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Defence mechanisms of olfactory neuro-epithelium: mucosa regeneration, metabolising enzymes and transporters

J.-B. Watelet, M. Strolin-Benedetti** and R. Whomsley****

*Department of Otorhinolaryngology, Ghent University, Belgium; **UCB sa, Colombes, France; ***Shire Pharmaceuticals, Basingstoke, Hampshire, UK

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Abstract. *Defence mechanisms of olfactory neuro-epithelium: mucosa regeneration, metabolising enzymes and transporters.* The olfactory neuro-epithelium is highly sensitive to chemicals and its direct microbiological environment. It also plays a role as an interface between the airways and the nervous system, and so it has developed several defence instruments for rapid regeneration or for the detoxification of the immediate environment. This review illustrates three of these defence mechanisms: regeneration of the epithelium, local production of metabolising enzymes and xenobiotic transporters.

Toxicants can inflict damage by a direct toxic response. Alternatively, they may require metabolic activation to produce the proximate toxicant. In addition to detoxifying inhaled and systemically derived xenobiotics, the local olfactory metabolism may fulfil multiple functions such as the modification of inhaled odorant, the modulation of endogenous signalling molecules and the protection of other tissues such as the CNS and lungs from inhaled toxicants. Finally, the permeability of nasal and olfactory mucosa is an important efficacy parameter for some anti-allergic drugs delivered by intranasal administration or inhalation. Efflux or update transporters expressed in these tissues may therefore significantly influence the pharmacokinetics of drugs administered topically.

Introduction

Inspired air is brought high into the nasal cavity to come in contact with the olfactory nerves, triggering the sense of smell, which is intimately associated with taste.[1] The neuro-epithelium has several instruments for ensuring optimal olfactory function. The olfactory neuro-epithelium is highly sensitive to the chemical or microbiological environment and it acts as an interface between the airways and the nervous system. As a result, it is suspected that it facilitates the direct transfection of bacteria or viruses[2] or the transportation of chemicals[3,4] into the dura and nervous system. On the other hand, the defence mechanisms deployed by the olfactory neuro-epithelium for rapid regeneration or for detoxification of the immediate environment, such as enzymatic control or transporter activation, have been the subject of several recent publications.

This review will provide an overview of three major defence mechanisms for toxic or chemical insults to the olfactory mucosa.

1. Chemical injury of the olfactory epithelium

This section does not look at physical (e.g. irradiation...) or pharmacological injuries to the olfactory mucosa. Many of the toxicity mechanisms are still under investigation and the literature is mainly based on case reports, making any generalisation hazardous.

The limited accessibility to biopsies of human olfactory epithelium, in conjunction with their small size, has resulted in an almost complete lack of information about how toxic compounds affect this highly differentiated mucosa. Most of what we know about the toxic action of inhaled compounds is derived from experimental animal studies.

1.1. Epidemiology

The inhalation of a number of environmental and industrial chemicals can lead to olfactory dysfunction. Even if the list of theoretically toxic chemicals is impressive, they are thought to be responsible for smell disorders in only 2% of patients.[5] The degree of olfactory damage seems to be related to the timing and duration of the exposure, the concentration of the agent, and the intrinsic toxicity of the agent. It therefore remains difficult to establish a complete list of environmental toxicants. Furthermore, the multiplicity of cofactors makes this analysis even more complex.

Table 1
Major toxicants and industrial agents that may induce smell disorders

Classes	Agents	Reported effect
Major toxic chemicals	Benzene or benzol	Hyposmia/anosmia
	Butyl acetate	Hyposmia/anosmia
	Carbon disulphide	Hyposmia/anosmia
	Chlorine	Hyposmia
	Ethyl acetate	Hyposmia/anosmia
	Formaldehyde	Hyposmia
	Hydrogen	Hyposmia/anosmia
	Selenide	Hyposmia
	Paint solvents : *acetone* *mineral turpentine (turps)* *true turpentine* *naphtha* *toluene* *white spirit* *xylene* *methyl ethyl ketone*	Hyposmia/anosmia
	Sulphuric acid	Hyposmia
	Trichloroethylene	Hyposmia/anosmia
Major industrial agents	Ashes	Hyposmia
	Cadmium	Hyposmia/anosmia
	Chalk	Hyposmia
	Chromium	Hyposmia
	Iron carboxyl	Hyposmia
	Lead	Hyposmia
	Nickel	Hyposmia/anosmia
	Ozone	Temporary hyposmia
	Silicone dioxide	Hyposmia

Table 1 lists the major environmental toxicants and their clinical consequences.

1.2. Clinical presentation

1.2.1. Acute toxicity

The relationship between toxicants and accidental smell disorders is easier to identify when the exposure is massive and sudden. The smell disorder can occur within the first seconds or the first hours after exposure and can lead to hyposmia or a transitory or permanent anosmia.

1.2.2. Chronic toxicity

On the other hand, when doses are lower and presented for a long period of time, the diagnosis can be more difficult to establish and, because of the long course of events, many confounding factors also need to be taken into account: e.g. age, viral infections, traumas...

Chronic exposure to low levels of benzene, butyl acetate, formaldehyde, and paint solvent has been commonly reported with associated olfactory dysfunction. Many dusts produced in industry have also been associated with olfactory dysfunction, including grain, silicone, cotton, paper, cement, lead, coal, chromium, and nickel.

Chronic metal toxicity was clearly illustrated in the case of cadmium during the 1950s. Up to 60% of people exposed to cadmium for more than 10 years presented an olfactory disorder.[6] The olfactory toxicity of solvents (e.g. acetone, acetophenone, benzenes...) is mainly due to their high lipophilicity. Olfactory deficit increases with cumulative exposure. Finally, chronic exposure to gases (such as carbon disulphide, carbon monoxide, sulphur dioxide, formaldehyde...) can also directly affect the olfactory mucosa. For example, exposure to ozone induces an increase in olfactory thresholds in healthy controls[7] but there is an adaptation phase after the initial experiments.

