PRESENCE AND INDUCTION OF EPOXIDE HYDRASE IN CULTURED HUMAN LEUKOCYTES

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SUMMARY. Epoxide hydrase (EH), a microsomal enzyme, is present and inducible in cultured human leukocytes. Its base levels ranged between 0.05 and 0.20 nmoles of diol among 12 individuals tested. It is inhibited by trichloropropene oxide (TCPO) and cyclohexene oxide (CHO). EH was inducible up to 1.6 times resting levels by 3-methylcholanthrene and up to 2.0 times by phenobarbital in a 24 hr period. Other hydrocarbons--dibenz(a,h)anthracene, benz(a)anthracene and benzo(a)pyrene--gave either weak or no measurable induction. The magnitudes of induction of aryl hydrocarbon hydroxylase (AHH) and EH by the same inducing agent showed a high correlation, suggesting that the mechanism for the induction of the two enzymes is the same.

K-region epoxides have been detected as microsomal metabolites of various polycyclic hydrocarbons such as dibenz(a,h)anthracene (1), phenanthrene, benz(a)-anthracene (2), pyrene, benzo(a)pyrene (3) and 7,12-dimethylbenz(a)anthracene (4). They appear to be products of reactions which are catalyzed by the NADPH-dependent mixed function oxidases. Subsequently, they rearrange to phenols, are converted enzymatically to dihydrodiols and glutathione conjugates (5) and react with macromolecular cell constituents (6,7).

In experiments measuring the levels of binding to proteins, DNA and RNA of hamster embryo cells, the K-region epoxides were bound to the macromolecules to a much greater extent than were the parent hydrocarbons, the phenols, or the cis- and trans-dihydrodiols (7).

In view of these findings and the role that epoxides may play in hydrocarbon carcinogenesis *in vivo*, a study of the epoxide-metabolizing enzymes is of obvious interest. The aryl hydrocarbon hydroxylase (AHH), which produces the epoxides,

can be detected and induced in cultured human leukocytes (8,9). Recent findings (10,11) suggest a coupled monooxygenase-epoxide hydrase system in hepatic microsomes which is inducible by 3-methylcholanthrene (3-MC). Such a system might have some significance to the carcinogenic properties of intermediate epoxides derived from polycyclic hydrocarbons. We therefore undertook to develop a method for assaying for the epoxide hydrase (EH) in a readily accessible human tissue, *i.e.*, cultured leukocytes.

METHODS. Venous blood was drawn from healthy volunteers and added to an equal volume of 4% dextran in normal saline containing 20 units/ml of heparin. None of the tested subjects was taking drugs. After sedimentation of the erythrocytes the leukocyte-rich supernatant was collected, centrifuged and suspended in a tris-buffered ammonium chloride solution (0.83% NH_4Cl + 0.2% tris, pH 7.3) for 15 min at 37° in order to lyse the erythrocytes. After additional centrifugation cells were resuspended in a small volume of culture medium and 3-4 million leukocytes were added to 5 ml of medium in capped culture tubes. Each tube included about 1.5-2 million polymorphonuclear cells, the remainder being lymphocytes and a few monocytes (12). Leukocytes were cultured in modified Eagle's minimal essential medium (F-13) supplemented with fetal calf serum (20%), phytohemagglutinin (1%), pokeweed mitogen (1%) (all from GIBCO) and heparin (60 units/ml).

After incubation for 72 hrs at 37° the cells received 5 µl of 1.5 mM 3-MC in acetone for an additional 24 hrs and were assayed for epoxide hydrase activity by a modification of the method of Oesch *et al.* (13,14). We employed their radiometric assay based on the conversion of [7-^{3}H] styrene oxide to [7-^{3}H] styrene glycol. Incubation mixtures contained 0.2 ml of 0.5 M tris buffer (pH 9.0) with 0.1% (w/w) Tween 80, water to make the final volume 0.4 ml, 0.05 ml acetonitrile containing 2 µmole of [^{3}H] styrene oxide (1.4×10^6 dpm) and 0.1 ml cell preparation. After incubation for 15 min at 37°, unreacted product was extracted into hexane and petroleum ether. Finally the glycol was extracted into ethyl acetate for assay by scintillation spectrometry.

Assay values presented here were corrected for recovery (80-85%) of

enzymatically synthesized [^{3}H] styrene glycol. Nonenzymatic conversion of oxide to glycol was neglected in the calculations of assay values since it accounted for less than 10 per cent with 15 min incubation time. Specific activity is expressed in nmoles styrene glycol formed in 5 min per mg N (13).

AHH in cultured leukocytes from the same subjects was assayed by a modification of the method of Nebert and Gelboin as previously described (15).

RESULTS AND DISCUSSION. Epoxide hydrase is present and inducible in cultured human leukocytes. Though its activity is low it is still measurable. The base levels of 12 individuals checked for epoxide hydrase activity in their leukocytes ranged between 0.05 and 0.20 nmoles of diol. The enzymatic reaction was linear for at least 20 min and was inhibited by 2.0 mM 1,1,1-trichloro-2-propene oxide (TCPO) (10) and 2.0 mM cyclohexene (CHO) (16); both are potent inhibitors of epoxide hydrase. Further, it exhibited linear kinetics with regard to cell concentration. The characteristics of the cell system used to detect the hydrase enzyme are the same as for the AHH (12). The lymphoblast is probably the principal cell type responsible for EH-activity since the majority (80-90%) of the cells counted after 72 hrs of incubation were the larger blast cells. This is further evidenced by the requirement for mitogen. EH activity could not be detected in leukocytes from freshly drawn blood. The cultured cells were only responsive to EH induction after 48-72 hrs of incubation, paralleling the appearance of blast cells in the cultures. EH activity was also detectable (0.5-5 nmoles/mg N/5min) in human placental tissue (unpublished observations).

The most potent inducers for EH in cultured leukocytes were 3-MC (1.5 μM) and phenobarbital (2.0 mM). Other hydrocarbons--dibenz(a,h)anthracene, benz(a)anthracene, chrysene, benzo(a)pyrene--were even at high concentrations either weak inducers or gave no measurable induction in a 24 hr period. The extent of induction by 3-MC varied between 1.1 and 1.6 times control levels, while phenobarbital produced from 1.2 to 2.0 times among 12 subjects tested. These results are in good agreement with those obtained by Oesch *et al.* (13) who found an increase of 150% in EH activity by pretreatment of male rats with 3-MC, and of 300% by pre-

treatment with phenobarbital. In contrast, no significant differences were found in the hepatic EH activities of control and 3-MC treated inbred or hybrid mice (17).

Some of the subjects were tested for EH induction in cultured leukocytes repeatedly over a period of several weeks. The deviations in extent of induction were less than 10% for leukocytes from the same individuals, probably indicating, as for AHH, a genetic control of inducibility.

The observed variations in extent of induction of EH activity were, as we have shown for AHH (15), not due to different culture conditions or different batches or concentrations of mitogens. Unlike the base levels, which varied according to culture conditions, the magnitudes of induction were relatively constant for the same individual.

Inducibility of AHH by 3-MC shows genetic variation in man (9). We therefore undertook to compare the inducibilities of EH and AHH by 3-MC in the cultured leukocyte system. Our data show a high correlation (>.98) between the two magnitudes. No exception was found thus far, suggesting that the extent of induction for these two enzymes or of the coupled monooxygenase-hydrase system is under the same genetic control. Studies are in progress to elucidate these findings and to determine their genetic basis.

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