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3-METHYLCHOLANTHRENE INDUCIBILITY OF ARYL HYDROCARBON HYDROXYLASE IN CULTURED HUMAN LYMPHOCYTES DEPENDS UPON THE EXTENT OF BLAST TRANSFORMATION

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Aryl hydrocarbon hydroxylase (AHH) is an enzyme of the microsomal mixed function oxidase system which is involved in the metabolism of various compounds including drugs, carcinogens, steroids and insecticides. Freshly isolated human lymphocytes do not contain measurable amounts of AHH activity; however after culture in the presence of mitogens, the resulting blast cells contain appreciable amounts of AHH, which is further inducible by polycyclic aromatic hydrocarbons such as 3-methylcholanthrene (3-MC)[1]. It has been suggested that the induction of AHH in cultured human lymphocytes by 3-MC is under the control of a single gene (2 alleles)[2] and that inducibility may be used as an index of susceptibility to chemical carcinogenesis[3] and as a measure of the genetic contribution to rates of drug metabolism[4]. We and others[5] have found a lack of reproducibility of induction ratios after a fixed time in culture. Our culture conditions were such that we measured induction ratios up to 3 fold greater than previously reported[2,6,7]. We now report evidence that the extent of induction of AHH as measured in lymphocyte culture varies with the length of culture and is directly dependent upon the blastogenic state of the cells at the time of harvest.

The 3-MC induced AHH activity was followed at daily intervals over a period of 2 to 7 days in culture in lymphocytes from 4 normal healthy male individuals aged between 29 and 35 years. After isolation on a hypaqueficoll gradient, peripheral venous lymphocytes were suspended at approximately 1.2×10^6 cells/ml in RPMI 1640 medium (Commonwealth Serum Laboratories, Melbourne) containing 5µg/ml phythohaemagglutinin (Burroughs-Wellcome), 1% pokeweed mitogen (v/v) (GIBCO) and 10% heat inactivated homologous AB human sera. For each time point, six 5 ml cultures (3 control, 3 induced) were set up and incubated at 37°C in

5% CO_2. Twenty-four hours prior to harvest, 1.5 µM 3-MC in methanol (or 10 µl methanol only) was added. Cells were collected by centrifugation, washed and resuspended in 1 ml of 50 mM tris-HCl buffer, pH 7.5, containing 3 mM $MgCl_2$ and 0.2 M sucrose[7]. After disruption by freeze thawing or ultrasonication, the cells were incubated at 37°C in the dark for 60 minutes with 25 µg benzo (a)pyrene (BP) (Sigma) and 1 mg NADPH (Sigma). The reaction was stopped by the addition of 4 mls of a cold acetone hexane solution (3/1) and the alkali soluble products of BP were measured fluorometrically at activation and emission wavelengths of 466 nm and 522 nm respectively[8]. The enzyme activity was expressed as pmoles of 3 OH BP/10^6 viable cells/min. DNA synthesis as measured by ^{3}H-thymidine incorporation was measured simultaneously in the blast transforming cells by the addition of 1µCi ^{3}H-methyl thymidine (TRK 120, Radiochemical Centre) to 0.2ml aliquots (performed in quintuplicate) of the initial suspensions (initially 1.2 x 10^6 cells/ml) for 24 hours before harvesting. The cells were then collected on fibre glass filters (GFA Whatman) using an automatic harvester (Otto Hiller), washed automatically three times with saline, precipitated with 5% trichloroacetic acid, dried with methanol, and counted in a toluene based scintillant.

The control AHH activity in cells from all 4 individuals varied little with increased time in culture, while the 3-MC induced AHH activity increased sharply to a maximum (Fig. 1). This maximal activity occurred after widely variable times in culture for lymphocytes from different individuals (Fig.2). Therefore, the time of assay relative to the time of maximally induced AHH activity considerably alters the observed extent of AHH induction.

During culture, DNA synthesis also increased sharply to a maximum,and for each individual this occurred at the same time as maximally induced AHH enzyme activity (Fig.3). Therefore the variation in the extent of induced AHH activity for different individuals reflects variation in the degree of blast transformation. Alteration in the extent of blast transformation of lymphocytes from an individual when re-studied would cause an apparent alteration of enzyme induction and would explain in turn a change in induction ratio. Whether this degree of blast transformation is an inherent property of the individual's lymphocytes or whether it reflects variation in assay conditions remains to be determined.

