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# INDUCTION OF ARYLHYDROCARBON HYDROXYLASE AND BLAST TRANSFORMATION IN HUMAN BLOOD LYMPHOCYTES

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Summary: Variation in arylhydrocarbon hydroxylase(AHH) inducibility in human peripheral blood lymphocytes was studied. AHH inducibility and the degree of blast transformation were determined simultaneously using [$^3$H]benzo(a)pyrene and [$^3$H]thymidine, respectively. AHH inducibility in terms of induction ratio(induced level to basal) or induction ratio per unit of blast transformation varied at different culture time and at different phytohemagglutinin concentrations within the same individuals. However, the ratio of absolute induced AHH activity and unit of blast transformation gave persistent value for the same individuals, indicating that AHH inducibility of human lymphocytes should be expressed in this manner in the study of cancer susceptibility.

## INTRODUCTION

Kellermann et al.(1) have reported that high inducibility of arylhydrocarbon hydroxylase(AHH) in peripheral blood lymphocytes was significantly more frequent among the patients with lung cancer than among the healthy individuals. Confirmation of this finding had been the subject of intense study in several laboratories(2,3), but most of the recent studies failed to confirm this observation(4-7). Since the difficulties encountered in these studies were the lack of reproducibility of AHH activity, efforts have been made to minimize the variation in the assay(8,9). However, even with improved assay system, reproducibility is still a major problem at present.

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Abbreviations used are: AHH, arylhydrocarbon hydroxylase, FCS, fetal calf serum, BA, benzanthracene, PHA, phytohemagglutinin, DMSO, demethyl sulfoxide, TCA, trichloroacetic acid, B(a)P, benzo(a)pyrene.

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One of the important factors influencing the reproducibility might be mitogen response, for the use of mitogen stimulated lymphocytes could not be excluded. Several reports have indicated the dependency of AHH inducibility on the extent of blast transformation of lymphocytes(7,9,10). If this is the case, some measure of the degree of blast transformation must be incorporated in any attempts to standardize the AHH assay in human lymphocytes.

For this purpose, we have studied the correlation between the blast transformation and AHH inducibility under different culture conditions. Standardization of the AHH assay was first done using radiometric method instead of the conventional fluorometric method. The measure of the extent of blast transformation was incorporated in the assessment of AHH inducibility to discover whether such expression of AHH inducibility might give a persistent value for the same samples from an individual.

## MATERIALS AND METHODS

Lymphocyte culture. Lymphocytes were separated from human venous heparinized blood by Ficoll-Paque(Pharmacia Fine Chem.,Upsala,Sweden) gradient solution sedimentation(11). The cells were cultured in RPMI 1640 medium containing 10% fetal calf serum(FCS), antibiotics and phytohemagglutinin (PHA, GIBCO,U.S.A.) and in a 5% $CO_2$-atmosphere at 37°C. Benzanthracene(BA, 2 µg/ml-medium) was added 24 hrs before enzyme assay for AHH induction. The cells were harvested by centrifugation at 1,200 rpm for 5 min and were resuspended in a fresh medium. Aliquots of the cell suspension were used for AHH assay and [$^3$H]thymidine incorporation.

AHH assay. AHH assay was performed by our modification of the radiometric method described by Cantfort et al.(12). Standardization of the method was done in respect of the inducer type, time dependency and cell concentration. The reaction was started by the addition of 1 µCi of [$^3$H]benzo(a)pyrene(100 mCi/mmole, The Radiochemical Centre,Amersham,England) to the lymphocytes culture in 1 ml of RPMI 1640 with 10% FCS. The incubation was done for 5 hrs at 37°C and stopped by the addition of 2 ml of KOH(0.15 M in 85% DMSO). Unmetabolized substrates were extracted with 5 ml of hexane three times. An aliquot(750 µl) of remaining aqueous layer was acidified, diluted with 10 ml of Insta Gel(Packard Instrument,S.A.,Rungis,France) and its radioactivity was counted in a scintilation counter. AHH induction ratio was calculated as the ratio of AHH levels in BA-induced cells and basal levels.

Assay of [$^3$H]thymidine incorporation. Lymphocytes in 1 ml of RPMI 1640 medium with 10% FCS were exposed to 1 µCi of [$^3$H]thymidine (26 Ci/mmole, C.E.A., France) for 2 hrs at 37°C. The reaction was stopped by adding 1 ml of ice-cold RPMI 1640 medium and the cell cultures were centrifuged at 1,200 rpm for 10 min. The incorporation of [$^3$H]thymidine was determined by glass fiber method(13). After washing the filters(Whatmann GF/C,24 mm) with 5% TCA-0.05 M tetrasodium diphosphate and ethanol, the filters were put into

counting vials, 0.5 ml of Soluene(Packard Instrument,S.A.) was added and was stand for 60 min at room temperature. A scintilation mixture Dimilium-30 (Packard Instrument,S.A.) was added to the vials and counting was done in a scintilation counter.