1.3. Principles underlying damage to the olfactory mucosa

Smell disorders secondary to toxic exposure can be due to multiple pathogenesis mechanisms: inflammation of the nasal mucosa leading to obstruction, neurosensorial lesions, central disorders or a combination of the above.

This review will focus mainly on peripheral neurosensorial disorders related to toxic injuries to the neuro-epithelium. However, it is possible that some toxicants could also have a central impact on the smell function, even though the supporting data is very fragmentary.

1.3.1. Histological damage

Attempts to link clinical anosmia to olfactory membrane abnor-

malities have provided relevant information about the clinical consequences of olfactory tissue damage.[8]

After exposure to olfactotoxins, cytotoxicity at the level of the olfactory mucosa is seen almost immediately (in less than 24 h). The tissue damage due to perchloroethylene gas is more persistent in the nasal mucosa of the olfactory region than in the respiratory region. Two weeks after exposure, ciliated epithelial cells, as well as a pseudostratified nonciliated columnar epithelium, begin to appear in the area previously covered by olfactory epithelium and remain for up to 3 months after exposure. A basement membrane is also present under the ciliated epithelium, suggesting a possible persistence of basal cells. The olfactory epithelium may therefore be replaced by ciliated respiratory epithelium. The lamina propria of the olfactory mucosa, however, loses its normal structure, with atrophy of the olfactory nerves and Bowman's glands.[9] Interestingly, whereas 2,5-methylsulfonyl-substituted dichlorobenzene (diCl-MeSO2-B) induces no signs of toxicity in the olfactory mucosa at doses as high as 130 mg/kg (i.p. injection), necrosis of Bowman's glands must be considered as the first sign of 2,6-(diCl-MeSO2-B)-induced toxicity at higher doses (Figure 1a), followed by degeneration of the neuro-epithelium. This implies that, for some toxicants with 2,6-positioned chlorine atoms and an electron-withdrawing substituent in the primary position at least, Bowman's glands may be the primary site of toxicity and degeneration of the neuro-epithelium may be a secondary effect.[10,11]

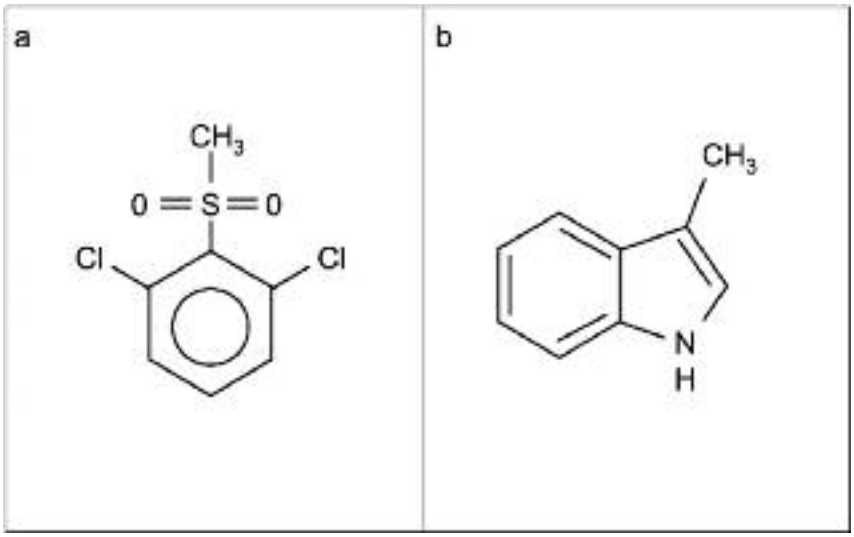


Figure 1
Chemical structure of 2,6-methylsulphonyl-substituted dichlorobenzene (a) and 3-methylindole (b), regularly used for anosmia models.

1.3.2. Direct or indirect toxicity

Toxicants can exert their damaging properties in different ways. Some agents, such as methylbromide and 3-methylindole (Figure 1b), produce a direct toxic response.[12,13] Other compounds, such as dimethylamine and 3-methylsulphate, require metabolic activation to produce the proximate toxicant.[14-17]

Finally, in addition their direct toxic effects, some toxicants can also have a strong impact on the enzymatic cascade. Cytochrome P450 (CYP) is a very large and diverse super-family of haemoproteins using a plethora of both exogenous and endogenous compounds as substrates. Twenty-eight hours after a single dose (12 mg/kg) of dichlobenil, characteristic regions of the olfactory mucosa showed signs of necrosis in haematoxyllin eosin-stained sections. This was accompanied by a dramatic reduction in CYP immunoreactivity in Bowman's glands, and an apparent redistribution of CYP immunoreactivity within sustentacular cells. The treatment of mice with metyrapone, a CYP inhibitor, at 10 min prior to and 2, 4, 6, and 8 h after a single dichlobenil injection, provided some protection against the damaging effects of dichlobenil.[18] Complementary results suggested that, in mice, 3-aminobenzamide, another inhibitor of CYP, can also reduce the local toxicity of dichlobenil in the olfactory mucosa, probably because of a reduction in the metabolic activation of dichlobenil at this site.[19]

Human data are more incomplete but it appears that individuals with olfactory loss caused by epithelial damage, as in chronic rhinosinusitis, display evidence of nerve fascicle degeneration and intra-epithelial neuromas.[20]

1.4. Defence mechanisms

The olfactory mucosa has mechanisms that can limit tissue lesions

secondary to inhaled toxicants. The olfactory epithelium can secrete many proteins or enzymes that act directly against the pathogens or irritants. Furthermore, the trigeminal reflex has a direct influence on the local respiratory and secretory physiology of the nasal cavities, reducing the risk of olfactory damage. However, chronic exposure to irritants such as trichloroethylenes,[21,22] tobacco smoke, carbon monoxide and chloromethanes can reduce the trigeminal defence reflex.

