/ml aprotinin, 5 mg/ml leupeptin, 1 mg/ml pepstatin), the cell suspension was rocked at 4 °C for 1 h and then centrifuged at 16,000 rpm for 10 min. The supernatant was recovered and stored in small aliquots at −80°C.

2.5. UV-radiation and cisplatin treatment

Cells were treated with 254 nm wavelength UV-radiation at a dose rate from 0.5 to 60 J/m2 with a UV lamp with energy emission of 300 J/m2/s (Bionovo, Legnica, Poland). During irradiation open, 12-well plates with cells were placed on ice, 15 cm from the source of light while the plates were rocked to give an even consistency over the surface. Alternatively, cells were incubated with 1 μM cis-diaminedichloroplatinum (II) (CDDP) (Sigma–Aldrich, Poznan, Poland) in growth medium for 1 h at 37°C. After being incubated with cisplatin or treated with UV-radiation, the cells were washed three times with ice-cold phosphate-buffered saline and resuspended in buffer 1.

2.6. MITT assay

The MITT test was used to quantitatively determine the viability of healthy lymphocytes, HNSCC lymphocytes and HTB-43 as well as SCC-25 cancer cell lines that had been treated by UV-radiation at dose range from 0.5 to 60 J/m2. Cells were plated briefly onto 96-well plates and after a 200 μl proper growth medium and after 72 h of incubation 20 μl of 10 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent was added to each well. After 4 h of incubation at 37°C, the supernatant was removed and an aliquot of 200 μl solution containing 10% SDS and 0.04% HCl was added to dissolve the water-insoluble formazan. After 1 hour later, the difference OD650 nm – OD570 nm was measured with an ELISA reader (Bio-Rad, Hercules, CA, USA). Based on the OD value, cell resistance to UV-radiation was presented as a percentage of the viability of untreated cells, estimated as 100% of the controls.

2.7. Comet assay

UV-radiation-induced DNA damage and its repair was measured by the single cell electrophoresis method. The healthy lymphocytes, HNSCC lymphocytes and HTB-43 as well as SCC-25 cancer cell lines were treated with UV-radiation at 30 J/m2. The time course of the repair of DNA damage was measured immediately after the cells’ exposure to UV-radiation and then after 15, 30, 60, 120, 240 min of post-treatment repair incubation in a growth medium in 37°C. The comet assay was performed under alkaline conditions according to the procedure of Singh et al. (1988) with modifications by Klade et al. (1996) [16,17]. The final concentration of the cells was adjusted to (1–3) × 106 cells/ml by adding a growth medium. A suspension of cells in 0.75% LMP agarose dissolved in PBS was spread onto microscope slides (Superior, Germany) pre-coated with 0.3% normal-melting agarose. The cells were then lysed for 1 h at 4°C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10. After lysis, the slides were placed in an electrophoresis unit. The DNA was allowed to unwind for 40 min in the electrophoretic solution consisting of 300 mM NaOH, 1 mM EDTA, pH >13. Electrophoresis was conducted at 4°C (the temperature of the running buffer did not exceed 12°C) for 30 min at the electric field strength of 0.73 V/cm (30 mA). The slides were then neutralized with 0.4 M Tris, pH 7.5, stained with 2 μg/ml DAPI and covered with cover slips. The objects were observed at 200× magnification in an Eclipse fluorescence microscope.
from were lesions (Nikon, Tokyo, Japan) attached to COHU 4910 video camera (Cohu, San Diego, CA, USA) equipped with a UV-1 filter block (an excitation filter of 359 nm and a barrier filter of 461 nm) and connected to a personal computer based image analysis system Lucia-Comet v. 4.51 (Laboratory Imaging, Prague, Czech Republic).

