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INDUCTION OF EPIDERMAL NAD(P)H:QUINONE REDUCTASE BY CHEMICAL CARCINOGENS: A POSSIBLE MECHANISM FOR THE DETOXIFICATION

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NAD(P)H:quinone reductase, which plays an important role in the detoxification of carcinogenic metabolites as well as oxidative cellular damage, was found to be present in epidermal cytosol where its specific activity far exceeds (140-160%) the corresponding hepatic value. The effect of topical application of crude coal tar, 3-methylcholanthrene and polychlorinated biphenyl Aroclor 1254, on epidermal and hepatic cytosolic NAD(P)H:quinone reductase activities was investigated in neonatal rats, Sencar and athymic nude mice. A single topical application of each agent resulted in significant increases in epidermal (185%-389%) and hepatic (150-255%) enzyme activities. This inducible enzyme may play an important role in the detoxification of reactive quinone species during the course of malignant neoplasia and against oxidative cellular damage in skin.

Skin is the largest body organ which serves as a major portal of entry for topically applied drugs and airborne environmental pollutants (1). The metabolism of drugs and other foreign compounds known as xenobiotics, often occurs by more than one sequential reactions (2). The initial reaction is generally oxidative and is catalyzed by the cytochrome P-450 dependent mixed function oxidase system known as phase I reactions (2). The metabolic products may then be further metabolized by conjugation with glutathione, sulfate or glucuronides and reduction mechanisms resulting in detoxification of the reactive intermediates (3). These are termed as phase II reactions. Topical application of several polycyclic aromatic hydrocarbons, nitrated polycyclic aromatic hydrocarbons, polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin to animals (4-7) and of therapeutic preparations containing crude coal tar to animals (8) and humans (9) results in variable induction of several phase I activities in skin and liver. In liver and other extrahepatic tissues increased formation of reactive intermediates of these xenobiotics generated by the phase I reactions are usually detoxified by cytosolic glutathione S-transferase(s) (3). It has

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been shown that glutathione S-transferase in skin is not induced by exposure to the xenobiotics including chemical carcinogens (10). It is therefore possible that other inducible pathway(s) may be present in cutaneous tissue to detoxify the reactive metabolite(s) generated by phase I reactions. Most of the phase I as well as phase II enzymes, which are known to be present in cutaneous tissue generally exhibit catalytic activity at 1-10% of corresponding levels in the liver.

NAD(P)H:quinone reductase (EC 1.6.99.2), also known as DT-diaphorase, is a widely distributed enzyme which promotes two-electron reductions of quinones. This property has led to the suggestion that this enzyme participates in protection against oxidative cellular damage (11,12). Reduction of quinones by NAD(P)H:quinone reductase leads to the formation of rather stable hydroquinones, which subsequently may undergo conjugation reactions (13,14). On the contrary, other flavoproteins which oxidize NAD(P)H, catalyze one-electron transfer leading to the generation of semiquinones which upon autooxidation produce superoxide radical (11). In this communication we report the presence and inducibility of cytosolic NAD(P)H:quinone reductase in rodent skin cytosol and demonstrate that the specific activity of the epidermal enzyme is significantly higher than that in liver.

MATERIALS AND METHODS

Chemicals: 3-Methylcholanthrene was purchased from Aldrich Chemical Co., Milwaukee, WI and polychlorinated biphenyl Aroclor 1254, was from Monsanto, St. Louis, MO. Therapeutic crude coal tar used was of USP grade. 2,6-Dichlorophenol indophenol (DCPIP), flavin adenine dinucleotide, NADPH and Tween-20 were obtained from Sigma Chemical Co., St. Louis, MO. All other chemicals used were of highest purity commercially available.

Animals and Treatment: Pregnant Sprague-Dawley rats were obtained from Holtzman Rat Farm, Madison, WI and were housed in individual plastic cages. Neonatal rats were allowed to suckle until the 4th day. Female Sencar mice (4-6 weeks old) were obtained from NCI-Frederick Cancer Research Facility (Bethesda, MD). Athymic nude mice (Balb/C) were obtained from Harlan Industries, Indianapolis, IN and housed in a pathogen free environment. In case of Sencar mice, they were shaved with electric clippers and Nair depilatory was applied 24 hrs before the treatment. All animals were treated topically with 3-methylcholanthrene (1 mg/10g body wt), Aroclor 1254 (1 mg/10g body wt.) in 0.2 ml acetone or with 0.2 ml crude coal tar. Animals receiving 0.2 ml of acetone alone served as control.