## RESULTS

Standardization of AHH assay and [$^3$H]thymidine incorporation.

Under the conditions described in the Materials and Methods section, AHH activity was linear with respect to time of incubation(0 to 5 hrs and 0 to 8 hrs for BA-induced and non-induced activities, respectively, with $1x10^6$ cells/ml) and with respect to cell concentration($0.25x10^6$ to $1.5x10^6$ cells/ml at 5 hrs incubation for both of the cell cultures). As for [$^3$H]thymidine incorporation, linearity was observed with time (0 to 2 hrs for PHA-stimulated cells with $0.5x10^6$ cells/ml) and with cell concentration ($0.2x10^6$ to $1.0x10^6$ cells/ml for 2 hrs incubation).

AHH inducibility and [$^3$H]thymidine incorporation in time-course study.

Lymphocytes were cultured at a cell concentration of $1x10^6$ cells/ml in a medium with 1% PHA. AHH activity and [$^3$H]thymidine incorporation were determined with BA-induced and non-induced cells as a function of culture time up to 5 days. AHH activity and [$^3$H]thymidine incorporation reached their maximum around 3 days of culture in most of human blood samples. The induction ratio(induced to basal) of AHH was varied in lymphocytes from the same individuals as the time of culture progressed.

In Fig. 1 are shown two representative experiments. Correlation was not obtained between AHH induction ratio and [$^3$H]thymidine incorporation(Fig.1a) but a close correlation was obtained between AHH induced activity and [$^3$H] thymidine incorporation(Fig.1b). These indicate that AHH inducibility when expressed in terms of the induced activity per unit of [$^3$H]thymidine incorporation but not of AHH induction ratio per unit of [$^3$H]thymidine incorporation gives persistent value within the same individuals.

AHH induced activity and [$^3$H]thymidine incorporation in different PHA stimulation. Lymphocytes were cultured in medium containing different concent-

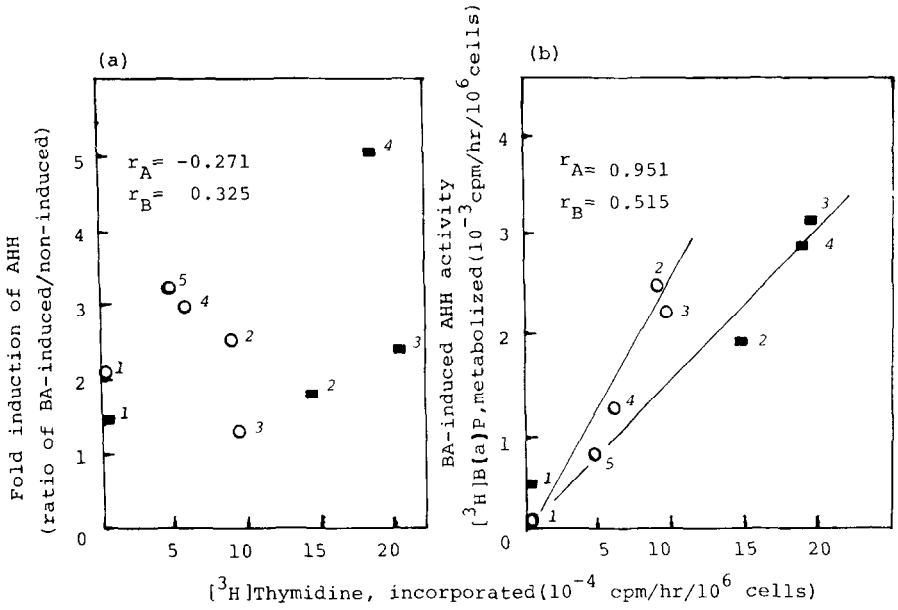


Fig. 1. Relationship between AHH induction ratio(a) or BA-induced activity(b) and the degree of [$^3$H]thymidine incorporation. Lymphocytes from subject A (○) and B (■) were cultured at the presence of 1% PHA. Each point represents AHH activity(induction ratio or induced activity) and [$^3$H]thymidine incorporation with numbers indicating the day of culture. Correlation coefficients are indicated as $r_A$ and $r_B$ for subject A and B, respectively.

rations of PHA(0.2% to 2.0%). On day 2 and 3, BA was added and AHH activity and [$^3$H]thymidine incorporation were determined on day 3 and 4(Fig.2). In 3 day's culture, induced activity of AHH and [$^3$H]thymidine incorporation increased as the PHA concentration increased. On the contrary, in 4 day's culture, both parameters rather decreased as the PHA concentration increased (Fig.2a). In both cultures, close correlation was also obtained between the AHH induced activity and [$^3$H]thymidine incorporation under the different concentrations of PHA.