2.8. NER assay

The 2961-bp plasmid pBS (pBluescript II KS-; Stratagene, CA, USA) and the 4202-bp plasmid pCGJ (Fermentas, Ontario, Canada) were prepared by alkaline lysis from the Escherichia coli strain XLI Blue. Both plasmids were recovered from 1% agarose after electrophoresis and purified using a gel-extraction kit (Qiagen). The pCGJ plasmid was treated with UV-radiation at 0.55 mJ/m² (pCGJ-UV) producing about 10 UV lesions per plasmid [18]. 1/5 vol. of 10× Nth protein buffer as well as the appropriate amount of Nth enzyme (Endonuclease III) was added and incubated at 37 °C for 30 min followed by inactivation at 65 °C for 2 min. This step was performed to remove pyrimidine hydrate photoproducts from the substrate. After electrophoresis in 1% agarose, only closed circular forms of both plasmids were recovered from the gel and purified by use of a gel-extraction kit (Qiagen). Plasmids were stored in –20 °C.

The functional NER assay was performed in vitro as described elsewhere with some modifications, Fig. 1 [18]. The reaction mixture (50 µl) containing 250 ng of each pCGJ-UV and untreated pBS closed circular plasmids, 0.37 Mβg (4.5 µCi) [γ⁻²⁵P]-dATP (4500 Ci/mmol), 200 µg cell-extract proteins and 70 mM potassium glutamate, was added to the reaction buffer (45 mM HEPES–KOH [pH 7.8]; 7.4 mM MgCl₂; 0.9 mM EDTA; 2 mM ATP; 20 µM each of dCTP, dGTP, and dTTP; 4 µM dATP; 40 mM phosphocreatine; 2.5 µg phosphocreatine kinase, 3.4% glycerol; and 18 µg bovine serum albumin) and incubated for 3 h at 30 °C. Before electrophoresis on a 1% agarose gel containing 0.5 µg/ml ethidium bromide, the plasmid DNA was purified from reaction mixtures and linearized with EcoRI. After electrophoresis, gels were dried onto Whatman 3MM filter paper under vacuum at 80 °C for about 1.5 h. Dried gel was put into a cassette with intensifying screens, exposed to pre-flashed Xray film and placed at ~80 °C for 6 h. Photographs of the ethidium bromide-stained gels were scanned and bands were excised from the exposed gel, together with the attached filter paper, under UV-light. The radioactivity (in cpm) was then determined in a scintillation counter (TRI-CARB-2900TR Liquid Scintillation Analyzer; IL, USA). The relative density of the bands of both plasmids on the negative of the photograph of the ethidium bromide-stained gel was quantified by densitometry [In Genius Bio Imaging; Synegen, UK]. The specific incorporation of [γ⁻²⁵P]-dAMP was expressed as an incorporation of the radiolabel in the damaged plasmid versus the radiolabel in the undamaged plasmid. The value of the [γ⁻²⁵P]-dAMP incorporation was normalized to the amount of total DNA.

2.9. Apoptosis assay

Annexin V–FITC (Annexin V–FITC Apoptosis Detection Kit I, BD Pharmingen, San Jose, CA, USA) assay, specific for phosphatidylserine was used to quantitatively determine the percentage of cells actively undergoing apoptosis within a population. For staining procedure cells were washed twice with cold PBS and then re-suspended in a binding buffer at a concentration of 1 × 10⁶ cells/ml. 100 µl of solution (1 × 10⁶ cells/ml) was transferred to 5 ml culture tube. 5 µl of Annexin V–FITC and 5 µl of propidium iodide were added. The cells were gently vortexed and incubated for 15 min at RT (25 °C) in the dark. After incubation, 400 µl of 1× binding buffer was added to each tube. The cells were analysed with a flow cytometer (FACSCalibur; BD Biosciences, San Jose, CA, USA), using the CellQuest program. Then the percentage of necrotic cells, apoptotic cells and cells with no apoptosis was measured.

