is not known. One explanation could be that heparin inhibits the toxicity of stimulated PMN by its binding to receptors present on hepatocyte plasma membrane [8]. This explanation seems unlikely since we observed that, when hepatocytes were preincubated with heparin, and then washed before the addition of PMN, they were not protected from PMN toxicity (45.2 ± 2.9% ALT release after preincubation without heparin vs 42.0 ± 7.1% after preincubation with 50 μg/ml heparin). The likely explanation is that the protective effect of heparin is due to its binding to proteinases released by stimulated PMN. Actually, it is well established that heparin is able to bind to and to inactivate proteinases released from human PMN, e.g. chymotrypsin- and elastase-like enzymes [1, 3].

PMN are known to play a major role in acute inflammatory reaction [13], and it has been suggested that heparin, besides its anticoagulant effect, had anti-inflammatory properties [12]. It is tempting to speculate that this effect of heparin might be in part explained by the inactivation of mediators released by PMN.

In summary, the effect of heparin was studied on the proteinase-mediated toxicity of human PMN against isolated rat hepatocytes. Opsonized zymosan-stimulated PMN were markedly toxic to hepatocytes and this cytotoxicity was inhibited by 32 to 55% by concentrations of heparin ranging from 2 μg/ml to 50 μg/ml. This effect was not due to an inhibition of the stimulation of PMN since heparin did not decrease the release of two neutrophil lysosomal enzymes, *N*-acetyl-β-glucosaminidase and β-glucuronidase. It was explained by the inhibition of the post-secretory step of PMN toxicity since heparin reduced by 60% the toxicity of a proteinase-containing PMN lysate and by 69% that of a preparation of PMN granule proteins.

Acknowledgements—We are greatly indebted to Ms L. Rosario for typing the manuscript.

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Biochemical Pharmacology, Vol. 38, No. 11, pp. 1867–1869, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Metabolism of diethylnitrosamine by microsomes of human respiratory nasal mucosa and liver

(*Received* 12 *October* 1988; *accepted* 2 *January* 1989)

Although the incidence of nasal tumors in the human population is low, certain individuals such as the workers in the leather or wood industry experience a higher risk of nasal cancer [1].

Nitrosamines can cause tumors in different tissues including the nasal cavity of experimental rodents [2–4]. They may also have a role in inducing cancer in the human nose as well as other respiratory tracts. The volatile diethylnitrosamine (DEN) present in air, water, foods and tobacco smoke [5] can be inhaled or absorbed from other tissues and readily passed to the nose. In rodents DEN, in addition to other nitrosamines administered i.p., reaches the nose in a few minutes [4] and is metabolically activated via the cytochrome P-450 dependent monooxygenases system [4, 6, 7] in the nasal mucosa.

We have recently described the presence of drug-metabolizing enzymes in human respiratory nasal mucosa [8]. In the present study we have investigated whether DEN can be deethylated by human nasal mucosa microsomal enzymes. The results have been compared with those obtained with microsomal preparations from human liver.

Methods

Nasal microsomes were prepared as previously described [6, 8] from nasal respiratory membranes obtained after surgical removal for therapeutic purposes from male and female humans affected by hypertrophy of the inferior turbinates. Nasal tissues were removed from patients and frozen in liquid N_2 within 10–15 min after removal and stored at −80° until assayed.

Washed microsomes were resuspended in 20% glycerol/Tris buffer (50 mM, pH 7.4), 1 mM EDTA. Wedge biopsies of livers were obtained from patients undergoing cholecystectomy. The surplus of the material required for histological analysis was made available for our studies. Tissue (about 0.5 g) was promptly frozen and stored at −80°.

Liver and mucosa microsomal fractions were prepared as previously described for rat liver [6, 9]. The extent of oxidative deethylation of DEN was determined by measuring acetaldehyde formation by HPLC according to Farrelly [10] as previously reported in detail [6]. Microsomal proteins were assayed by the method of Lowry *et al.* [11].

Results and discussion

Previous studies have shown that the major metabolite of DEN is acetaldehyde in both rodent [6, 7, 12] and human [13] liver. Acetaldehyde is formed by oxidation of DEN at the methylene carbon atom to form the α-hydroxynitrosamine. This compound has a very short lifetime under physiological conditions and it rearranges to ethyldiazohydroxide which can react with cellular constituents or with water to form ethyl alcohol or ethylene [7].

The results summarized in Table 1 show the presence of DEN-deethylase (DENd) activity in microsomes prepared from human nasal mucosa. No acetaldehyde formation was observed either when nasal mucosa microsomes previously boiled for 5 min in a boiling water bath were used in the complete incubation system or when NADPH or its regenerating system was omitted from the incubation mixture.

Table 2 shows the oxidative dealkylation of DEN by liver monooxygenases using two substrate concentrations.