Preparation of Cytosol and Microsomes from Skin and Liver: At desired time points after the topical treatment, the animals were killed and skin and livers removed. The tissues were washed with ice cold 0.15 M KCl containing 10 mM EDTA (pH 7.4). The skin was cleaned of fat by gentle scraping with sharp scalpel blade (Bard-Parker No 20). Dermis and epidermis from the whole skin of neonatal rats was separated as described earlier (15). The epidermis from Sencar and athymic nude mice was separated by immersion in sterile water at 55°C for 30 sec as described earlier (16). The cytosolic and microsomal fractions from tissue homogenates were prepared according to the established procedure described earlier (15).

Enzyme Assays: Aryl hydrocarbon hydroxylase, 7-ethoxycoumarin-O-deethylase, 7-ethoxyresorufin O-deethylase, epoxide hydrolase (towards benzo(a)pyrene 4,5-oxide as substrate) and glutathione-S-transferase (towards benzo(a)pyrene 4,5-oxide as substrate) activities were determined as described earlier (4,5,15). NAD(P)H:quinone reductase activity was determined according to the method of Ernster (17) as modified by Benson et al (18). A typical reaction mixture contained 25 mM Tris-HCl (pH 7.4), 0.7 mg of crystalline bovine serum albumin 0.01% (v/v) Tween-20, 5 μM flavin adenine dinucleotide, 0.2 mM NADPH and an appropriate aliquot of the enzyme source in a final volume of 3.0 ml. Electron acceptor DCPIP (40 μM) in 20 μl of water was added to initiate the reaction and the initial velocity of the reduction of DCPIP was measured spectrophotometerically at 600 nm using a molar extinction coefficient of 2.1 x 10^4 $M^{-1}cm^{-1}$. Protein was determined by the method of Lowry et al (19) using bovine serum albumin as reference standard.

RESULTS AND DISCUSSION

Limited studies have been carried out to assess the capacity of skin to detoxify the toxic metabolites of carcinogens, drugs, and chemicals generated by the phase I enzymes of xenobiotic metabolism. Data in table 1 illustrates that in cutaneous tissue NAD(P)H:quinone reductase is present at levels far exceeding the corresponding liver levels and further show that the enzyme activity is predominantly located in the epidermis. Specific enzyme activities in whole skin, dermis, epidermis and liver are expressed in several ways. When the data are considered as nmol product/min/mg protein, the highest activity occurred in epidermis and followed the pattern epidermis> liver > whole skin > dermis. Similar pattern is observed when enzyme activity is expressed as nmol product/min/g tissue. Prior studies

Table 1. NAD(P)H:quinone reductase activity in whole skin, dermis, epidermis and liver of neonatal rats

	Enzyme Activity		
	Specific activity (nmol DCPIP reduced/ min/mg protein)	Organ activity (nmol DCPIP reduced/ min/g tissue)	Total organ activity (nmol DCPIP reduced/ min/organ)
Whole Skin	23.4 ± 2.5	427 ± 25	624 ± 30
Dermis	10.0 ± 1.3	165 ± 12	248 ± 15
Epidermis	63.5 ± 4.2	1327 ± 40	411 ± 20
Liver	53.5 ± 4.1	511 ± 32	665 ± 50

Tissues from four day old neonatal rats were pooled and processed for subcellular fractionation and enzyme determination. Data represent mean ± S.E. of 4 values. For each value tissues from 4 animals were pooled. DCPIP, dichlorophenol indophenol.

Table 2. Effect of a single topical application of known inducers of mixed function oxidases on epidermal and hepatic NAD(P)H:quinone reductase activities in neonatal rats, Sencar and athymic nude mice