This review looks at three complementary defence mechanisms engaged in the protection of the highly sensitive olfactory neuro-epithelium: regeneration of the epithelium and the roles played by metabolising enzymes and transporters.

2. Regeneration of olfactory epithelium

The regeneration of the olfactory neuro-epithelium has also been a subject of debate.[23] The precise events in the repair process are still largely unknown and the existing data about the inducing signals is sparse.

2.1. Structural recovery of olfactory tissue

After exposure to many olfactotoxins, at least in rodents, dead cells slough off, and during the next 30 days, the olfactory epithelium is restored. With other chemicals, the return to a mature olfactory epithelium is less rapid, especially when the lamina propria and Bowman's glands appear to be severely damaged.[24] Recovery of Bowman's glands is accompanied by a return of olfactory function, suggesting that damage to Bowman's glands should be seen as a key element in the development of olfactory deficits in humans.

In studies exploring the long-term response in the olfactory mucosa of mice after exposure to the olfactory toxicants dichlobenil (a herbicide) or methimazole (an antithyroid drug), it appeared that, three and six months after exposure to dichlobenil ($2\times$ or 1×25 mg/kg i.p.), the dorsomedial part of the olfactory region showed a respiratory metaplasia with abundant invaginations and a fibrotic lamina propria. By contrast, 3 months after exposure to a toxic dose of methimazole (2×50 mg/kg i.p.), the olfactory neuro-epithelium and lamina propria had been restored. An intact lamina propria is thought to be a prerequisite for the repopulation of the neuro-epithelium after toxicant-induced injury.[25]

Recently, round or oval openings with a diameter of 50 to 500 microns were observed on the surface of the olfactory epithelium.[26] These olfactory pits are blind pouches lined with olfactory epithelium, presenting as invaginations of the neuro-epithelium into the connective tissue to depths varying between 150 and 200 microns. The function of the pit specialisation is unclear, but it appears to be a feature of normal, young epithelium. The configuration of the blind pouches may prolong odorant association with the olfactory receptor neurons, or they may contain specialised neurons that have not yet been recognised. These findings could also serve to identify fully functional epithelium or newly regenerated epithelium with *restitutio ad integrum*.

2.2. Growth factors and olfactory marker proteins in olfactory mucosa repair

Some biological factors involved in the repair process have been identified. As in other repair processes, the role played by growth factors and enzymes is determinant. The development of human olfactory mucosa shows that epidermal growth factor-receptor, transforming growth factor-alpha and nerve growth factor-beta proteins are reliable markers for developing or regenerating olfactory epithelium.[27] Mucus covering the human olfactory epithelium contains insulin growth factor-I and insulin growth-factor-binding proteins, suggesting that these factors have a role in the activity of the olfactory mucosa. The amounts are reduced in the mucus of patients with neurodegenerative diseases, possibly reflecting a dysfunction of the mucosa itself.[28] On the other hand, a lack of beta 2-microglobulin, and presumably class I, may be a general phenotype of neuronal cells regardless of their mitotic state or exposure to environmental antigens.[29]

Recently, in mice, another potential differentiation marker has been identified: the olfactory marker protein (OMP). Cells expressing OMP have been located in the anterior/ dorsal region of the nose quite distant from the regio olfactoria. The cells are arranged in ganglion-like clusters during perinatal stages and appear to persist in adult animals. Although OMP is present in cells of the central nervous system and the cribriform mesenchyme,[30-39] it is considered a valuable general marker for mature olfactory sensory neurons in nasal neuro-

epithelia, and growing evidence has been accumulated that it is indeed a participant in olfactory function.[37,40-45] Based on the concept that cells expressing OMP typically operate as chemosensors, it seems conceivable that the newly discovered OMP cells may have a chemosensory function as well, but could also serve as indicators of fully functional neuroepithelium.

2.3. Metabolising enzymes during repair of olfactory mucosa

Several metabolising enzymes have been considered as indicators of the degree of olfactory regeneration. In the peripheral olfactory organ, continual olfactory receptor neuron (ORN) turnover exposes neighbouring cells to potentially damaging cellular debris such as free radicals. These, in turn, may be inactivated by binding directly to glutathione (GSH) or by enzymatic conjugation with glutathione S-transferase (GST). The recovery of GST activity and widespread GST immunoreactivity during regeneration indicates the modulation of neuroprotective, developmental, and/or physiological processes by GST.[46] In mice with unilateral naris closure for 3, 4, or 5 months, CYP immunoreactivity was clearly reduced in rostral regions of the open-side olfactory mucosa, where losses of receptor neurons resulted after 3 to 5 months of closure. Closed-side immunoreactivity was similar to controls. After 4 months of closure in animals that had regrown their receptor neurons, open-side immunoreactivity for CYP was comparable to controls. Furthermore, olfactory bulbectomy also depressed CYP immunoreactivity in mice. The presence or absence of receptor neurons may markedly affect CYP expression in non-neuronal cells of the olfactory mucosa.[47] Finally, the precursor nature of the highest reductase-expressing cells suggests that differentiation-specific mechanisms regulate cytochrome P450 reductase gene transcription during organogenesis.[48]

2.4. Metal chelators during the repair of olfactory mucosa

Finally, other biological factors involved in repair are an important source of information about the local defence and repair mechanisms of the olfactory mucosa. Metallothionein (MT) is a family of cysteine-rich, low molecular weight (MW ranging from 3500 to 14000 Da) proteins. MTs have the capacity to bind both physiological (Zn, Cu, Se...) and xenobiotic (Cd, Hg, Ag...) heavy metals through the thiol group of its cysteine residues, which represents nearly 30% of its amino acid residues. MT function is not clear, but experimental data suggest it may provide protection against metal toxicity, be involved in the regulation of physiological metals (Zn and Cu) and provide protection against oxidative stress. There are four main isoforms expressed in humans. When exploring the expression of the MT1 and MT2 isoforms of metallothionein in the mouse olfactory mucosa, it appeared that, in untreated mice, both were strongly expressed in supporting cells, acinar cells of Bowman's glands, and olfactory neurons. Irrigation with irritative solution caused exfoliation of the olfactory epithelium and, during the resultant regeneration, metallothionein immunoreactivity was associated with the proliferating basal cells.[49]

3. *Xenobiotic-metabolising enzymes and enzymes involved in the metabolism of reactive oxygen species*

Drug-metabolising enzymes, which are theoretically supposed to participate in detoxication phenomena, can also activate some toxicants (Table 2).