2.10. Statistical analysis

The values in this study were expressed as a mean ± SD or ±SEM as described in the figure legends for three separate experiments from each of the analysed patients and controls. In comet assay the DNA repair capacity was measured for each of the 20 cases and 20 controls, then the mean value of DNA percentage in comet tail as well as appropriate statistical error was given in figures. The percentage of cells viability and MTT assay were scored for the 20 cases and 20 controls, either. However, due to small amount of biological material obtained from HNSCC patients and healthy donors we were pooled 20 samples taken from each of examined groups in BER in vitro assay. In order to calculate statistical error, both the patients and the controls pooled group was triplicated, then the mean of percentage values and statistical error was given in figures, as well. The autoradiogram pictures show representative gel from the analysis of pooled samples in BER in vitro assay. Blinded replicate samples were used for quality control (QC). Variation (CV) within samples was never as large as in group independent samples of patients or controls, assuming high QC. If no significant differences between the variations were found, as assessed by the Snedecor–Fisher test, the differences between means were evaluated by applying Student’s t-test. Otherwise, the Cochran–Cox test was used. The data were analysed using the statistical package STATISTICA (StatSoft, Tulsa, OK).

![Fig. 1. Nucleotide excision repair (NER) reaction scheme with substrate of UV-radiation induced cyclobutane pyrimidine dimmers indicated as black stars in pCGJ plasmid (pCGJ-CPD) after Nth digestions performed to remove pyrimidine hydrate photoproducts from the intermediate of pCGJ-UV plasmid. An untreated pBS plasmid at closed circular structure was used as a reference substrate for repair reaction.](image-url)

3. Results

3.1. Cell viability

There was a dose-dependent decrease in cell viability after UV-radiation exposure from 0.5 to 60 J/m² and for the highest dose of 60 J/m², 30% of the cell population was dead. No differences in cell viabilities were found between analysed lymphocytes of HNSCC patients, healthy donors and HTB-43 cells (P>0.05). In the concentrations of cisplatin used in the experiment, the average viability of the cells was over 80%. The measurement of the viability of both healthy controls and patients' cells in each subsequent preparation always gave a viability above 80% (data not shown).

3.2. Genotoxic treatment

![Fig. 2. Shows the mean percentage of lymphocytes' viability from HNSCC patients and healthy donors as well as from HTB-43 larynx cancer cells and SCC-25 tongue cancer cells. The viability was measured by an MTT test 72 h after exposure to an increasing dose of UV-radiation from 0.5 to 60 J/m². Lymphocytes from healthy donors were more sensitive to genotoxic treatment than those from HNSCC patients. It was also found that UV-radiation induced a statistically significant viability decrease at a dose range from 2.5 to 60 J/m² (P<0.001). The average viability of the control lymphocytes for 15 J/m² was 58.0%, for 30 J/m² – 46.0% and for 60 J/m² it was 41.0% while for HNSCC lymphocytes the average viability was respectively 54.4%, 39.2% and 38.1%. Cancer cell lines were also sensitive to UV-radiation and it was found that increasing doses induced a statistically significant decrease in SCC-25 and HTB-43 viability at the dose range from 1 to 60 J/m² and at a dose range from 2.5 to 30 J/m² (P<0.001). The average viability of the cancer cell line HTB-43 after 5 J/m² was 19.3%, after 15 J/m² it was 17.5%](image-url)
and after 30 J/m² it was 14.5% while for SCC-25 the average viability was respectively 46.2%, 38.2% and 20.6%. The cancer cell lines HTB-43 and SCC-25 displayed a higher sensitivity to UV-radiation than both groups of lymphocytes.

3.3. DNA repair kinetics

Fig. 3 shows DNA damage presented as a percentage of DNA in comet tail (Tail DNA %) lymphocytes from healthy donors and from HNSCC patients, HTB-43 and SCC-25 cell lines after treatment with 15 J/m² of UV-radiation immediately after exposure as well as 30, 60, 120 and 240 min thereafter. In all cases, DNA damage in the control cells was constant, indicating that preparation and subsequent processing of the cells did not introduce significant damage to their DNA.