Group	NAD(P)H:Quinone Reductase Activity (nmol DCPIP reduced/min/mg protein)			
	Epidermis	% of control	Liver	% of control
Neonatal Rat				
Control (acetone)	63.5 ± 4.2	100	53.5 ± 4.1	100
Crude Coal Tar	201.6 ± 10.3	315	89.7 ± 5.6	167
3-Methylcholanthrene	240.7 ± 15.2	389	98.8 ± 3.5	185
Aroclor 1254	196.3 ± 12.5	315	132.0 ± 8.4	249
Sencar Mice				
Control (acetone)	47.7 ± 5.7	100	75.7 ± 5.3	100
Crude Coal Tar	149.0 ± 4.9	300	120.3 ± 9.3	160
3-Methylcholanthrene	153.8 ± 13.3	318	180.3 ± 11.7	240
Aroclor 1254	135.3 ± 6.5	281	114.5 ± 7.2	152
Athymic Nude Mice				
Control (acetone)	73.6 ± 5.7	100	107.7 ± 8.7	100
Crude Coal Tar	231.7 ± 4.9	310	196.3 ± 22.1	183
3-Methylcholanthrene	245.3 ± 14.3	312	172.5 ± 12.6	160
Aroclor 1254	139.9 ± 5.6	185	190.2 ± 17.3	177

Four day old neonatal rats, adult Sencar and athymic nude mice were topically applied with 0.2 ml acetone (control), 0.2 ml crude coal tar or 3-methylcholanthrene or Aroclor 1254 (1 mg/0.2 ml acetone/10 g body wt.). Animals were killed 24 hrs after the treatment and enzyme activities determined. For each determination tissues from 4 animals were pooled. Data represent mean ± S.E. of 4 individual values.

from our laboratory have shown that microsomal enzymes of phase I drug metabolizing system are predominantly present in epidermis (15) and this study further suggest that of the two skin compartments epidermis is the major site for the metabolism of xenobiotics. The substantial activity of this enzyme in epidermis may play a key role in protecting this tissue against the toxicity of a variety of reactive metabolites generated by polycyclic aromatic hydrocarbon and other chemicals (12,20).

The effect of a single topical application of 3-methylcholanthrene, Aroclor 1254 and crude coal tar to neonatal rats, Sencar and athymic nude mice on epidermal and hepatic NAD(P)H:quinone reductase activity is shown in table 2. Enzyme activity in the epidermis was significantly elevated in rats and mice by each of these agents. In each case higher induction effects were observed in epidermis than in liver. Maximum induction effects in epidermis occurred 36 hrs after topical application of crude coal tar and 3-methyl-

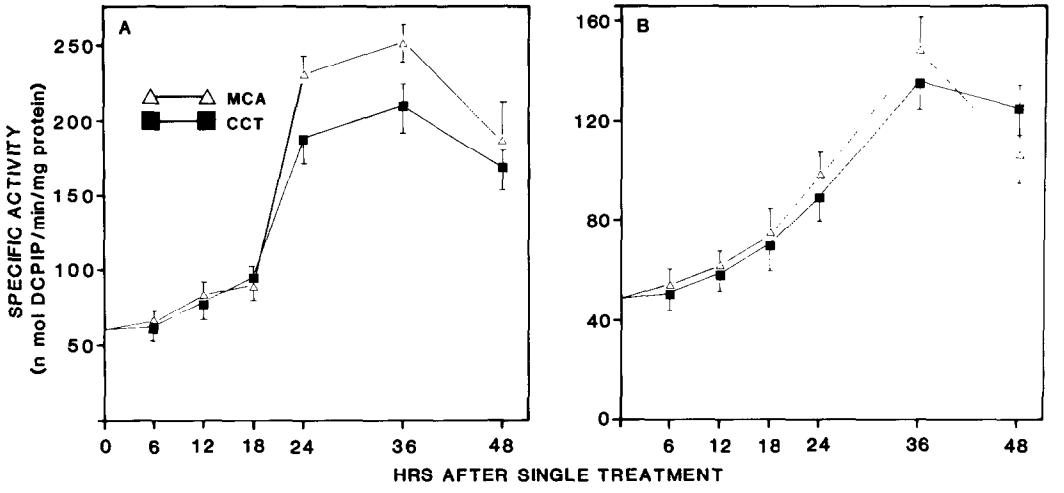

Figure 1. Effect of a single topical application of 3-methylcholanthrene (MCA) and crude coal tar (CCT) to neonatal rats on (A) epidermal and (B) hepatic NAD(P)H:quinone reductase activities. Data represent mean ± S.E. of 4 individual values. For each determination tissues from 4 animals were pooled.

cholanthrene (Figure 1). It has been reported that hepatic cytosolic azo reductase, which is believed to be identical with NAD(P)H:quinone reductase is significantly increased by 3-methylcholanthrene treatment (21). In an earlier study Hommes et al (22) reported increased hepatic NAD(P)H:quinone reductase activity after benzo(a)pyrene treatment of neonatal rats. Our data also suggest that crude coal tar, a complex mixture of polycyclic aromatic hydrocarbons is a potent inducer of skin and hepatic NAD(P)H:quinone reductase activity.