Drug-metabolising enzymes have been grouped into phase I reactions, in which enzymes control oxidation, reduction, or hydrolytic reactions, and phase II reactions, which involve the introduction of a hydrophilic endogenous species into the drug molecule.[50]

The phase I enzymes lead to the introduction of functional groups, such as -OH, -COOH, -SH, -O- or NH2 groups, resulting in a modification of the drug.

The phase II reactions may directly affect the parent compounds that contain appropriate structural motifs, or functional groups added or exposed by phase I oxidation.[51] Sulphation, glucuronidation, and glutathione conjugation are the three most prevalent classes of phase II metabolism. These conjugation reactions enhance the water solubility and molecular weight of the metabolite, and also add a negative charge to the molecule. Phase II reactions are generally cytosolic, with the exception of glucuronidation, which is microsomal. Conjugation reactions generally, although not always, terminate the biological activity of the drug. The catalytic rates of phase II reactions are generally significantly faster than the rates of the CYPs[50] and so the initial (phase I) oxidation reaction is normally rate-limiting.

Superoxide dismutases, metallozymes that catalyse the dismuta-

tion of superoxide anion radicals into hydrogen peroxide, are the cell's major enzymatic defence against cytotoxic reactive oxygen species and oxidative stress. There are two main forms of superoxide dismutases: the superoxide dismutase containing manganese, which is a mitochondrial enzyme, and the superoxide dismutase containing copper-zinc, which is located primarily in the cytosol and secondarily in the nucleus.[52]

3.1. Enzymes present in olfactory mucosa

Despite recent progress in the identification and characterisation of numerous nasal biotransformation enzymes in laboratory animals, the expression of biotransformation genes in human nasal mucosa remains difficult to study. Given the potential role of nasal biotransformation enzymes in the metabolism of airborne chemicals, including fragrance compounds and therapeutic agents, as well as the potential interspecies differences between laboratory animals and humans, several studies have attempted to identify the enzymatic content of human nasal mucosa.

Olfactory xenobiotic metabolism may be responsible for multiple functions:

1°/ the modification of inhaled odorant,

2°/ the modulation of endogenous signalling molecules,

3°/ the detoxification of inhaled and systematically derived xenobiotics, with some drugs having a potential influence on the development of smell disorders (Table 3),

4°/ the protection of other tissues such as CNS and lungs from inhaled toxicants.

3.1.1. In animals

The mammalian olfactory mucosa (OM) is unique among extrahepatic tissues in having high levels, and tissue-selective forms, of CYP enzymes. These enzymes may have important toxicological implications, as well as biological functions, in this chemosensory organ. In addition to tissue-selective, abundant expression of CYP1A2, CYP2A, and CYP2G1, some of the OM CYPs are also known to evince early developmental expression, a resistance to xenobiotic inducers, and a lack of responsiveness to circadian rhythm.[53] The respiratory and olfactory tissues are also the first line of contact with hazardous airborne chemicals.

In mice, CYP immunoreactivity in olfactory mucosa is observed only in Bowman's glands and supporting cells,[47] but not in receptor neurons or their progenitor basal cells. This localisation of CYP in the non-neuronal cells of the olfactory mucosa supports the view that one of these cells' major roles is the biotransformation of inhaled compounds.

In newborn rats, ultrastructural studies have shown that mitochondria are present in the apices portions of olfactory receptor neurons dendrites and of supporting cell apices, suggesting that these regions near the surfaces are metabolically the most active in odorant detection, signal processing and detoxification, the latter for supporting cells.[54] Several P450 isoforms, including CYP1A2, CYP2A, CYP2B, CYP2C, CYP2G1, and CYP3A, NADPH cytochrome P450-reductase, and microsomal epoxide hydrolase have been detected in the mouse vomeronasal organ (VNO), although their expression levels were much lower than those in the main olfactory epithelium. These findings reinforce the hypothesis that olfactory mucosal and VNO microsomal CYP enzymes are actively involved in maintaining cellular hormonal homeostasis and other perireceptor processes associated with olfactory chemosensory function.[55] These high levels of CYP present in the olfactory mucosa in mammalian animals may contribute to the known tissue-selective toxicity of numerous chemical compounds.

In rats, inspired acetaldehyde is metabolised inside the olfactory mucosa by aldehyde dehydrogenase (ALDH). However, concentration dependence upon uptake suggests that a saturable process is involved: at exposure concentrations of 300 ppm or greater, the delivered dosage rate may equal or exceed the capacity of this enzyme.[56]

In bullfrogs, carbonic anhydrase could play a role in the detection of CO_2 thanks to its location in olfactory neurons. However, only a small population of olfactory receptor neurons are CO_2-sensitive.[57]

3.1.2. In humans

The xenobiotic-metabolising enzymes present in nasal mucosa, especially the olfactory neuroepithelium, and in the lungs play important roles in the first-pass metabolism of anti-allergic drugs that are administered through nasal sprays, aerosols or puffs. Zhang *et al.*[58] have found transcripts for nine drug-metabolising enzymes [ALDH6, ALDH7, CYP1B1, CYP2E1, CYP2F1, CYP4B1, flavin-containing monooxygenase 1 (FMO1), GSTP1,

