SELECTIVE MODIFICATION OF REPLICATING DNA BY BENZ(a)PYRENE

N. M. Mironov, L. V. Dmitrieva,
E. I. Kupriyanova, and V. S. Shapot

UDC 616.018-02-092.9

KEY WORDS: benz(a)pyrene; DNA; replication; mechanism of chemical carcinogenesis.

The problem of whether metabolites of chemical carcinogens interact with the genetic material of the cell in random fashion, or whether targets modified by carcinogens with the greatest intensity exist in DNA is of the utmost importance for the understanding of the initial stages of chemical carcinogenesis, for it is in these targets that the probability of damage to the cell oncogens is determined. A complex multilevel system of DNA organization exists in the cells of eukaryotes, and each of its levels evidently can make its own contribution to the specificity of interaction between carcinogens and genetic material. For instance, the probability of interaction of the benz(a)pyrene metabolite antibenz(a)-pyrene 7,8-diol 9,10-epoxide with guanyl bases of protein-free DNA (leading to a break in the DNA) is determined by the nature of the neighboring bases [7]. Complex formation by DNA with histones, forming nucleosomal structures, is the reason why the highest levels of modification of DNA by benz(a)pyrene are located in the internucleosomal regions [6]. Finally, at the next level of intranuclear DNA organization, where nucleosomal structures interact with nonhistone proteins and are attached to the nuclear matrix, regions of DNA nearest to the nuclear matrix, enriched with transcription-active genes and with certain potential oncogenes, were found to undergo maximal modification in undividing cells [2].

Since DNA, in the course of its replication, is attached to the nuclear membrane [3, 12], it was decided to study whether such DNA, like active genes, is most accessible for injury by benz(a)pyrene metabolites. An alternative to this possibility might be to consider that replicating DNA, interacting with replication enzymes and proteins of the nuclear matrix, is protected by them from the action of carcinogens more reliably than nonreplicating DNA.

EXPERIMENTAL METHOD

Nuclei of regenerating rat liver cells were isolated 24 h after partial hepatectomy, incubated with ^{3}H-benz(a)pyrene in the presence of NADPH, and divided into four fractions: 1) escaping from the nuclei during incubation; 2) extracted by a solution with low ionic strength (0.2 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.6); 3) extracted by a solution with high ionic strength (2 M NaCl); and 4) the nuclear matrix, as described in detail in [2]. DNA was isolated from each fraction by the phenolic method, with treatment by protein kinase K and ribonuclease (RNase). Finally, the DNA was washed twice with ethanol and once with acetone. These procedures were shown to remove 99% of the benz(a)pyrene not covalently bound. The concentration of benz(a)pyrene metabolites covalently bound with the different RNA fractions was determined in conventional units, taking the concentration of adducts in fraction 2 as 1 unit. A culture of mouse embryonic (C3HA) fibroblasts was obtained by treatment of minced 11-12-day embryos with 0.3% tryosin solution at 37°C for 45 min. The material was then fragmented in medium 199 on a magnetic mixer for 5-10 min. Cells of the first passage, growing on medium 199 with 10% bovine serum and ^{14}C-thymidine (56 mCi/mmole) were used in the experiments. The cells were synchronized by double thymidine block in accordance with the scheme: 16 h + 8 h + 16 h (2 mM thymidine). Next, ^{3}H-benz(a) pyrene (0.16 μg/ml medium, 2.8 Ci/mmole; or ^{3}H-thymidine (51 Ci/mmole, in control samples) was added at 2.5 h in the S phase. The cells were collected, washed to remove thymidine and benz(a)pyrene, the nuclei were isolated in a 0.5% solution of Triton X-100 and DNA was isolated from the nuclei as indicated above. The DNA was fractionated electrophoretically in 0.8% agarose gel under denaturing conditions for 3.5 h [8]. All the lanes were cut into 25

Laboratory of Biochemistry of Tumors, Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Shapot.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 106, No. 8, pp. 217-220, August, 1988. Original article submitted June 21, 1987.