Prior studies have shown that the microsomal fraction of skin contains the heme-protein cytochrome P-450 and that drug metabolizing enzymes such as aryl hydrocarbon hydroxylase, 7-ethoxycoumarin-O-deethylase, 7-ethoxyresorufin-O-deethylase, aminopyrine-N-demethylase, epoxide hydrolase and glutathione-S-transferase(s) are present in cutaneous tissue (4,5,15,23) at a level of 1-10% of hepatic activities. In recent years our laboratory has attempted to characterize the capacity of skin to metabolize drugs and foreign chemicals including carcinogens. These studies have shown that the activity of cutaneous drug metabolizing enzymes are altered by skin exposure to environmental chemicals (15,24,25). The data in table 3 compare the specific activities of phase I and phase II drug metabolizing enzymes in epidermis and liver of neonatal rats. Whereas all enzyme activities

Table 3. Comparative levels of xenobiotic metabolizing enzyme activities in neonatal rat epidermis and liver

Enzyme	Epidermis	Liver	$\frac{\text{Epidermis} \times 100}{\text{liver}}$
Aryl hydrocarbon hydroxylase (pmol 3 OH BP/min/mg protein)	1.16 ± 0.04	56.46 ± 3.52	2.1
7-Ethoxyresorufin-O-Deethylase (pmol resorufin /min/mg protein)	0.42 ± 0.08	6.61 ± 0.70	6.4
7-Ethoxycoumarin-O-Deethylase (pmol 7-HC/min/mg protein)	0.96 ± 0.04	73.82 ± 2.31	1.3
Epoxide hydrolase (pmol BP 4,5 diol/min/mg protein)	163 ± 8	2208 ± 75	7.4
Glutathione-S-transferase (nmol conjugate/min/mg protein)	0.22 ± 0.02	3.68 ± 0.30	6.0
NAD(P)H:Quinone reductase (nmol DCPIP reduced/min/mg protein)	63.5 ± 4.2	53.5 ± 4.1	118.7

Tissues from four day old neonatal rats were pooled and processed for subcellular fractionation and enzyme determinations. For each determination tissues from 4 animals were pooled. Data represent mean ± S.E. of 4 individual values. 3 OH-BP, 3-hydroxy-benzo(a)pyrene; 7-HC, 7-hydroxycoumarin; BP 4,5 diol, benzo(a)pyrene 4,5-diol, DCPIP, dichlorophenol indophenol.

in epidermis ranged from 1-10% of the corresponding liver activities, NAD(P)H:quinone reductase was found to occur at levels far exceeding that in liver.

The induction of the phase I enzymes of the mixed function oxidase system results in the formation of reactive metabolites (2,26) which must be detoxified. Since other detoxification enzymes in skin such as glutathione-S-transferase, are not induced by mixed function oxidase inducers (10,25), any oncogenic reactive intermediate formed by the phase I enzymes must be inactivated or effectively scavenged to abrogate the toxic response. Inducible NAD(P)H:quinone reductase may offer a protective role against these toxic metabolites. Furthermore, other important function of NAD(P)H:quinone reductase is the study of Lind et al (11) which showed that the induced levels of the enzyme significantly inhibited the generation of O_2^- and directed the formation of stable hydroquinones from quinones of ubiquitous environmental pollutant, benzo(a)pyrene. These results re-emphasize that NAD(P)H:quinone reductase is an important cellular control mechanism for preventing the formation of semiquinone and O_2^-.