In view of these results, the observed induction of AHH by 3-MC in cultured human lymphocytes depends largely on the degree of blast transformation of the

cells at the time of harvest. Therefore, the variation due to time and extent of blast transformation must be standardised if the degree of inducibility in cultured lymphocytes is to be used as a genetic marker for chemical carcinogenesis and drug metabolism.

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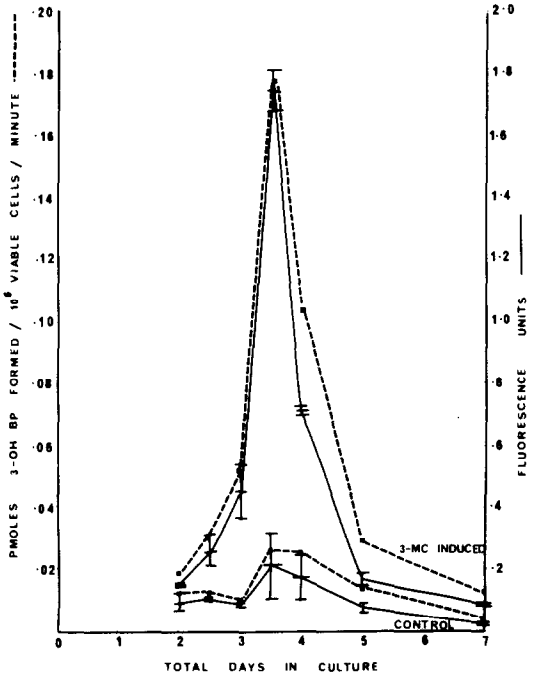

Fig. 1. AHH activity (—fluorescence units, -----pmoles 3-OH benzo(a)pyrene formed/10^6 viable cells/minute) during increased time in culture in blast transformed lymphocytes from a single individual. 3-MC (1.5 μM) was added 24 hours prior to harvest. AHH activity was assayed fluorometrically at excitation and emission wavelengths of 466 nm and 522 nm respectively. Each time point on the unbroken lines represents mean ±S.D. of triplicate cultures. The broken line represents conversion of fluorescence units to pmoles 3 OH BP from a standard curve and expressed as a function of viable cell numbers (as measured by eosin exclusion)/minute of assay. Since there was little change in AHH activity for control cells, the changes in induction ratio closely followed observed changes in 3-MC induced AHH activity.

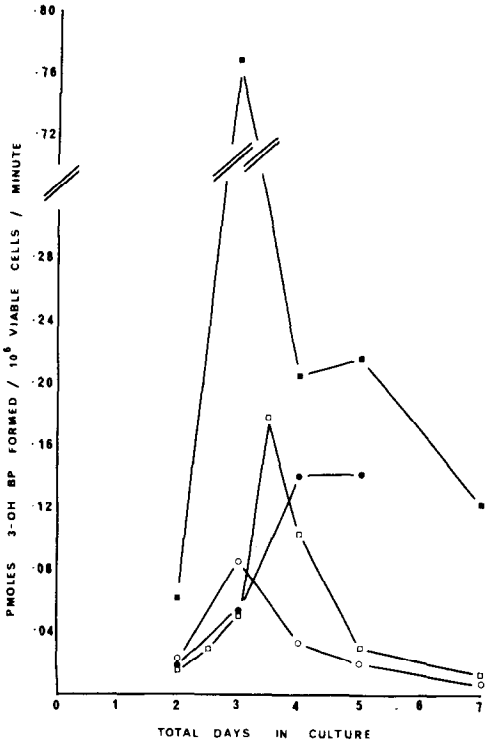

Fig. 2. 3-MC induced AHH activity in cultured lymphocytes from all four individuals studied. Axes are as for Fig. 1. The pattern of AHH activity in control cultures in each individual was similar to that in Fig. 1.

■ individual No. 1; O No. 2; ▫ No. 3; ● No. 4.

