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PRELIMINARY COMMUNICATIONS

VARIABLE EFFECTS OF CIGARETTE SMOKING ON ARYL HYDROCARBON HYDROXYLASE, EPOXIDE HYDRATASE AND UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES IN RAT LUNG, KIDNEY AND SMALL INTESTINAL MUCOSA

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Cigarette smoke is known to be carcinogenic for the respiratory tract (1). The smoke consists of many components including aromatic polycyclic hydrocarbons (2) which are metabolized by microsomal aryl hydrocarbon hydroxylase (AHH) to intermediate epoxides. Epoxides can be metabolized further by epoxide hydratase to dihydrodiols or by glutathione S-epoxidetransferase to glutathione conjugates or also converted nonenzymatically to phenols (3). The dihydrodiols can be conjugated with UDP-glucuronic acid (4) or metabolized by AHH to diolepoxides (5). The active intermediates of polycyclic hydrocarbons consist of both certain epoxides and also dioepoxides which react covalently with nucleoproteins and have a high mutagenic activity and probably are responsible for the carcinogenic effects obtained (6-11).

The activity of AHH can be induced several-fold in different tissues (12). Exposure to the cigarette smoke has been reported to induce the AHH activity in the rat lung (13,14) and kidney (14), but the inducibility by the cigarette smoke in the rat bowel and liver has been reported to be inconsistent (14,15).

The mutagenic effect of active metabolites of benzo(a)pyrene, which is also present in cigarette smoke (1), is effectively controlled by epoxide hydratase which metabolizes epoxides to much less reactive dihydrodiols (16). Although epoxide hydratase is functionally and structurally coupled with AHH, recent analyses have revealed that these key enzymes in regard to the chemical carcinogenesis by aromatic polycyclic hydrocarbons are under different genetic control (17,18). The effects of cigarette smoking on epoxide hydratase, inactivating epoxides, and UDP-glucuronosyltransferase catalyzing glucuronidation have not been reported earlier.

The aim of the present study was to investigate the effects of cigarette smoke on the AHH, epoxide hydratase and UDP-glucuronosyltransferase activities in the main extrahepatic drug-metabolizing tissues of the rat. Male adult Wistar rats (weighing 260-300 g) were exposed to cigarette smoke in an inhalation chamber with a total volume of 23 l. Four or five rats were allowed to inhale at the same time the smoke from 10 commercial filter cigarettes (1 mg nicotine and 16 mg tar per cigarette, as guoted by the manufacturer) consecutively during one hour. The air flow through the chamber was controlled to about 16 l/min by a suction pump. Only a small portion of the air passed through the cigarette, which was burnt in 3 min. to 1 cm from the filter. Control rats were exposed to the air flow in an identical chamber, the cigarette being replaced by a filter.

The rats were killed by a blow on the head 12 hr after the exposure, bled and the lungs, kidneys and small intestine removed and cooled in ice-cold aqueous 0.25 M sucrose solution. The small intestinal mucosa were scraped off and homogenized in 0.25 molar sucrose in a Potter-Elvehjem homogenizer. The microsomes were isolated by $CaCl_2$-aggregation (19,20) as described earlier (21). Digitonin treatment of the microsomes was carried out according to Hänninen (22). Protein was determined by the biuret method (23).

The activity of AHH was determined fluorimetrically utilizing 3,4-benzpyrene (Koch-Light Laboratories Ltd., Colnbrook, England) (24) as previously described (25). UDP-Glucuronosyltransferase activity was assayed fluorimetrically with 4-methylumbelliferone (BDH Chemicals, Ltd., Poole, England) as the aglycone (26,27). For the measurement of epoxide hydratase activity, a microassay developed by Oesch et al. (28) was used. The incubation mixture consisted of (final concentrations): 0.5 mM of styrene oxide (Koch-Light) in acetonitrile containing about 10^6 dpm $\left[7\text{-}^3H\right]$ styrene oxide (NEN Chemicals Gmbh, Dreieichenhain, West Germany), 0.10 M Tris-HCl buffer, pH 9.0, containing 0.06% w/v Tween 80, and microsomes in a total volume of 0.3 ml.