UGT2A1] in human foetal nasal mucosa. These observations, combined with the previous detection of other enzymes in human nasal mucosa (CYP2A6, CYP2A13, CYP2B6, CYP2C, CYP2J2, CYP3A, NADPH-cytochrome P450 reductase, microsomal epoxide hydrolase, GSTA, GSTP1, and UGT2A1),[59,60] provide strong support for the idea that both human foetal and adult olfactory mucosa play an active role in the biotransformation of numerous xenobiotics. Olfactory toxicity in the perinatal period may have a greater impact on behaviour, growth, and development than in adults.[61] Prenatal expression of xenobiotic-bioactivating CYP enzymes in human OM suggests that the human foetal OM may be a preferred target tissue for the toxicity of maternally derived chemical compounds that are activated by the CYP enzymes.[61]

In the foetus, the level of CYP2A13 mRNA is much higher than that of CYP2A6 mRNA, as has been found previously in adult nasal mucosa. Immunohistochemical studies confirm that, in the foetus, the CYP2A proteins are expressed in the supporting cells in the olfactory epithelium and in Bowman's glands in the lamina propria.

3.1.3. In human diseases

Reactive oxygen species, which induce the expression of superoxide dismutases, have been implicated in the neurodegeneration associated with Alzheimer's disease (AD).[52] Individuals with AD exhibit early, severe deficits in olfactory ability. The pronounced increase in superoxide dismutase immunoreactivity in the olfactory epithelium of AD subjects suggests that oxidative stress may be responsible, at least in part, for the olfactory deficits in subjects with AD.[52]

3.2. Inhibition/induction of enzymes present in animal and/or human olfactory mucosa by xenobiotics, and the biological consequences

In rats, low-level m-xylene exposure results in the organ-selective alteration of CYP isozyme activities and subsequent 1-nitronaphthalene-induced toxicity.[62] Furthermore, coumarin (50 μM) inhibits the metabolism of three widely used gasoline oxygenates (ethyl tert-butyl ether and tert-amyl methyl ether) by approximately 87%, these ethers being predominately metabolised through CYP of the rat olfactory mucosa.[63] Inhibition of CYP activities with either metyrapone or carbon tetrachloride eliminates or significantly reduces the olfactory toxicity of beta,beta'-iminodipropionitrile in the rat.[64] The dichlobenil-induced olfactory damage is accompanied by a dramatic reduction in CYP immunoreactivity in Bowman's glands of the olfactory mucosa. Finally, treatment of mice with metyrapone or 3-aminobenzamide (two CYP inhibitors) after a single dichlobenil injection provides some protection against the damaging effects of dichlobenil.[18,19]

In vitro, with nasal and hepatic microsomes from rats and rabbits, carbon monoxide binding and hexamethylphosphoramide N-demethylase activity have been found to be most sensitive to alkyl-substituted dioxolanes. Mono-oxygenase activity in the nasal mucosa is inhibited more readily than that in the liver.[65]

The administration of 3,5-diethoxycarbonyl-4-ethyl-1,4-dihydro-2,6-dimethylpyridine (4-ethyl-DDC) to hamsters results in a marked loss of CYP-dependent reactions (peroxidase, 7-ethoxycoumarin O-deethylase, and 7-ethoxyresorufin O-deethylase) in both the liver and the olfactory epithelium.[66]

Hydrogen sulphide (H_2S) is an important brain, lung, and nose toxicant. Inhibition of cytochrome oxidase is the primary biochemical effect associated with lethal H_2S exposure. In adult male rats, immediately after 3 hours of exposure to H_2S (10, 30, 80, 200, and 400 ppm), decreased cytochrome oxidase activity is observed in the respiratory and olfactory epithelium following exposure to > or = 30 ppm H_2S. Increased olfactory epithelial sulphide concentrations are observed following exposure to 400 ppm H_2S. Cytochrome oxidase inhibition may be considered to be a sensitive biomarker of H_2S exposure in target tissues like the nose.[67]

When investigating the role of metabolic activation in the olfactory toxicity of methyl iodide (MeI), adult male rats were exposed via nose-only inhalation to 100 ppm MeI for 0-6 h, and non-protein sulphydryl (NP-SH) concentrations determined in selected tissues. Depletion of NP-SH occurred in all tissues, but was most marked and rapid in the respiratory epithelium of the nasal cavity and the kidney. Olfactory, lung and liver NP-SH levels were affected to a lesser extent. In order to inhibit CYPs, animals were pretreated with cobalt protoporphyrin IX. This reduced hepatic CYP concentrations by >90%, but when animals were then exposed to 100 ppm MeI for four hours there was no effect on the severity of the olfactory lesion. The

Table 2

Instances in which nasal metabolism probably results in (a) detoxication or in (b) activation. Modified from Ding[59]

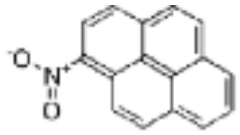
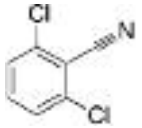
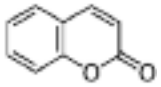
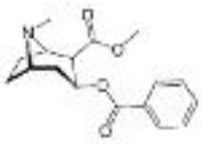
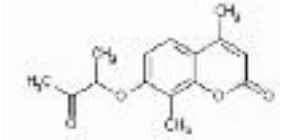
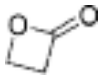
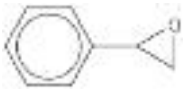
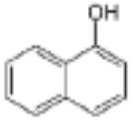
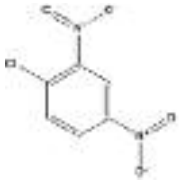
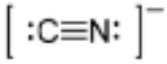
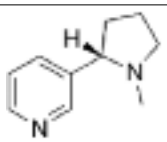
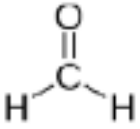
Substrates	*Chemical structures*	*Enzyme systems or enzymatic reactions*
A. Instances in which nasal metabolism probably results in detoxication		
Nitropyrenes		Oxidases and hydroxylases
2,6-dichlorobenzonitrile		Hydroxylases
Coumarin		7-hydroxylase
Cocaine		Demethylation
Alkoxycoumarins		Dealkylation
Lactones		Carbooxylesterases
Styrene oxide		Epoxide hydrolases
Naphthol		Transferases
Chlorodinitrobenzene		Transferases
Cyanide		S-transferases (rhodanese)
Nicotine		Demethylases
Formaldehyde		Aldehyde deshydrogenases

