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Influence of chronic UV-light exposure on hepatic and cutaneous monooxygenases

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Summary. Hairless female Ng/-mice were irradiated by UV-light for 16 h daily over a period of 24 weeks. Monooxygenase activities were measured in liver and skin, and an induction of the aryl-hydrocarbon hydroxylase was detected in liver by both fluorometric and radiochemical methods, whereas no induction of this enzyme could be demonstrated in the skin.

Monooxygenases are membrane-bound enzymes which play an important role in the oxidative metabolism of numerous chemical carcinogens, drugs and lipophilic endogenous substrates. They have been found in the microsomal fractions of different tissues in various mammalian species. Apart from genetic factors[1], environmental agents may also be capable of influencing the levels of monooxygenases and their inducibility.

To our knowledge, the effects of radiation on the activity of different monoxygenases, especially aryl-hydrocarbon hydroxylase (AHH; EC 1.14.14.2), have not yet been published. Tredger and Chhabra[2] postulated that the light cycle may be related to the circadian variations in monooxygenase activities found in the liver, lungs and other extrahepatic tissues of experimental animals. An increased 7-ethoxycoumarin-deethylase(7-EOC-D)-activity was found in the skin of female hairless mice after exposure to short-wave UV-radiation (254 nm) and to a sun-lamp (280–750 nm)[3].

In the present study, we report the effects of chronic UV-light exposure on the activity of cutaneous and hepatic monooxygenases in hairless mice.

Materials and methods. Adult female hairless Ng/-mice (mean weight 33 g) were kept at a constant room temperature of 21 °C and a relative humidity of 55% and were allowed free access to food and water. 10 mice were irradiated for 16 h daily, using assemblies of TL 40/W 09 (Phillips) fluorescence tubes (mean daily doses: UVA = 106 J/cm^2; UVB = 0.62 J/cm^2). A detailed description of the experimental procedure has been published elsewhere[4]. 24 weeks after the start of the experiment, irradiated and nonirradiated control animals (10 mice) were sacrificed by decapitation, and liver and skin microsomes were prepared as previously described[5]. In the microsomal fractions, the following parameters were determined: protein content[6], cytochrome P-450 content[7], 7-EOC-D[8], AHH[9] and aminopyrine-N-demethylase(ADM)[10]. The benzo(a)pyrene metabolism and AHH-activity were studied by the radiometric assay described by Van Cantfort et al.[11].

Results and discussion. The results of our investigation are shown in tables 1 and 2. The values of all measured parameters in the skin microsomes of the irradiated animals revealed no significant differences as compared to controls. On the contrary, in the liver of the irradiated animals, a significant rise in the protein and cytochrome

Table 1. Protein, cytochrome P-450 content and cytochrome P-450-dependent enzyme activities in skin microsomes (values represent a pool of 10 animals) and in liver microsomes (values represent means and SD ($\bar{x} \pm$ SD)) of 8 animals

	Protein[a]	Cytochrome P-450[b] content	7-EOC-D[c]	AHH[d]	ADM[e]
Skin					
Irradiated	2.7	n.d.	0.02	0.2	n.a.
Controls	2.9	n.d.	0.02	0.2	n.a.
Liver					
Irradiated	22.1 ± 0.4	0.78 ± 0.01	2.70 ± 0.66	5.7 ± 0.2	210.0 ± 10.0
Controls	13.1 ± 0.4	0.37 ± 0.02	1.00 ± 0.21	1.6 ± 0.1	66.9 ± 11.2
p-Values	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

n.d., not detectable; n.a., not analyzed. [a]mg × g tissue^{-1}; [b]nM cytochrome P-450 × mg protein^{-1}; [c]nM umbelliferone × min^{-1} × mg protein^{-1}; [d]nM 3-OH-benzo(a)pyrene × 20 min^{-1} × mg protein^{-1}; [e]nM formaldehyde × min^{-1} × g tissue^{-1}.

P-450 content of the microsomes was observed. In addition, the cytochrome P-450-dependent activities of AHH, ADM and 7-EOC-D were also significantly increased. The pattern of the in vitro inhibition of 7-EOC-D by metyrapone and α-naphthoflavone after irradiation was similar to that found in the controls. Finally, the metabolism of benzo(a)-pyrene in the irradiated mice was clearly enhanced, as shown by the increased amounts of benzo(a)pyrene metabolites found in the liver microsomes. These results indicate that, under the experimental conditions described above, UV-irradiation leads to an induction of the hepatic monooxygenases, particularly AHH, whereas it has no apparent effect on the cutaneous monooxygenases. Pohl and Fouts[3] measured an increased activity of 7-EOC-D in the skin of female hairless mice (strain HRS/J); however, they used short-wave UV-radiation (254 nm) or a sunlamp (280–750 nm), and the 7-EOD-activity was the only parameter they determined.