Effectiveness of DNA repair was estimated in relation to DNA damage level in UV-irradiated cells and was the greatest after 30 min of post-treatment incubation for lymphocytes from healthy donors (17.7%) and HNSCC patients (17.2%). The highest level of DNA damage in SCC-25 and HTB-43 cancer cell lines was 40.9% after 60 min and 38.3% after 120 min, respectively. Lymphocytes from healthy donors treated with UV-radiation were able to completely repair DNA damage after 60 min of post-treatment incubation repair ($P > 0.05$) while lymphocytes from HNSCC patients were able to completely repair DNA damage after 120 min of post-treatment incubation ($P > 0.05$). The level of DNA damage in HNSCC lymphocytes exposed to UV-radiation after 60 min of post-treatment incubation was 19.2%. The HTB-43 and SCC-25 cells were not able to completely recover either after 60 min or after 120 min of post-treatment incubation ($P < 0.001$) but recovered after 240 min ($P > 0.05$). The level of DNA damage in SCC-25 and HTB-43 cells exposed to UV-radiation after 60 min of post-treatment incubation was 33.1, 40.9 and after 120 min was 34.6%, 38.3%, respectively.

3.4. In vitro functional NER assay

To evaluate the efficiency of NER activity, an in vitro DNA-repair assay was used. It was performed on whole-cell extracts of lymphocytes from healthy donors, lymphocytes from HNSCC patients and HTB-43 cancer cells treated with cisplatin or UV-radiation (Fig. 4). It was estimated that lymphocytes from healthy donors displayed the most effective NER repair. The HTB-43 cells displayed a 31.51% ($P < 0.001$) significant decrease in DNA repair efficiency without any treatment, and lymphocytes from HNSCC
patients displayed a decrease of 62.15% (P<0.001), compared to an allocated 100% displayed by control lymphocytes from healthy donors. There were no statistically significant differences in DNA repair efficiency compared to not treated controls after healthy lymphocytes were treated with UV-radiation – 97.49% (P>0.05) or cisplatin – 91.76% (P>0.05). However, cancer cells displayed a statistically significant decrease in NER repair after UV-radiation treatment – 44% for HNSCC lymphocytes (P<0.001) and 15.76% for HTB-43 cells (P<0.001) according to the 97.49% efficiency estimated for healthy lymphocytes. Significant decrease in NER repair for HNSCC lymphocytes 48.14% (P<0.001) and for HTB-43 cells 9.76% (P<0.001) compared to the 91.76% for healthy lymphocytes was also observed after treatment with cisplatin.

3.5. Apoptosis induction

The data from the apoptosis analysis are shown in Fig. 5. In each experiment, the percentage of living cells not treated with UV-radiation was over 84% and the percentage of necrotic cells was below 0.2%. For the HTB-43 and SCC-25 cancer cell lines the percentage of living cells not treated with UV-radiation was over 78% and the percentage of necrotic cells was below 0.4%.

There were no differences in the percentage of apoptotic cells before treatment with UV-radiation, between 14% of healthy lymphocytes and 16.3% of HNSCC lymphocytes (P>0.05) and 18.4% of HTB-43 (P>0.05) and 17.1% of SCC-25 cancer cells. In all cases after UV-radiation treatment, the percentage of apoptotic cells rose in a dose-dependent manner. It was observed that UV-radiation in a dose of both 15 and 30 J/m² induced a statistically significant increase in the activation of apoptosis in HNSCC lymphocytes compared to healthy lymphocytes. The data analysis of cells within a population that were actively undergoing apoptosis after 15 J/m² of UV-radiation showed 19.2% of control lymphocytes and 30.1% of HNSCC lymphocytes (P<0.05). After treatment with 30 J/m² it showed 35% and 44% (P<0.05), respectively. It was also shown that UV-radiation induced a statistically significant increase in apoptosis for a dose of both 15 J/m² and 30 J/m² in HTB-43 and SCC-25 cancer cells compared to healthy lymphocytes. The percentage of apoptotic cells after 15 J/m² of UV irradiation for HTB-43 was 53.6% (P<0.001) and for SCC-25 it was 55.2% (P<0.001) and after 30 J/m² it was 70.6% (P<0.001) and 77.0% (P<0.001), respectively.

4. Discussion

Epidemiological studies show that cancer risks may differ among populations and these differences are associated with the lifestyle, diet, environment, gender, race, ethnicity and genetic predispositions [19]. DNA damage usually leads to mutagenesis and genomic instability resulting in a cancerous transformation. Thus, the variations in the DNA repair capacity among individuals are suggested to be a major risk factor of genetic predisposition to cancer occurrence. It may also contribute to resistance against anti-cancer treatment [19–21]. It was also reported that various human cancers, including lung, ovary and breast cancers might be associated with an altered nucleotide excision repair pathway [22,23].