The induction of AHH activity in lung was over 6-fold, in kidney, 8-fold and in small intestinal mucosa, 4-fold, when compared with control animals (Table 1.) However, the epoxide hydratase activity increased only in the small intestinal mucosa (2-fold). Despite high induction of AHH in lung and kidney, epoxide hydratase activity was decreased in lung microsomes (54% of the control level), and in kidney it was at control level. Many of the

metabolites of xenobiotics present in the cigarette smoke are capable of being conjugated with glucuronic acid (4). UDP-Glucuronosyltransferase activity increased slightly in the lung and small intestinal mucosa. The pulmonary activity after smoking was 42% and 27% above the control values for native and digitonin-treated microsomes, respectively (Table 2). In small intestinal mucosa the glucuronidation rate appeared to double after the exposure.

Table 1. The effect of exposure to cigarette smoke on aryl hydrocarbon hydroxylase (AHH) and epoxide hydratase activities in microsomes of different rat tissues.

	AHH (pmoles/hr / mg micr.prot.)				EPOXIDE HYDRATASE (nmoles/hr / mg micr.prot.)			
LUNG								
Control	11 ± 2	(5)			10 ± 2	(10)		
Smoking	73 ± 12	(5)	660%[a]	xxx	5,4 ± 1	(10)	54%	x
SMALL INTESTINAL MUCOSA								
Control	16 ± 1	(8)			5,6 ± 1	(10)		
Smoking	63 ± 15	(8)	390%	x	11 ± 1	(10)	200%	xx
KIDNEY								
Control	32 ± 5	(10)			35 ± 2	(5)		
Smoking	270 ± 34	(10)	840%	xxx	40 ± 2	(5)	110%	NS

The means and standard errors of the means are indicated. Numbers of animals are in parentheses. The significances of the differences between control and smoking rats are indicated by the following symbols: xxx = $2P < 0{,}001$, xx = $2P < 0{,}01$, x = $2P < 0{,}05$ and NS = not significant (Student T-test).

[a] Per cent of the control activity.

Table 2. The effect of exposure to cigarette smoke on UDP-glucuronosyltransferase activities in microsomes of different rat tissues (nmoles 4-methylumbelliferone conjugated/hr/mg prot). For explanation see Table 1.

	UDP-GLUCURONOSYLTRANSFERASE					
	(native)			(digitonin activated)		
LUNG						
Control	10,0 ± 1,1	(15)		17,1 ± 1,4	(10)	
Smoking	14,2 ± 2,6	(15)	142% NS	21,8 ± 2,2	(10)	127% $1P<0,05$
SMALL INTESTINAL MUCOSA						
Control	14,0 ± 4,1	(8)		41,0 ± 6,4	(8)	
Smoking	28,1 ± 5,8	(8)	201% $1P<0,05$	40,9 ± 10	(8)	100% NS
KIDNEY						
Control	56,8 ± 3,9	(10)		517 ± 34	(10)	
Smoking	58,4 ± 3,4	(10)	103% NS	578 ± 40	(10)	112% NS

It is of great interest to note that whereas pulmonary AHH activity has highly increased after the exposure of rats to the cigarette smoke, epoxide hydratase activity shows a simultaneous decrease. In spite of the high induction of AHH in the kidney, the epoxide hydratase activity in this organ did not change. Under these circumstances, highly electrophilic epoxides have probably more time to react with nucleophilic macromolecules. Though epoxide hydratase activity was measured with styrene oxide as substrate, the current results are also valid for the hydration of the electrophilic epoxides of polycyclic hydrocarbons, since purified epoxide hydratase preparation is known to catalyze the hydration of both styrene oxide and several K-region arene oxides (29,30). In the small intestinal mucosa, where the induction of AHH was rather low, epoxide hydratase appeared also to be enhanced. Thus the balance between activating - inactivating enzymes after smoking which may result in the protection of the tissues against carcinogenesis was at its

most advantageous in the small intestine rather than the lung and kidney. Our present results provide an addition to the knowledge about the mechanism of the carcinogenic effects of cigarette smoking.

These observations might be explained as follows: only in lungs are the concentrations of the inhibitory components of the smoke high enough to decrease epoxide hydratase activity. These could be for example epoxidized metabolites of smoke constituents. Cigarette smoke could also cause some tissue damage in the lung. Various tissues apparently have different sensitivities to epoxide hydratase induction. Perhaps the components responsible for epoxide hydratase induction cannot be absorbed into the circulation and therefore are not transported to the kidney, whereas those components causing the induction in the small intestine have reached it through the swallowing the smoke.

In any case further studies are necessary to clarify the effect of long-term smoking on AHH vs. epoxide hydratase activities in different tissues. This work is in progress.

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