Use of nasal cells in micronucleus assays and other genotoxicity studies

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Genotoxicity experiments with exfoliated nasal mucosa cells are a promising minimally invasive approach for the detection of DNA-damaging compounds in ambient air. Results of single cell gel electrophoresis (SCGE) assays with individual cells and organ cultures from biopptic material show that DNA damage caused by compounds such as nitrosamines, polycyclic aromatic hydrocarbons and pesticides can be detected. Biochemical studies indicate that enzymes involved in the metabolism of environmental mutagens are represented in nasal cells. Several protocols for experiments with nasal cells have been developed and it was shown that formaldehyde, metals, styrene and crystalline silica induce DNA damage in SCGE and/or in micronucleus studies; furthermore, it was also found that polluted urban air causes DNA instability in nasal epithelial cells. Comparisons of these data with results obtained in lymphocytes and buccal cells indicate that nasal cells are in general equally sensitive. Broad variations in the baseline levels, differences of results obtained in various studies as well as the lack of information concerning the impact of confounding factors on the outcome of experiments with these cells indicate the need for further standardisation of the experimental protocols.

Introduction

The cells of nasal tissues are the first that come into contact with gaseous genotoxins in ambient air. Therefore, they may be more suitable indicators for the detection of atmospheric toxins than blood cells (1). It has been postulated that exfoliated mucosa cells may have a high predictive value for the detection of carcinogens since >90% of human tumours are of epithelial origin (2). Apart from the potential value of nasal cells for the prediction of cancer risks in inner organs, compounds may be detected, which cause tumours in the nasal epithelia. It is known that factors such as wood dust and formaldehyde cause nasal tumours and it was also shown that occupational exposures, for example, in textile and leather industries are associated with increased nasal cancer (3–6).

This article gives an overview on the detection of genotoxic effects in nasal cells. The first paragraph describes morphological characteristics of the nose and provides information on drug-metabolising enzymes. Subsequent sections concern the use of cultured nasal cells in genotoxicity studies, results of animal experiments and data from human trials. In the last chapter, attempts are made to summarise the current state of knowledge and significant achievements in genotoxicity research with nasal epithelia to identify knowledge gaps and provide recommendations for future research. It is notable that the nasal mucosa has been also used in cytological studies in which the transition of the cells from normal to the metaplastic status was monitored, but such investigations are not described in this overview (for reviews, see refs. (7,8)).

Morphological, anatomical and histological characteristics of the human nose and the nasal mucosa

Information concerning the anatomy of the nose and of histological characteristics of the mucosa can be found in medical textbooks (9,10). This chapter focuses on issues, which are relevant for genotoxicity studies. Figure 1 provides an illustration of the anatomy of the human nose. The turbinates are lined with squamous olfactory or respiratory mucosa (Figure 1a–c). Due to the presence of columnal epithelia, the nasal turbinates share the same lining as the rest of the respiratory tract. The nasal cavity is the initial site of injury induced by gaseous irritants (11,12). The mucous layer has an important function in regard to the conditioning of inhaled air and provides a sticky surface for the deposition of inhaled particles (13–17) and for the absorption of gases (18,19). Due to the surface enlargement by three turbinates, the epithelium covers an area of ~120 cm² (20). The lower and middle turbinates are characterised by respiratory mucosa with stratified columnal ciliated epithelium (Figure 1b). Basal cells are positioned on the lower parts followed by intermediate layers containing non-ciliated cells, while those in the superficial layer are columnated, ciliated and mature. Interspersed are columnar non-ciliated goblet cells containing mucin. The apical region of ciliated cells is anchored to adjacent ciliated cells and goblet cells. The upper part of the cavity and the lower part of septum are covered by the olfactory mucosa, which contains primary sensory cells (Figure 1c).

In general, the commonest cell type (75% of all cells) detected in the nasal smear samples represented columnar surface epithelium (goblet cells), other epithelial cells (14%) and neutrophils (11%). Eosinophils and lymphocytes are very rare (on average 0.07 and 0.2%, respectively) (21). The cell types can be distinguished by the size of their nuclei and shape (Figure 1d), both are smaller than exfoliated buccal cells and possess larger nuclei; ciliae are rarely seen and may be destroyed during sampling and slide preparation.
The most common acute toxic effects seen by microscopy are loss of the ciliae, alterations of mucin production, changes in cell shape, dysplasia and metaplasia (22). The inferior and middle turbinates come into most intense contact with toxins contained in inhaled air. At this site, non-ciliated cuboidal cells may become metaplastic (7).