In order to estimate the role of DNA repair capacity in the pathogenesis of head and neck cancer, the NER efficiencies in peripheral lymphocytes from HNSCC patients as well as HTB-43 larynx cancer and SCC-25 oral cancer cell lines were evaluated. Previously, a case control study was performed on a group of 47 patients with head and neck squamous cell carcinoma and on 38 healthy controls [24]. The extent of DNA damage, including oxidative lesions, and the efficiency of repair after genotoxic treatment with hydrogen peroxide were examined using the alkaline comet assay in peripheral blood lymphocytes and cancer cells from tissue biopsies. Impaired DNA repair in patients with head and neck cancer was reported. The comet assay has also been used to examine the association of DNA repair capacity in peripheral lymphocytes with the risk of HNSCC [25]. These studies showed increased baseline damage and decreased repair in HNSCC patients. The authors also reported high variability in background DNA damage, which might suggest genetic instability in HNSCC cells. Iwakawa et al. (2005) compared residual DNA damage in 10 healthy controls and 87 HNSCC patients. The mean residual damage after 15 min of repair was significantly higher in HNSCC patients than in healthy controls [26]. Saha et al. (2008) examined peripheral white blood cells and also showed that, during the first 15 min, repair was slower in HNSCC patients than in healthy controls [27]. By the comet assay, during 30 min of post-treatment repair Mohankumar et al. (1999) showed a decrease in the extent of DNA repair after UV radiation of smokers’ lymphocytes [19].

While, tobacco smoking is considered as a high risk factor in pathogenesis of head and neck cancer, UV-radiation was used in our study to activate NER which is the main pathway involved in DNA lesions removal caused by tobacco chemicals such as BPDE-dG adducts. UV-radiation is very well known DNA-damaging agent resulting in wide range of mutagenic and cytotoxic lesions such as cyclobutane-pyrimidine dimers, 6-4 photoproducts as well as strand breaks which has been used in a carcinogenesis study for many years [28]. By interfering the genome integrity both, tobacco
chemicals and UV-radiation cause an alternation to DNA structure which triggers NER. NER is also critical for intra- or interstrand crosslink adducts removal from DNA caused by anticancer drug of cisplatin which was examined in our study. In this study, we analysed the time-course of DNA repair within 240 min of incubation after treating peripheral blood lymphocytes and cancer HTB-43 or SCC-25 cells with UV-radiation. The time of DNA recovery in HNSCC lymphocytes was 240 min in comparison to the 60 min time of DNA recovery of healthy lymphocytes. This suggested that some abnormalities of the repair mechanism might occur. The most interesting data reflected a polymerization and re-synthesis of a new strand of DNA in comparison to the base excision level during the NER pathway in HTB-43 and SCC-25 cancer cells. Both cancer cells displayed a characteristic shift in the time of excision start up, 60 min for HTB-43 and 120 min for SCC-25 in comparison to healthy lymphocytes.

According to literature data, it is reported that the most lesions in normal cells were being cut out during the first 30 min of NER [29]. Thus, in this study, it is suggested that re-synthesis of a new strand of DNA, rather than excision, could be defective in HNSCC. This hypothesis is supported by our further results of an in vitro functional NER assay by Sliwinski et al. [30]. In this approach, the NER capability was assessed in the cell extracts during DNA re-synthesis with UV-irradiated plasmid as a substrate. The level of \([\gamma^{32}P]-dAMP\) incorporation during the phase of DNA re-synthesis was detected by autoradiography and quantified using densitometry. It has been shown that there is a decrease in \([\gamma^{32}P]-dAMP\) incorporation in the cell extracts obtained from HNSCC patients compared to the extracts from healthy donors. Moreover, it was found that the HNSCC cancer cells displayed a lower efficiency of repairing the DNA damage caused by UV-radiation or cisplatin, measured by the DNA re-synthesis ability in the NER pathway. This was not observed for the cells from healthy donors.