The interpretation of these findings is difficult. The possibility that the enzyme induction in the liver might be due to a direct effect of the radiation on the hepatocytes seems unlikely, though some photons could have reached the superficial parts of the liver through the extremely thin mouse skin. Alternatively, the UV-radiation may have led to the formation of an inducing substance in the skin or in the cellular elements of the peripheral blood, and the enzyme induction could have been mediated by excited states of oxygen in a sequence of molecular events similar to that suggested by Paine to explain monooxygenase induction in liver cell cultures after exposure to light[12]. The apparent lack of AHH induction in the irradiated skin should be considered with caution, since it may be a temporary and not a representative event. Indeed it is possible that the activity of the cutaneous monoxygenases under UV-irradiation also reveals marked fluctuations, as previously reported with regard to other enzymes[13]. Further studies are now warranted in order to define the behavior of monooxygenase activity in the liver and skin at various intervals under UV-irradiation and also after UV-tumorigenesis has developed.

Table 2. AHH-activity in cpm water-soluble ^{14}C-benzo(a)pyrene/mg protein/min, measured in liver and skin of adult female hairless Ng/-mice by radiometric assay

Organ	Control	Irradiated animals
Liver	504.3	1431.5
Skin	71.5	64.3

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Peroxidase activity and thiocyanate accumulation in salivary glands

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Summary. Salivary glands with high, low, or no peroxidase activity do not differ in [$S^{14}CN^-$] after the i.v. injection of $KS^{14}CN$, nor do the glands differ from blood and muscle in [$S^{14}CN^-$]. The content of SCN^- in a salivary gland does not mirror the gland's participation in the peroxidase-mediated antimicrobial mechanism.

Saliva and tears of some mammals contain the thiocyanate ion SCN^-. It is oxidized by H_2O_2 and lactoperoxidase (LP, E.C.1.11.1.7.) to the antimicrobial hypothiocyanite ion $SCNO^-$ [1,2]. This reaction is supposed to occur in the oral cavity, LP being delivered by the saliva and H_2O_2 by the normal microbial flora[3]. The thiocyanate ion is synthesized by the enzyme thiosulphate cyanide sulphotransferase (E.C.2.8.1.1.). The enzyme is found in many, possibly most, tissues but mainly in the liver of the rabbit[4], the rat[4], and the guinea-pig[5]. There is also a high activity in the kidney[4]. In the guinea-pig the activity of the enzyme in the salivary glands has been reported as $\frac{1}{3}$–$\frac{1}{2}$ of that in the liver and the kidney[5]. After the i.v. injection of thiocyanate the major part is excreted via the kidneys and, as CO_2, via the lungs. A small proportion of injected $^{35}SCN^-$ appears as ^{35}sulphate whereas $^{14}CO_2$ accounts for 30% of $S^{14}CN^-$ [6]. There may also be some contribution of SCN^- from hydrolyzed mustard oil and other thiocyanates of alimentary origin.

The extent of coincidence between peroxidase and SCN^-

Table 1. Lactoperoxidase activity in some excretory glands of 3 rodents, recalculated to give $\Delta A_{470} \times sec^{-1} \times g^{-1}$ wet wt. Four animals per group

	Submandibular gland	Sublingual gland	Parotid gland	Lacrimatory gland
Rat[a]	0.4±0.2	0.6±0.2	0.5±0.3	15±1
Guinea-pig	12 ±3	13 ±2	4 ±1	
Rabbit	0	0	0	

[a]Two strains.

Table 2. Mean salivary gland, lacrimatory gland, and muscle [$S^{14}CN$]/Serum[$S^{14}CN$] ratios, after i.v. injection of 10 µCi $KS^{14}CN$/rat and guinea-pig, and 20 µCi $KS^{14}CN$/rabbit. A, 0–25 min after and B, 25–50 min after the injection

	Rat[a]		Guinea-pig		Rabbit	
	A	B	A	B	A	B
Submandibular gland	0.58	0.36	0.37	0.40	0.62	0.79
Sublingual gland	0.36	0.37	0.27	0.36	0.49	0.49
Parotide gland	0.26	0.56	0.32	0.27	0.32	0.35
Lacrimatory gland	0.34	0.31				
Muscle tissue	0.23	0.22	0.39	0.38	0.36	0.32

[a]2 strains.