Biochemical Pharmacology, Vol. 34, No. 3, pp. 385–386, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

THE BINDING OF THE BENZO/A/PYRENE METABOLITES TO THE DNA OF ISOLATED RAT LIVER NUCLEI AND NUCLEAR MATRIX

W.Baer-Dubowska, K.Alexandrov [x/]

Department of Biochemistry, Academy of Medicine
ul.Grunwaldzka 6, 60-780 Poznań, Poland
[x/]Institut de Recherches Scientifiques sur le Cancer
BP N° 8, 94802 Villejuif, France

INTRODUCTION

The formation of benzo/a/pyrene /BP/ : DNA adducts is the result of the action of the cytochrome P-450 monooxygenase system and related enzymes, which are inducible by different compounds. This activation and induction takes place both in the endoplasmic reticulum and in the nucleus. No information is available concerning the comparison of the DNA bound adducts in the isolated nuclei from livers of 3-methylcholanthrene /MC/ pretreated /induced/ and untreated /uninduced/ rats. The preferential binding to matrix DNA has been detected in vivo /1/. Similar results were observed in vitro with rat liver nuclei from induced rats /2,3/.

In this study the nature of modified nucleosides formed by uninduced and induced nuclei and the amount of modified nucleosides in the nuclear subfractions of uninduced and induced nuclei with special reference to the matrix DNA has been compared.

RESULTS AND DISCUSSION

Incubation of [^{3}H] BP with isolated rat liver nuclei resulted in the binding of BP-derived species to nuclear DNA, as shown in Fig. 1.

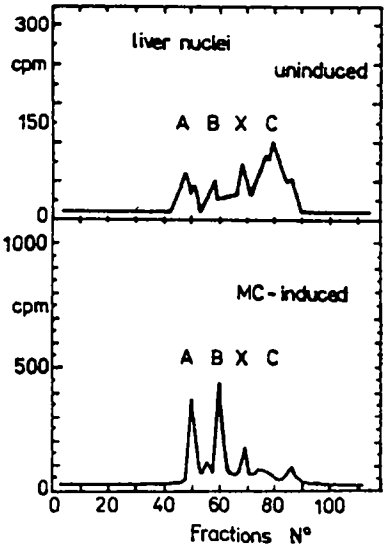

Fig.1. HPLC analysis of BP:DNA adducts formed in uninduced and MC-induced nuclei. Nuclei / average 10 mg DNA / were incubated with [^{3}H] BP / 4.8 Ci/mmol ; 200 nmol and NADPH/10 mg / in 10 ml of 0.25 M sucrose buffer / 0.05 M Tris-HCl pH 7.4 , 5 mM $MgCl_2$ / for 30 min. The isolated DNA was hydrolysed enzymically to deoxyribonucleosides, which were purified on short Chrompack C18 columns. Unhydrolysed DNA and unmodified bases were removed by elution with water. The less polar hydrocarbon-deoxyribonucleoside adducts were eluted with methanol and analysed by HPLC using an Altex ultrasphere ODS column and linear gradient of 40-100% water:methanol at a flow rate of 1 ml/min .

In both uninduced nuclei and these obtained from pretreated rats the nature of adducts was identical, however pretreatment with 3-methylcholanthrene resulted in a quantitatively higher level of binding. The major hydrocarbon-deoxyribonucleo-

side adduct found in the MC-induced nuclei cochromatographed with that obtained from the reaction of diol-epoxide (±) 7β,8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydrobenzo/a/pyrene /BPDE I/ with DNA /peak B/. Smaller amounts of other adducts were also present including: a/ a more polar product which cochromatographed with 9-hydroxybenzo/a/pyrene-4,5-oxide adduct /peak A/, b/ that obtained following reaction of 4,5-benzo/a/pyrene oxide with DNA /peak C/ and c/ unknown product /peak X/. The major DNA-bound product found in the control nuclei cochromatographed with that obtained following reaction of 4,5-benzo/a/pyrene oxide with DNA. The incubation of liver nuclei with [^{3}H] BP resulted also in a much higher total binding level to the matrix-bound DNA in comparison with the other subnuclear fractions, both in the uninduced and MC-induced nuclei /Table I/. Similar results were obtained concerning the amounts of modified deoxyribonucleosides.

Table I. [^{3}H] Benzo/a/pyrene covalently bound to DNA of the nuclear subfractions / pmol/mg DNA /. The nuclei were incubated as described under Fig.1. and subfractionated by the methods of Pardoll et al. /4/ and Berezney et al. /5/ .

Nuclei	Fraction	Total binding	Ratio 1/	Modified 2/ nucleosides	Ratio 1/
Uninduced	Intact nuclei	11.5	-	7.6	-
	Low Mg chromatin	3.5	1	1.2	1
	High salt chromatin	16.9	4.8	17.6	14.7
	Nuclear matrix	83.8	23.9	52.2	43.5
MC-induced	Intact nuclei	41.5	-	33.1	-
	Low Mg chromatin	19.7	1	11.6	1
	High salt chromatin	50.7	2.6	30.1	2.6
	Nuclear matrix	91.1	4.6	59.1	5.1

1/ The ratios are BP bound / mg DNA values compared to Low Mg chromatin.

2/ The nucleosides eluted with methanol from a short Chrompack C_{18} column.

From these experiments it appears that nuclear activation of BP may be to large extent dependent on the regiospecificity of the type of cytochrome P-450. The formation of BP adducts in the uninduced nuclei is consistent with K-region oxygenation of benzo/a/pyrene being mediated by cytochrome P-450. In the MC-induced nuclei the non-K-region oxygenation catalysed by cytochrome P-448 prevails. The different pattern of metabolic activation and increased total metabolism of BP in MC-induced nuclei could explain also the difference in the subnuclear distribution of BP bound to DNA observed with the two nuclear preparations.

REFERENCES

1. Hemminki K., Vaino H., /1979/ Cancer Letters, 6, 167-173
2. Ueyama H., Matsuura T., Shinhachiro N., Nakayasu M., Ueda K., /1981/ Life Sciences, 29, 655-661
3. Mironov N.M., Grover P.L., Sims P., /1983/ Carcinogenesis, 4, 189-193
4. Pardoll D.M., Vogelstein B., Coffey D.S., /1980/ Cell, 19, 527-536
5. Berezney R., Coffey D.S., /1977/ J.Cell Biol., 73,616-637