The complex of the DNA repair pathway is critical for genotoxic susceptibility and is strongly associated with the predisposition to cancer development [3]. Analysis of a patient’s genotoxic susceptibility is very useful for searching host factors in the pathogenesis of cancer. On the other hand, genotoxic susceptibility may limit the effectiveness of anticancer treatment, which is considered for cancer patients. There are many biological differences between normal and cancer cells, however DNA repair in head and neck cancer has not been fully elucidated. In our study using different methods of single cells electrophoresis assay and in vitro cells extract assay after UV treatment we were able to examine the excision and the polymerization during NER pathway. It is very interesting because these steps are critical for DNA lesions repair caused by tobacco chemicals and we found that the polymerization was defective in HNSCC cells. Our results suggest that treatment with DNA-reactive drugs, including cisplatin, might be considered an effective strategy of therapy for patients with head and neck cancer. Recent studies have shown that cisplatin can be used as an effective anticancer agent in treatment of HNSCC patients [31–33]. Rampino et al. (2010) concluded that concomitant adjuvant chemoradiotherapy with weekly low-dose cisplatin for high-risk squamous cell carcinoma of the head and neck might be able to improve the patients’ outcome presented in the phase II prospective study [33].

In our study, we have reported that the molecular basis of the head and neck squamous cell carcinomas might be their higher genotoxic susceptibility as a consequence of an impaired NER pathway. Results presented in this work are in accordance with these obtained by Cheng et al. (1998), Wei et al. (2000) and Wang et al. (2010) [34–36]. Their results provide evidence that low DNA repair capacity (DRC) is associated with an increased risk of head and neck as well as lung cancer. They suggest that the DNA repair capacity is an independent susceptibility biomarker for head and neck cancer risk. However, they investigated only transcription-coupled DNA repair, whereas in our work we extended these studies and, for the first time, investigated the whole DNA repair as well as global genome nucleotide excision repair (GG-NER) activity.
in this context. Furthermore, we have found a higher induction of apoptosis after genotoxic treatment of HNSSC lymphocytes as well as HTB-43 and SCC-25 cells compared to healthy donors. A higher rate of apoptosis in HNSSC cancer cells was positively associated with their genotoxic sensitivity and finally accompanied by less effective DNA repair. That might confirm that NER could be a critical factor in the pathogenesis of head and neck cancer.

According to data obtained from HNSSC patients in this study, it should also be dispose of the risk of reverse causality when assessing DNA repair in cancer patients compared to healthy controls. The more effective DNA repair may cause drug resistance against anticancer treatment which was demonstrated in a large number of our previous studies with human leukemia [37–39]. Other abnormalities including growth factor independent proliferation and/or apoptosis inhibition may be also associated with an activated DNA repair in cancer cells [40,41]. It is suggested that effective DNA repair in tumor tissue may impair clinical response by promoting the removal of DNA adducts formed by chemotherapeutic drugs like cisplatin [42]. Interestingly, Bosken et al. (2002) hypothesized that patients with non-small-cell lung cancer (NSCLC) who had effective NER would have poorer survival than patients with suboptimal NER. He also suggested that the association between NER effectiveness and survival would be most marked in patients receiving chemotherapy [43]. Alternatively, in our study we estimated an association of impaired NER repair with a higher level of apoptosis induction and a lower cells survival under genotoxic treatment, what in turn may be connected to better cancer response to chemotherapy treatment.

Finally, we suggest that impaired DNA repair in patients with squamous cell carcinoma of head and neck may have serious biological consequences that must be fully elucidated in order to better understand the molecular basis of this disease and hence the therapeutic possibilities. In our study using lymphocytes, we evaluated general DNA repair of patients in comparison to healthy subject but it is also very interesting to investigate NER efficiency in advanced stages of HNSSC, then tissue samples from primary tumors and metastasis should be examined. Although, the size of study groups is limited, our results demonstrate a potential significance of NER in pathogenesis of head and neck cancer. This study is to be continued in order to draw a solid conclusion based on a larger population.

Conflict of interest

Nothing to declare.

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References