## DISCUSSION

The lack of reproducibility in the study of AHH inducibility in human peripheral lymphocytes is caused by many factors(8,9). One of the factors might be the assay method of AHH. Most workers(1-6,9) used fluorometric assay, which has several defects such as a rapid decomposition of the meta-

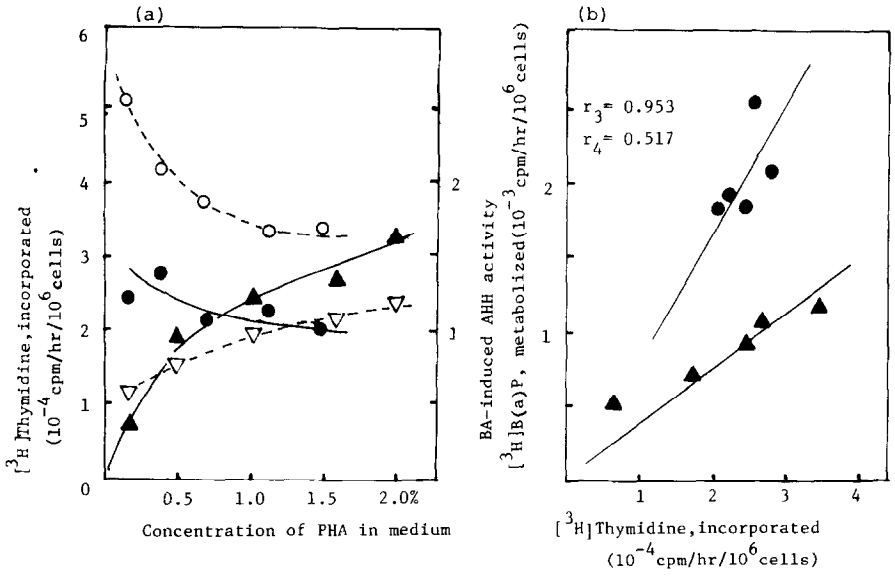


Fig. 2. Effects of PHA concentration on induced AHH activity and [$^3$H]thymidine incorporation. Lymphocytes from subject C(▲▽) and D(● ○) were cultured at different concentrations of PHA for 3 days and 4 days, respectively. BA-induced AHH activity(▽ ○) and [$^3$H]thymidine incorporation(▲● ) are plotted(a). The correlation between these two parameters are shown (b) with correlation coefficients $r_3$ and $r_4$ for 3 day's and 4 day's culture.

bolic products, determination of only a part of the metabolites which are non-carcinogenic,etc.(14). Some workers(7,15,16) have employed radiometric assay for cultured cells but the incubation as long as 1 to 3 days(15,16) was required. Values obtained by such a long period-incubation sometimes fails to reflect true enzyme levels *in situ* , especially in case of mitogen induced lymphocytes that might change their enzyme levels in the course of culture. We have standardized the method of Cantfort *et al.*(12) for the measurement of human lymphocyte AHH activity and could measure even basal levels with maximum of 5 hrs incubation time.

One of the other factors influencing the reproducibility of AHH assay might be the degree of mitogen-induced blast transformation of lymphocytes (7,9,10). We have first expressed AHH inducibility in terms of AHH induction ratio per unit of blast transformation, which would give a persistent value of AHH inducibility if the dependency of AHH induction on mitogen response exists. The results of time-course study(Fig.1), however, demonstrated that

neither AHH inducibility thus expressed nor the simple ratio of induced AHH levels to basal levels gave a persistent value for the same individuals. Further, PHA-stimulation study(Fig.2) has demonstrated that lymphocytes responded to the contrary manner in respect of PHA concentration when BA-induction was performed before and after the occurence of the peak AHH activity. These indicate that cell responsibility to AHH inducers and mitogens might change in the course of culture and as a function of PHA concentration. The facts that the peak activity of AHH induction and blast transformation occur over the narrow range of time and after variable times in lymphocyte culture raised the difficulties in measuring these parameters in the same stage of mitogen response of lymphocytes from different individuals. The difficulties arefurther substantiated by the facts that mitogen-induced transformation of lymphocytes was found altered in patients with lung cancer(17-20), under bacterial and viral infections(21,22) and under drug treatment(23).

The present study, on the other hand, has shown that BA-induced activity of AHH was closely related to the degree of blast transformation under various culture conditions. Hence we could conclude that AHH inducibility when expressed as the ratio of AHH induced activity and the degree of mitogen response would give a persistent value for each individual's sample and the study of the AHH inducibility in human peripheral lymphocytes in respect of susceptibility to lung cancer, if it exists, should be done using the parameter thus expressed.

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