Table 2
Continuation

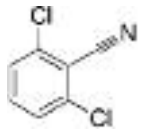
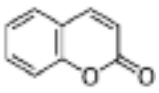
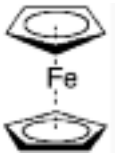
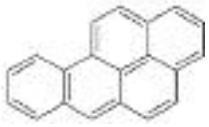
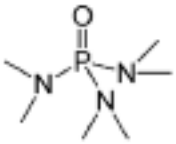
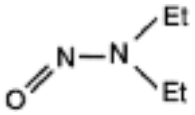
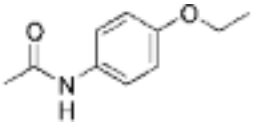
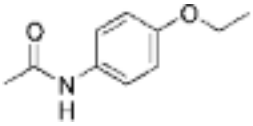
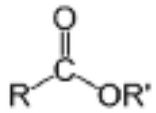
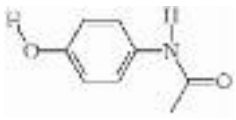
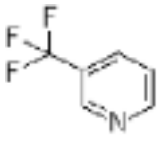
B. Instances in which nasal metabolism probably results in activation		
2,6-dichlorobenzonitrile		Epoxygenase
Coumarin		Epoxygenase Ferrocene
		Oxidases
Benzo(a)pyrene		Oxidases
Hexamethylphosphoramide Ferrocene		Demethylases
Diethylnitrosamine		Deethylases
Organonitriles		Oxidases
Phenacetin		Oxidases
Esters		Carboxylesterases
Acetaminophen		Oxidases
Trifluoromethylpyridine		N-oxidases

Table 3
Major therapeutic agents susceptible to induce smell and taste disturbances

Groups	Agents
Antibiotics	Ampicillin Azithromycin Ciprofloxacin Clarithromycin Griseofulvin Metronidazole Ofloxacin Tetracycline
Anticonvulsants	Carbamazepine Phenytoin
Antidepressants	Amitriptyline Clomipramine Desipramine Doxepin Imipramine Nortriptyline
Antihistamines and decongestants	Chlorpheniramine Loratadine Pseudoephedrine
Antihypertensives and cardiac medications	Acetazolamide Amiloride Betaxolol Captopril Diltiazem Enalapril Hydrochlorothiazide and combinations Nifedipine Nitroglycerin Propranolol Spironolactone
Anti-inflammatory agents	Auranofin Colchicine Dexamethasone Gold Hydrocortisone Penicillamine
Antimanic drugs	Lithium
Antineoplastics	Cisplatin Doxorubicin Methotrexate Vincristine
Antiparkinsonian agents	Levodopa
Antipsychotics	Clozapine Trifluoperazine
Antithyroid agents	Methimazole Propylthiouracil
Lipid-lowering agents	Fluvastatin Lovastatin Pravastatin
Muscle relaxants	Baclofen Dantrolene

authors' conclusion was that GSH conjugation of MeI is a detoxification pathway, and that there is no role for CYP in the development of toxicity, at least within the nasal cavity. They suggest that, as MeI is extremely effective at depleting GSH, it is possible that those tissues which suffer extensive GSH depletion and have slow rates of GSH turnover may become vulnerable to oxidative insult due to prolonged GSH depletion.[68]

The prenatal human expression of the CYP2A proteins in the olfactory mucosa provides evidence for the potential risks of developmental toxicity associated with maternally derived xenobiotics, since both CYP2A6 and CYP2A13 are known to be efficient in the metabolic activation of tobacco-specific nitrosamines and other respiratory toxicants.[69] Furthermore, recent kinetic, immune-inhibition, and immunoblot data have confirmed that CYP2A13 is a functional enzyme and the catalyst of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) alpha-hydroxylation in human foetal nasal mucosa. This reaction is a key bio-activation pathway in NNK-induced carcinogenesis. The results are also the first demonstration of high-efficiency NNK alpha-hydroxylation in human tissue.[70] Reports indicate that a significant level of GST activity is located in the cytosol of olfactory and respiratory nasal mucosa in humans. The specific activity of this enzyme system in human nasal mucosa appears to be higher (77 ± 21 nmol/min/mg, 1-chloro-2,4-dinitrobenzene used as substrate)[71] than that reported for a number of extrahepatic tissues, suggesting the potential role of nasal mucosa in the protection of the body against the toxic effect of

compounds present in the inhaled air.

On the other hand, by initially giving low doses of compounds that induce CYP metabolic enzymes, a protective status is induced and, subsequently, higher exposures produce little or no toxicity. For example, in humans, it has been shown that smokers are less susceptible to solvent-induced olfactory deficits than people who have never smoked.[72-74]

3.3. Drugs used in the treatment of allergic rhinitis and administered by nasal route as subtrates/inhibitors/inducers of xenobiotic-metabolising enzymes present in the olfactory mucosa

The reason for the absence of an extensive first-pass effect in the nasal mucosa for some drugs (e.g. propranolol) is probably the fact that only a few specific isozymes (phase I or phase II) are present in the nasal mucosa and that the others are not present or not developed.