**Representation of drug-metabolising enzymes in the nasal mucosa**

Several studies concerning the activation of xenobiotics in nasal cells of humans and rodents (23–27) demonstrate that enzymes involved in the activation and detoxification of carcinogens are present (24). The overall P450 cytochrome superfamily content in human respiratory epithelium is twice as high as in the lung but only 5% of that of the liver (24). It has been shown that nitrosamines in tobacco smoke (N-nitrosodiethylamine (NDEA), N′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) are more efficiently converted to DNA-reactive metabolites in nasal cells than in the liver (28,29).

Gene expression studies revealed differences in the activation capacity of nasal tissue and other organs, e.g. CYP21A3 is expressed in human nasal mucosa but not in the liver (30). Notable species differences include the lack of CYP2G1 in humans, which is present in nasal tissue of most other mammals; another example is the expression of CYP2F. This interspecies variability may explain differences in the carcinogenicity of styrene and naphthalene in humans and rodents (30).

Gender and age differences have been studied in regard to enzyme expression. No sex differences were seen at the transcription level of CYP2G1 in rats (31) or in the levels of CYP2A protein in humans and mice (32,33), but many CYPs were found to be expressed in nasal mucosa of mammals earlier during development than in the liver.

A variety of Phase II enzymes [epoxide hydroxylase (EH), glutathione-S-transferase (GST), DT-diaphorase, benzadehyde dehydrogenase and carbonyl transferase] have been detected in nasal cells of humans and other mammals (24). Their activities are lower as in hepatic tissue, but in contrast to Phase I enzymes, the differences are less dramatic, i.e. the most significant difference in humans are a 10-fold higher activity of GST and a 2-fold higher level of EH in the liver (24). UDP-glucurononyltransferase activity was not detected in human samples (24), but the enzyme was found in the mucosa of monkeys (26). It is also notable that strong differences between
humans and monkeys were observed in the activities of other enzymes, indicating that the monkey is not an ideal model for metabolism studies of the upper respiratory tract (24).

The activities of Phase I and Phase II enzymes are not evenly distributed in the nasal mucosa and the levels of them are higher in olfactory than in respiratory mucosa (27,34–36). Some Phase I and Phase II enzymes are represented in nasal cells in inducible form and differences between their inducibility in nasal and hepatic cells were found with 3-methylcholanthrene, phenobarbital and ethanol (25,30).

**Genotoxicity assays with nasal cells**

In most studies with nasal cells, either comet or micronucleus (MN) formation was monitored. Comet assays [single cell gel electrophoresis (SCGE) assays] are based on the determination of DNA migration in an electric field. They are conducted with cells from various tissues and are cost- and time-effective (37,38). SCGE measurements allow under alkaline conditions the detection of single- and double-strand breaks and apurinic sites (38). The use of the enzymes endonuclease III (endoIII) and formamidopyrimidine glycosylase allows a measure of the endogenous formation of oxidatively damaged bases (39); treatment of the cells with hydrogen peroxide or radiation allows the monitoring of changes in sensitivity towards oxidative DNA damage. Recently, protocols have been developed that enable the investigation of DNA repair processes (40) and of changes in the sensitivity of the cells towards chemical carcinogens (41).

MNi are extranuclear DNA-containing bodies, which can be evaluated microscopically (42). They are formed as a consequence of chromosomal breakage (clastogenicity) and/or chromosome loss; by use of pancentromeric and/or specific chromosome fluorescence in situ hybridisation probes, it is possible to find out if they contain whole chromosomes or only acentric fragments (43).

While comets reflect changes of the DNA, which disappear as a consequence of repair processes, MNi reflect persisting chromosomal aberrations, which arise due to defects in chromosome segregation or due tomsirepair of DNA breaks. In regard to the interpretation of the results, it is important that the consequences of comet formation are not fully understood while it was shown recently in a comprehensive analysis by Bonassi et al. (44) that increased MN levels in lymphocytes are associated prospectively with cancer risks. However, it is not known if MN in exfoliated cells reflect cancer risks and an international project concerning the validation of assays with buccal cells is in progress (45).

**In vitro studies**

No studies with stable cell lines derived from nasal mucosa have been published, but a number of investigations have been conducted with primary cells and in all of them, DNA migration was monitored in SCGE assays. The cells were isolated from human biopsy samples or from rodents by enzyme (collagenase, hyaluridase and pronase) treatment (46,47).