In human liver DENd activities obtained at a concentration of either 1 mM or 50 mM DEN, are similar or higher than those previously reported [13] although our data show a stronger dependence of the rates on the DEN concentration. These data suggest that besides a human P-450j isozyme (inducible by ethanol) reported to work specifically at low DEN concentration [13], DEN is also appreciably metabolized in human liver by other P-450 forms. Comparison between the DEN dealkylation rates in nasal mucosa and liver suggests that the interindividual variation of the rate of acetaldehyde formation in human liver is small, whereas that in nasal mucosa is greater. The observed variation could reflect the different ability of respiratory mucosa to metabolize DEN as has been shown for other substrates of human nasal P-450 dependent monooxygenase [8]. However at low DEN concentration the interindividual variation of DENd was smaller. The low DENd activity at a DEN concentration of 1 mM can indicate that in the respiratory section of human nose a very low amount of P-450j occur constitutively. This P-450j has been suggested to metabolize selectively DEN at low concentration in human liver [13]. By contrast, rabbit nasal mucosa contain a substantial amount of P-450j [14]. Other P-450 forms with less affinity towards DEN would be responsible for DEN metabolism when added at 50 mM. A striking result is the similarity of DENd activity in nasal mucosa and human liver.

Keeping in mind that the P-450 content is reported to be about 0.2–0.8 nmol/mg protein in human liver [15] and 0.026 nmol/mg protein in human respiratory nasal mucosa [8], the DEN-deethylation activity of nasal mucosa if expressed per nmol P-450 is 10–25 times higher than that of human liver. In rabbit [7] and hamster [6] the nasal DENd activity was 9- and 2-fold higher, respectively, than in the liver. By contrast, nasal mucosa and liver of rat showed similar DENd activities [6].

In conclusion we have found that the human respiratory nasal mucosa is able to catalyse the oxidative deethylation of DEN at a rate similar to liver. Since human nasal metabolism in the olfactory region, or in certain specific cells, is expected to be higher than in the respiratory tract by analogy with what has been found for other mammalian species [3], a role of human nasal mucosa in the bioactivation *in situ* of DEN by direct alkylation through the

Table 1. DEN-deethylase activity of human nasal mucosa microsomes[a]

Patient number	Sex[b]	Age (years)	Smoking habits	Rates of CH_3CHO formation at DEN concentrations of	
				1 mM	50 mM
12	F	16	non-smoker	0.07	0.15
13	F	17	non-smoker	0.04	2.46
14	M	30	smoker	0.15	1.01
15	F	24	non-smoker	0.12	0.63

[a] The assay mixture contained respiratory nasal mucosa microsomes (1.5 mg protein/ml) and DEN as indicated in a total volume of 2.5 ml. After 30 min of incubation at 37°, acetaldehyde formed was determined by HPLC and expressed as nmol/mg protein per min.

[b] M: male; F: female.

Table 2. DEN-deethylase activity of human liver microsomes[a]

Patient number	Sex[b]	Age (years)	Rates of CH_3CHO formation at DEN concentrations of	
			1 mM	50 mM
1	F	48	n.d.	0.8
2	M	48	1.3	4.2
3	F	57	0.5	2.1
4	F	63	1.8	4.1
5	F	62	0.6	2.3

[a] The assay mixture contained liver microsomes (0.8 mg protein/ml) and DEN as indicated in a total volume of 2.0 ml. After 30 min of incubation at 37°, acetaldehyde formed was determined by HPLC and expressed as nmol/mg protein per min.

[b] M: male; F: female; n.d. = not determined.

ethylating intermediates or by the formation of the nasal carcinogens acetaldehyde [16] or ethylene seems possible.

Acknowledgements—This work was supported by progetto Finalizzato "Oncologia" of C.N.R.

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Biochemical Pharmacology, Vol. 38, No. 11, pp. 1869–1872, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Gonadotropin-dependent metabolism of 7,12-dimethylbenz(*a*)anthracene in the ovary of rhesus monkey

(*Received* 12 *October* 1988; *accepted* 10 *January* 1989)

7,12-Dimethylbenz(*a*)anthracene (DMBA)* is a potent inducer of skin and breast tumors in the rat. This compound affects several steroidogenic organs in another way. DMBA causes necrosis in the two inner zones of the rat adrenal cortex [1] and in the germinal epithelial cells in the seminiferous tubuli of the testis [2] in this same animal. The adrenal necrosis is dependent on the presence of a fully functional pituitary gland, probably related to ACTH-induced maturity of the middle layer of the adrenal cortex and does not occur in immature animals [3].

Cell death caused by DMBA treatment may be prevented by coadministration of certain inhibitors and inducers of cytochrome P-450 [4]. Antioxidants also prevent this cytotoxicity partially, as well as preventing the extensive cytotoxic effect of 7-hydroxymethyl-12-methylbenz(*a*)anthracene in rat adrenal cell cultures [5], suggesting the involvement of a peroxidative mechanism, generating reactive oxygen, in the development of cellular necrosis. In addition, covalent binding of DMBA-metabolites to adrenal microsomal protein was increased 7-fold in the presence of peroxidase, indicating that, using endogenous hydrogen peroxide, peroxidases may activate

* Abbreviations used: ACTH, adrenocorticotropic hormone; DMBA, 7,12-dimethylbenz(*a*)anthracene; Hepes, *N*-2-hydroxy-ethylpiperazin-*N*-2-ethane sulfonic acid; PAH, polycyclic aromatic hydrocarbons; PMSG, pregnant mare's serum gonadotropin.