Through an increased Na(+)/K(+) ATPase expression in the regenerated olfactory mucosa, dexamethasone contributes to the recovery of function after the morphological regeneration. This mechanism involves, at least in part, its receptor by regulating the ionic concentration in the olfactory mucosal micro-environment.[75] However, there is data to suggest that dexamethasone potentiates 3-methylindole (3-MI) olfactotoxicity during the first 2 weeks after insult. This effect may be due, at least in part, to the inducing action of dexamethasone on the CYP responsible for the metabolic bio-activation of 3-methylindole.[76] Furthermore, in an anosmia mouse model induced by injection of 3-MI, the thickness and cell numbers of olfactory neuro-epithelium and the expression of OMP were all reduced more significantly in the group treated with dexamethasone.[77] This characteristic of dexamethasone treatment was associated with further deterioration in olfactory injury by 3-MI and recovery was achieved using a combination treatment of dexamethasone and ginkgo biloba, probably explainable by anti-oxidant effects.

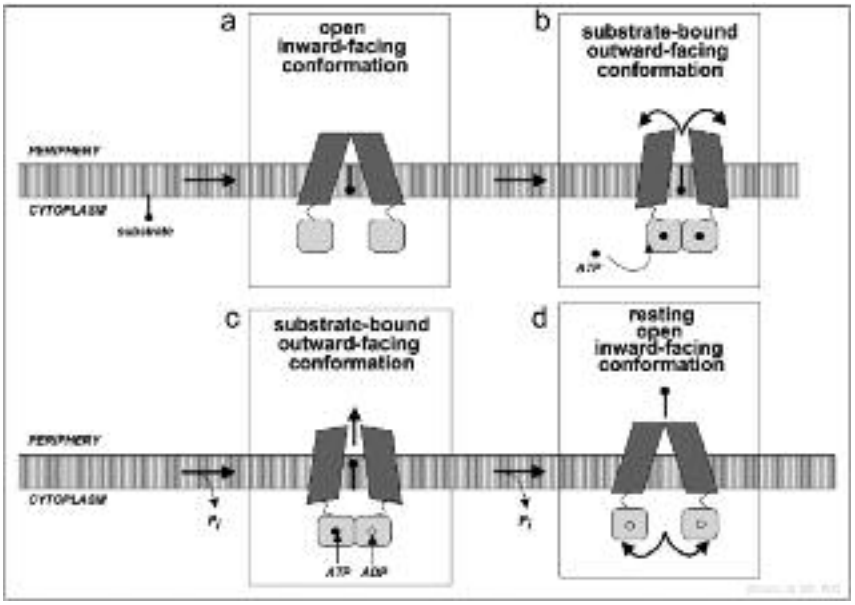


Figure 2
Suspected mechanisms for substrate transport by efflux transporters

4. *Efflux and uptake transporters*

Transporters are membrane proteins that can translocate endogenous compounds (such as bile acids, sugars, amino acids and hormones) and xenobiotics (such as drugs or toxicants) across biological membranes to maintain homeostasis and to detoxify any potentially harmful foreign substances. Transporters are either efflux pumps (*i.e.* they expel xenobiotics out of the cells) or uptake transporters (*i.e.* they transport xenobiotics from biological fluids into the cells). Permeability-glycoprotein (P-glycoprotein, P-gp, ABCB1 or MDR1) is an efflux pump, whereas organic anion transport polypeptides (OATPs), organic anion transporters (OATs) and organic cation transporters (OCTs) are uptake transporters[78] (Figure 2).

With some drugs, the affinity for transporters can confer positive aspects. For example, a high affinity for the P-gp efflux pump (which is also present at the blood-brain barrier (BBB)) might explain the absence of central nervous system side-effects associated with some H1 antihistamines. Differences in the ability of classical and modern antihistamines to interact with P-gp and other transport proteins at the BBB may determine their CNS penetration and as a consequence the presence or absence of central side-effects.[79-84] On the other hand, a high affinity for efflux transporters at the BBB may limit the

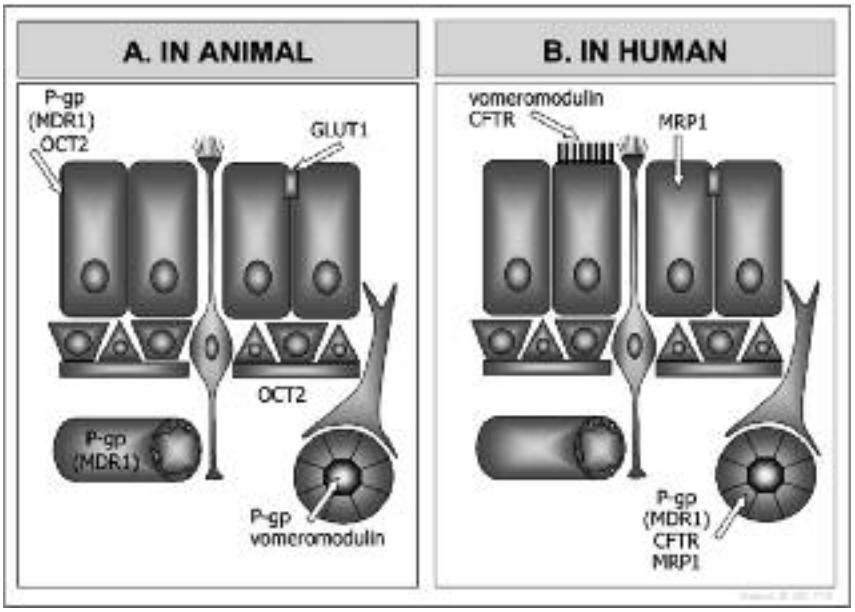


Figure 3
Localisation of transporters in the olfactory mucosa (a) in animals and (b) in humans

effectiveness of a drug when the intended site of action is the central nervous system.

The nasal mucosa and respiratory tract may be an important point of entry for some anti-allergic drugs delivered by intranasal administration or inhalation. Transporters expressed in these tissues may therefore influence the pharmacokinetics of drugs administered in this way.