In a number of studies, comet formation by carcinogens such as N-nitro-4-methylpiperazine (48), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), metal compounds and the pesticide lindane was observed in human cells (46,47). Interestingly, comparisons between the effects caused by the latter compound in colon mucosa and nasal cells show that cells from the gastrointestinal tract are less sensitive, while no differences were observed between the responses of rat and human nasal cells (46). Lindane was also tested by Tisch et al. (49) comparatively with other pesticides (transfluthrin, cyfluthrin and natural pyrethrum). The genotoxic activities of these agents were similar, but comparisons between DNA migration in cells collected from the middle and inferior conchae showed that the former tissue is more sensitive (49). The same group tested also a pesticide (permethrin), the repellent (N,N-diethyl-m-toluamide) and an organophosphate (diazinon) in the same system and detected more pronounced effects in cells from the middle turbinate (50). Also formaldehyde (51) and benzene (52) were studied with human nasal cells in vitro. Kleinsasser et al. (53) compared the effect of several compounds (dibutylphthalate, diisobutylphthalate, NDEA, benzo(a)pyrene and MNNG) in mucosa cells from larynx, oesophagus and inferior nasal turbinate and peripheral lymphocytes. Only poor correlations between the effects in blood and mucosa cells were observed. The same group also published results with several phthalates and stressed that nasal cells are more sensitive than those from the otopharynx (54,55). The time-dependent disappearance of comets induced by the NDEA in nasal cells and lymphocytes in SCGE experiments revealed no significant differences between the repair capacities of the two the cell types (54).

Kleinsasser et al. (55,56) published a protocol for mini organ cultures (25 × 5 mm) with nasal mucosa. The tissues are isolated by surgery and cultivated in bronchial epithelium basal medium after several washing steps. The authors reported that it is possible to detect with these models DNA damage in SCGE trials with compounds such as MNNG, NDEA and sodium dichromate (56). They also showed that the activity of the cytochrome P450 isozyme CYP2A6 is stable up to 11 days. On the basis of this observation, they stress that the use of organ cultures has the advantage that it reflects the metabolism of promutagens more adequately than experiments with single cells (57). They studied also the effect of repeated treatment of organ cultures with nitroso compounds and dichromate (56,58). While no increase of the response was seen with nitrosamines after repeated treatment, additive effects were found with MNNG and dichromate and the authors emphasise that this model reflects chronic exposure of humans to toxins better than other protocols.

Organ cultures of nasal mucosa cells were used in antigenotoxicity studies with comet assays (59) and quercetin, coenzyme Q10, ascorbic acid, zinc, dexamethasone, α-tocopherol and N-acetylcyesteine were found to be protective against oxidative DNA damage induced by H2O2 (59–61). An interesting study with nasal cells was published by Doolittle et al. (62). The authors conducted unscheduled DNA synthesis measurements to investigate induction of excision-repairable DNA lesions caused by N-nitrosodimethylamine inhalation in different tissues of rats. They found that liver cells are 10- to 15-fold more sensitive than cells from nasal and maxilloturbinates, from the ethmoid turbinate and from the trachea while in spermatocytes, the effects were 35- to 60-fold weaker than in nasal epithelium (62).

**Studies with laboratory rodents**

Only few results of SCGE studies with nasal cells from chemically treated rats and mice have been published. The cells were isolated with the techniques described above;
subsequently, DNA damage was monitored under alkaline conditions. Experiments with lindane showed that the pesticide causes in rats DNA migration in nasal cells after inhalation (46). Also with the rodent carcinogen N-nitroso-4-methylpiperazine (to which workers in the rubber industry are exposed), a positive result was obtained (48). Valverde et al. (63) studied DNA migration in tissues in mice after inhalation of lead. An effect in nasal mucosa cells was found after 4 weeks while in other organs (e.g. the brain), DNA damage was observed earlier.

**Human SCGE studies**

In total, 11 studies have been published (1,22,64–72). In all trials, DNA damage was measured under alkaline conditions, only in one ultraviolet DNA endonuclease was used additionally (72). The results of five investigations concerned the impact of urban air pollution, and in all significant, DNA damage was detected, which was attributed to ozone (65–67,70,71).