Skin is a major interface between the body and its environment and is a portal of entry for numerous environmental chemicals, drugs, and other agents, some of which exhibit local toxic and/or tumorigenic effects in cutaneous tissue whereas others have been shown to exert toxic effects in extracutaneous tissues (1). In recent years, it has become more evident that organ-specific toxicity including carcinogenesis is dependent upon metabolic events that occur at the target site. Introduction into cells of extraneous oxidation-reduction labile systems that have the capacity to undergo 1 and 2-electron valency changes may lead to the generation of reactive oxygen species and/or perturb the balance of oxidation-reduction substrates. Consistent with this assumption, it is reasonable to assume that the balance of toxication and detoxication enzyme pathways in the skin would be a major determinant of the availability of the carcinogenic metabolite for initiating tumorigenesis in cutaneous tissue. Thus the inducibility of epidermal NAD(P)H:quinone reductase by the inducers of the phase I enzymes could significantly influence the balance between enhancement of toxicity and detoxification in cutaneous tissue.

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REFERENCES

1. Bickers, D.R. (1980) In: "Current Concepts in Cutaneous Toxicity" (V.A. Drill and P. Lazar, eds) pp. 95-126. Academic Press, New York.
2. Conney, A.H. (1982) Cancer Res. 42, 4875-4917.
3. Bresnick, E. (1980) In: "Enzymatic Basis of Detoxication" (W.B. Jakoby, ed) pp 69-81. Academic Press, New York.
4. Mukhtar, H., Link, C.M., Cherniack, E., Kushner, D.M. and Bickers, D.R. (1982) Toxicol. Appl. Pharmacol. 64, 541-549.
5. Asokan, P., Das, M., Rosenkranz, H.S., Bickers, D.R. and Mukhtar, H. (1985) Biochem Biophys. Res. Commun. 129, 134-140.
6. Pohl, R.J., Philpot, R.M. and Fouts, J.R. (1976) Drug Metab. Dispos. 4, 442-450.
7. Kumaki, K., Jensen, N.M., Shire, J.G.M. and Nebert, D.W. (1977) J. Biol. Chem. 252 157-165.
8. Bickers, D.R., Wroblewski, D., Choudhury, T.D. and Mukhtar, H. (1982) J. Invest Dermatol. 78, 227-229.
9. Bickers, D.R. and Kappas, A. (1978) J. Clin. Invest. 62, 1061-1068.
10. Mukhtar, H. and Bresnick, E. (1976) J. Invest. Dermatol. 66, 161-164.
11. Lind, C., Mochestein, P. and Ernster, L. (1982) Arch. Biochem. Biophys. 216, 178-185.
12. Lind, C., Mochestein, P. and Ernster, L. (1979) in Third International Symposium on Oxidases and Related Redox-Systems. Albany, NY (King, T.E., Mason, H.S. and Morrison, M., eds) Pergamon Press, New York, pp. 313-339.

13. Chesis, P.L., Levin, D.E., Smith, M.T., Ernster, L. and Ames, B.N. (1984) Proc. Natl. Acad. Sci. USA 81, 1696-1700.
14. Talalay, P. and Benson, A.M. (1982) Adv. Enzyme Regul. 20, 287-300.
15. Bickers, D.R., Choudhury, T.D. and Mukhtar, H. (1982) Mol. Pharmacol. 21, 239-247.
16. Das, M., Khan, W.A., Asokan, P., Bickers, D.R. and Mukhtar, H. (1987) Cancer Res. 47, 767-773.
17. Ernster, L. (1967) Methods in Enzymol. 10, 309-317.
18. Benson, A.M., Hunkeler, M.J. and Talalay, P. (1980) Proc. Natl. Acad. Sci. USA 77, 5216-5220.
19. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
20. DeLong, M.J., Prochaska, H.J. and Talalay, P. (1986) Proc. Natl. Acad. Sci., USA 83, 787-791.
21. Haung, M.T., Miwa, G.T. and Lu, A.Y.H. (1979) J. Biol. Chem. 254, 3930-3940.
22. Hommes, F.A., Everts, R.S. and Hanninga, H. (1978) Biol. Neonat. 34, 248-252.
23. Mukhtar, H. and Bickers, D.R. (1981) Biochem. Pharmacol. 30, 3257-3260.
24. Mukhtar, H. and Bickers, D.R. (1981) Drug Metab. Dispos. 9, 311-314.
25. Mukhtar, H. and Bickers, D.R. (1983) Drug Metab. Dispos. 11, 562-567.
26. Jerina, D.M. and Bend, J.R. (1977) In: "Reactive Intermediates: Formation, Toxicity and Inactivation" (Jollow, D., Kocsis, J.J., Snyder, R. and Vainio, H., eds) pp. 207-215, Plenum Press, New York.