Drugs can be absorbed in the nasal mucosa and throughout the conducting airway from the trachea down to the bronchioles and ultimately in the distal lung across the alveolar epithelium. However, most agents of pharmacologic interest probably access the brain via the olfactory epithelium, which represents a more direct route of uptake.[85]

In the last 5-10 years, extensive literature has been published about hepatic, renal, intestinal and brain transporters,[78,86] but interesting information concerning the presence of transporters in the nose is increasingly available (Figure 3).

4.1. Transporters in olfactory mucosa

4.1.1. Animals

P-gp is present both in bovine olfactory and nasal respiratory mucosa, but expression seems to be greater in the olfactory epithelium than in the nasal respiratory epithelium.[85] It has also been demonstrated in other species.[87]

P-gp was localised in the epithelial cells, nasal glands, and the vascular endothelium of both the bovine olfactory and nasal respiratory mucosae, and the expressed P-gp was capable of effluxing etoposide. Rates of etoposide efflux were higher in the olfactory mucosa than in the nasal respiratory mucosa and the staining density observed using immunohistochemistry suggests that the expression of P-gp is greater in the olfactory epithelium than in the nasal respiratory epithelium.[88]

Recently, both OCT1 and OCT2 have been localised in nasal mucosa. They may provide a route for the systemic absorption of cationic drugs.[89] A novel putative transporter, mouse OAT6, is expressed predominantly in the olfactory mucosa, but not in the kidney or brain. Sequence comparisons and intron phasing analysis indicate that OAT6 is closely related to OAT1 and OAT3.

In rats, the glucose transporter GLUT1, which mediates the specific transfer of glucose across blood-brain, blood-cerebrospinal fluid and blood-nerve barriers, is abundant in occludin-positive cells. Both GLUT1 and tight junction protein occludin may serve as part of the machinery for the specific transfer of glucose in the olfactory system while preventing the non-specific entry of substances.[90]

Vomeromodulin, a glycoprotein synthesised by the lateral nasal glands, has been proposed as a pheromone transporter and functions as a chemosensory stimulus transporter associated with perireceptor processes in vomeronasal and olfactory transduction.[91] In the rat, vomeromodulin mRNA and protein have been localised in abundance in the glandular acini of the maxillary sinus component of the lateral nasal glands. In addition, the vomeronasal and posterior glands of the nasal septum, the mucus of the sensory and non-sensory epithelia of the vomeronasal organ, and the mucociliary complex of the olfactory, respiratory, and associated rat nasal epithelia also express vomeromodulin mRNA and protein.[92] Finally, in rats, the expression of vomeromodulin is dramatically upregulated by alachlor and butachlor, two chloracetanilide herbicides

that can induce olfactory tumours in rats.[93]

4.1.2. Humans

P-gp is present in human nasal respiratory mucosa, together with other transporters.[94,95] The high-affinity transporter PEPT2 has been found in the respiratory tract and is expressed in the bronchial epithelium and in alveolar type II pneumocytes in human airways.[96] It is possible, therefore, that this may represent a target for the delivery of peptidomimetic drugs and prodrugs. Its expression at the level of upper airways and olfactory mucosa is still under investigation.

In human nasal mucosa, vomeromodulin immunoreactivity is localised in the mucociliary complex of the vomeronasal and respiratory epithelia.[92]

Finally, although monoamine oxidase (MAO) has been reported to be present in human olfactory mucosa,[97] the limited extent of dopamine metabolism should be considered in conjunction with the strong activity of transporters observed at this level.[98] Consequently, when administered intranasally, dopamine can be transported in a nearly intact form through the BBB and then be metabolised exclusively in the central nervous system, which can be seen as the target organ here. These findings should encourage the researchers not to make an over-drastic distinction between their research on local metabolising enzymes and research looking at local transporter activity.

4.2. Drugs used in the treatment of allergic rhinitis and administered by nasal route as substrates/inhibitors/inducers of transporters present in the olfactory mucosa

Despite the presence of a number of protective barriers such as efflux transporters and metabolising enzymes in the olfactory system, lipophilic compounds such as hydroxyzine and triprolidine can access the CNS primarily by passive diffusion when administered via the nasal cavity.[99] It has been reported that the lipophilicity of compounds such as hydroxyzine and triprolidine, coupled with their ability to inhibit P-gp, enable them to permeate freely across bovine olfactory mucosa.[99] It has also been demonstrated that chlorpheniramine and chlorcyclizine are effluxed from the olfactory mucosa by efflux transporters such as P-gp.[100]

Furthermore, it has already been shown that topical steroids (e.g. budesonide) may increase the expression of P-gp in human nasal mucosa.[95]

It would be important to collect additional information, in humans in particular, about whether the drugs used in allergic rhinitis and administered by nasal routes are substrates/inhibitors/inducers of the transporters found in the nose, both to understand their nasal permeability and to predict possible drug interactions.[101,102]

Finally, in addition to transporters, other substances such as ionic surfactants (sodium cholate, sodium taurocholate, Tween 80 and Poloxamer F68) are potentially useful permeation enhancers for the nasal delivery of hydrophilic compounds such as fexofenadine HCl.[103]

Conclusions

Olfactory mucosa has different strategies for defending itself against toxic environments. The regeneration of the epithelium and the roles played by metabolising enzymes and transporters are only partially understood and much fundamental data is missing, especially in humans. Unfortunately, the animal models can only reflect some of the processes engaged by human olfactory mucosa because of the high inter-species variability in the content of metabolising enzymes and xenobiotic transporters. However, a better understanding of olfactory toxification/detoxification or the activation of membrane transporters could serve as a basis for the improvement of existing treatment such as intranasally administered drugs or for the development of novel therapeutic approaches.

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J.-B. Watelet, M.D., Ph.D.
Department of Otorhinolaryngology
Ghent University Hospital
UZ Gent, 1P1
De Pintelaan 185
B-9000 Ghent, Belgium
E-mail: jeanbaptiste.watelet@ugent.be