In several studies, DNA damage was measured comparatively in cells from different tissues. In studies with asthmatics and non-asthmatics, damage was seen in nasal cells but not in leukocytes (68,69). The authors hypothesise that the hyperactivity of the nasal epithelium may prevent systemic effects from pollutants and that the nasal cells of asthmatics are more sensitive to DNA damage than those of non-asthmatics (68,69). In this context, it is notable that in one air pollution study, DNA damage was found in nasal cells and lymphocytes but not in oral cells (71).

Only in few trials, sex and age effects were taken into consideration. DNA migration was higher in males than in females living in polluted areas (67); this observation was in agreement with the findings in lymphocytes (67,73). Interestingly, no age effect was found in a study concerning urban air pollution while in a study with children of various ages, the strongest effects were observed in the youngest individuals (70).

Glück and Gebers (1) conducted a study with smokers, which concerned associations between DNA migration and the formation of dysplastic and metaplastic cells, which are indicative for cancer risks. An association between these parameters was found and the authors conclude that their results confirm the hypothesis that changes detectable with conventional nasal cytology are associated with DNA damage. However, it is possible that the comets are formed in these cells as a consequence of incomplete ligation of newly synthesised DNA strands and reflect DNA synthesis and not necessarily damage of the genetic material. For details, see supplementary Table S1, available at *Mutagenesis* Online.

**Human MN studies**

In total, 16 studies have been published in which MN frequencies were evaluated (21,74–88). The majority (74–80) concerns the effect of formaldehyde. In most of them, induction of MNi was found but the effects varied over a broad range [between 25 (78,80) and 260% (75,77,89)].

Trials with chromium-exposed workers yielded negative results (21) while in other occupational studies, for example, with styrene (83), chromic acid and ethylene oxide (87), metals (81,82) and crystalline silica exposure (88), enhanced MN levels were observed; also in two studies concerning urban air pollution, positive results were obtained (84,85).

In none of the articles published so far, attempts have been made to characterise the cell types in which the MN and other anomalies were scored. However, some authors (21,80) mentioned that they found in nasal smears ~10% neutrophils and lymphocytes, which can be easily distinguished from epithelial cells by their size and morphology.

In several trials, positive results were obtained in nasal cells and also in lymphocytes and buccal cells; only in two studies with formaldehyde, a significant increase of the MNi levels was detected in buccal cells while no effect was seen in nasal tissue (78,80).

It is noteworthy that the experimental conditions varied strongly, i.e. different sampling methods were used, the number of evaluated cells varied between 1000 and 6000 and also the staining technique was not uniform. Most trials were conducted with DNA-specific stains, e.g. Feulgen (n = 9), acridine orange (n = 1) and DAPI (n = 3); in three studies, Wright’s stain and Giemsa were used.

The baseline MNi frequencies in untreated controls varied over a broad range [i.e. between 0.14 (85) and 2.84% (88)]. In nine trials, MNi rates were scored in parallel in buccal cells and/or in lymphocytes. In three of these studies, the MN frequencies in oral and nasal cells were similar (82,85,87), but the absolute frequencies varied substantially, i.e. between 0.14 and 0.99. In three investigations, the MN levels in nasal cells were consistently (2- to 9-fold) higher than in the mouth (77,78,80). Comparisons with the rates in lymphocytes yield also inconsistent results: in three investigations, similar frequencies were found (77,81,82) while in others, the levels in the blood cells were between 2- and 12-fold higher (78,83,88).

In three studies, MNi were analysed in nasal cells with pancentromeric probes; one with stainless steel production workers exposed to chromium (21) and two with formaldehyde-exposed students (80,86). In control subjects, 40–60% MN-stained centromeres positive indicating that these MN contain intact chromosomes and are formed as a consequence of aneuploidy (21,80). In chromium-exposed subjects, the number of MN+ was not altered (21) while in students exposed to formaldehyde (80), the number of MN+ (indicating chromosome breaks) was significantly elevated.

No studies have been conducted to evaluate the effects of smoking on MNi formation in nasal cells, but the impact of cigarette consumption on the outcome of occupational and other exposures was taken into consideration in several trials. In some of these studies, no increase of genotoxic effects was found in exposed subjects (81,87), but in two investigations, namely in workers exposed to silica dust and formaldehyde, smoking was associated with increased MN frequencies (76,88) while in unexposed control groups, no significant effects attributable to tobacco-smoking were detected (76,81,87). It is notable that also in studies with buccal cells, clear effects were only seen in heavy smokers with DNA-specific stains (90).