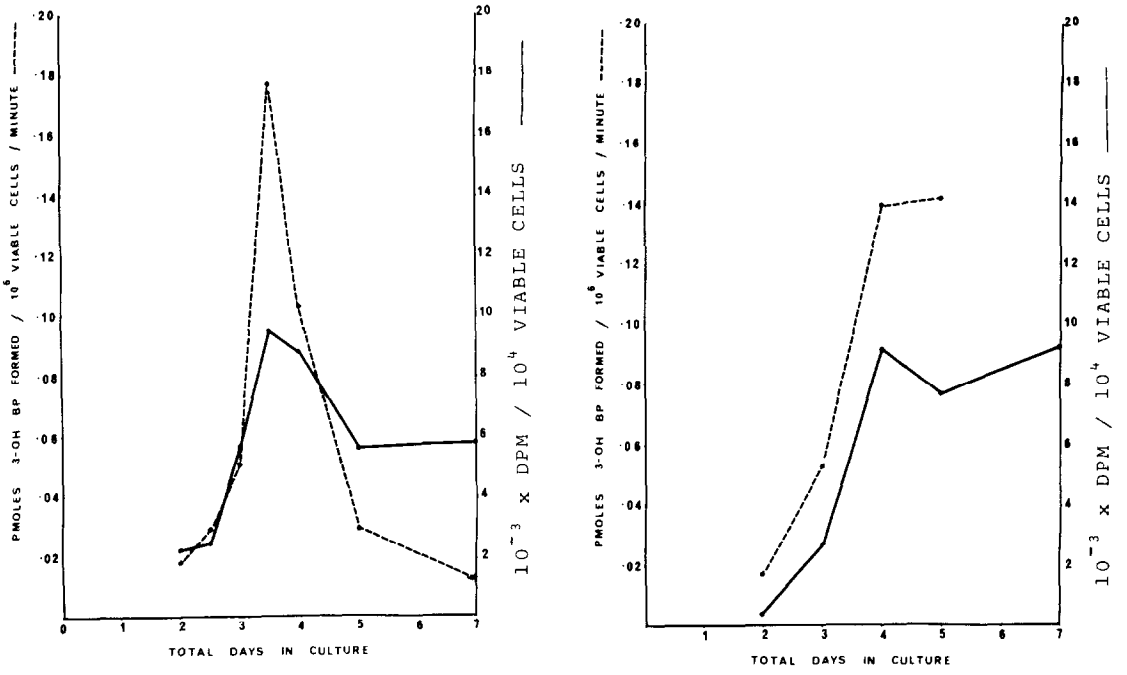

Fig. 3. Comparison of 24 hour ^{3}H-thymidine incorporation (continuous line) and the corresponding 3-MC induced AHH activity (interrupted line) in cultured cells from different individuals. Both parameters are plotted as a function of total time in culture; ^{3}H-thymidine and 3-MC were added simultaneously 24 hours prior to harvest. (a) and (b) are results from individuals No.3 and No. 4 respectively - 2 individuals with widely different times of expression of maximal 3-MC induced AHH activity.
Each time point on the AHH activity plots represents mean of triplicate cultures; each time point on the ^{3}H-thymidine incorporation figures represents mean of 5 identical cultures.

REFERENCES

1. J.P. Whitlock, H.L. Cooper and H.V. Gelboin, Science 177, 618 (1972)
2. G. Kellermann, M. Luyten-Kellermann and C.R. Shaw, Amer. J. Hum. Genet. 25, 327 (1973).
3. G. Kellermann, C.R. Shaw and M. Luyten-Kellermann, New Engl. J. Med. 289, 934 (1973).
4. G. Kellermann, M. Luyten-Kellermann, M.G. Horning and M. Stafford, Clin. Pharmac. Ther. 20, 72 (1976).
5. S.A. Atlas, E.S. Vesell and D.W. Nebert, Cancer Res. 36, 4619 (1976)
6 H.L. Gurtoo, N. Bejba and J. Minowada, Cancer Res. 35, 1235 (1975)
7. D.L. Busbee, C.R. Shaw and E.T. Cantrell, Science 178, 315 (1972)
8. K.M. Robie, Y.M. Chas, R.E. Talcott and J.B. Schenkman, Chem.-Biol. Interactions 12, 285 (1976)