Nuclear anomalies other than MNi (condensed chromatins, buds, binucleates, karyorrhexis and karyolysis) were recorded only in two investigations (84,85). These endpoints provide additional information on acute toxic and genotoxic effects in buccal cells (91,92). Comparisons between the frequencies of these anomalies in nasal cells with those recorded in oral cells of subjects living in polluted areas of Mexico City showed an increase in the former cell type (84,85).

The age of the participants was taken into consideration in only a few investigations. No impact on the baseline frequencies

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and on the study outcomes was found in two studies (76,81) while in a trial with silica dust-exposed workers, a significant association between MN frequencies and age was found (88). For details, see supplementary Table S2, available at Mutagenesis Online.

Conclusions and future requirements

The use of nasal cells for the detection of genotoxins in the air is promising since they are the first to come into contact with such agents (93). The cells possess a variety of drug-metabolising enzymes including those involved in the metabolism of DNA-reactive carcinogens and results from in vitro SCGE assays indicate that nasal epithelia are able to activate of promutagens (e.g. nitrosamines and polycyclic aromatic hydrocarbons). Furthermore, protocols for experiments with rodents have been published for the detection of DNA migration in nasal cells and it was shown that a number of genotoxic carcinogens induce damage (46).

In total, we found 27 human studies in which DNA stability was measured in nasal cells either in SCGE or in MN experiments. The results show that it is possible to detect the effects of occupational and environmental exposures to hazardous chemicals. Only in a few studies other cell types were evaluated in parallel and the findings suggest that nasal cells are in general equally sensitive.

The number of human studies with nasal cells is much lower compared to those conducted with other indicator cells. One of the reasons for the small number of studies may be that the collection of nasal cells requires more expertise. As a consequence of the paucity of studies, MN and SCGE studies with nasal cells are by far less validated and standardised than ones with blood and buccal cells (91,92,94). The impact of parameters such as age, gender, lifestyle and diet-related factors and smoking habits is still unclear. Other important issues are the sampling site and the timing of cell collection after exposure. The activities of drug-metabolising enzymes are higher in the olfactory than in the respiratory epithelium and in vitro results with material collected from different sites shows that cells from the median turbinates are more sensitive that cells from the lower parts of the nose. It takes 2–3 weeks for damaged cells from the oral cavity to migrate from the basal layer to the surface; during this period, the cells divide and MN are formed (91,95). Earlier sampling will not lead to measurable effects while after longer phases without exposure, the MNi frequencies decline. This information, which is essential for an adequate study design is not available for experiments with nasal cells.

As mentioned above, no attempts have been made to discriminate between different types of cells in which MNi and other nuclear anomalies were scored. The classification of different types of cells may be important step in the standardisation of the tests procedure. We showed in an earlier study with mouth cells that it is essential to use DNA-specific stains to obtain reliable results since keratin bodies may lead to misinterpretations (90). Since the nasal cells are of epithelial origin as well, this finding is also relevant for the evaluation of nuclear anomalies in these cells. The criteria for the identification of nuclear anomalies in buccal cells have been described in detail in a recent article of Thomas et al. (91). According to our experience, they can be also applied in studies with nasal cells; also in the studies of Gonsebatt et al. (84,85), the criteria for oral cells were employed.

A number of nuclear anomalies other than MN, which reflect acute toxic effects and DNA alterations can be scored in exfoliated cells (91,96). It has been shown in studies with buccal cells that these parameters provide relevant additional information (97,98). For example, a number of investigations indicated that binucleated cells and broken eggs are more sensitive biomarkers of genotoxic exposure than MN formation (97–99) but their mechanistic relationship to DNA damage events is unclear. These nuclear aberrations (98) can be also evaluated in nasal cells (85); furthermore, it is also possible to monitor phenomena attributable to toxic effects such as loss of cilia and formation of dysplastic and metaplastic cells, which may provide additional information for cancer risks and cannot be reliably evaluated in experiments with cells from other tissues (1,8).

The multiple endpoints, which can be monitored in with nasal cells as well as their ability to activate carcinogens underline their potential usefulness for the detection of hazardous compounds. However, the comparatively poor database and the divergence of the protocols highlight the urgent need for standardisation and harmonisation of the methods and for the validation of the sensitivity of this promising approach.

Supplementary data

Supplementary Table S1 and Table S2 are available at Mutagenesis Online.

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References