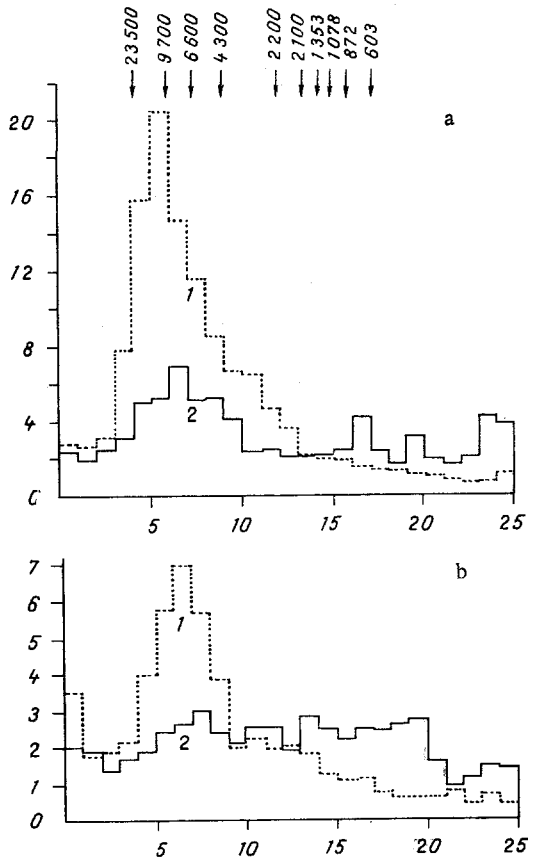

Fig. 1. Distribution of ^{3}H-benz(a)pyrene metabolites covalently bound with DNA (a) or of ^{3}H-thymidine incorporated during the S phase into DNA (b) of fibroblasts retention-labeled with ^{14}C-thymidine. Abscissa, No. of strips into which the corresponding lane of 0.8% agarose gel was cut (here and in Fig. 2): ordinate, radioactivity in these strips (cpm × 10^{-2}). 1) ^{14}C; 2) ^{3}H. Arrows indicate position of markers (here and in Fig. 2).

parts each 0.5 cm long, and radioactivity was counted in each part: ^{14}C (characterizing the total DNA content) and ^{3}H (characterizing the quantity of bound benz(a)pyrene or of newly synthesized DNA).

EXPERIMENTAL RESULTS

During fractionation of nuclei of regenerating rat liver cells [3] in solutions with low and high ionic strength, replicating DNA is found in the nuclear matrix fraction. However, to test whether this DNA fraction is modified by benz(a)pyrene metabolites more intensively than the others, we isolated the nuclei from regenerating rat liver cells, incubated them with benz(a)pyrene, fractionated them, and determined the concentrations of benz(a)pyrene metabolites covalently bound with DNA of the fractions. The highest concentration of benz(a)-pyrene metabolites was found in the nuclear matrix, namely 6.3 ± 1.5 conventional unit (c.u.), in the same fraction as that in which DNA replication took place. An increased concentration of metabolites also was found in the DNA fraction released on account of endonuclease hydrolysis during incubation of the nuclei with benz(a)pyrene, although in this case it was only about half as high as in DNA of the nuclear matrix (2.8 ± 0.1 c.u.). The increased concentration of benz(a)pyrene metabolites in this DNA fraction was evidently due to the presence in it of fragments of replicating DNA, released from the nucleus as a result of increased sensitivity of replicating DNA to the action of nucleases [4, 5]. The concentration of benz(a)-pyrene metabolites in the DNA fraction extracted by a solution with high ionic strength was 0.7 ± 0.1 c.u.

The results of this experiment thus indicate that replicating DNA is evidently modified by benz(a)pyrene more intensively than nonreplicating DNA.

To test this hypothesis further we compared the level of modifications of short DNA fragments in the process of replication and of long fragments of mature DNA. For this purpose embryonic mouse fibroblasts grown on medium with ^{14}C-thymidine and synchronized by double thymidine block were used. Control experiments showed that if ^{3}H-thymidine was added to the medium containing these cells during the S phase for 2.5 h, the highest concentration of newly synthesized DNA (the highest ^{3}H/^{14}C ratio) was found in the composition of the short fragment (Fig. 1b).

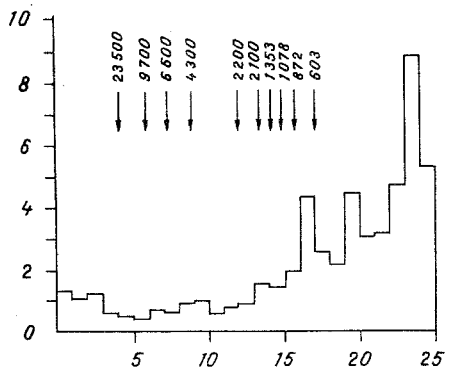

Fig. 2

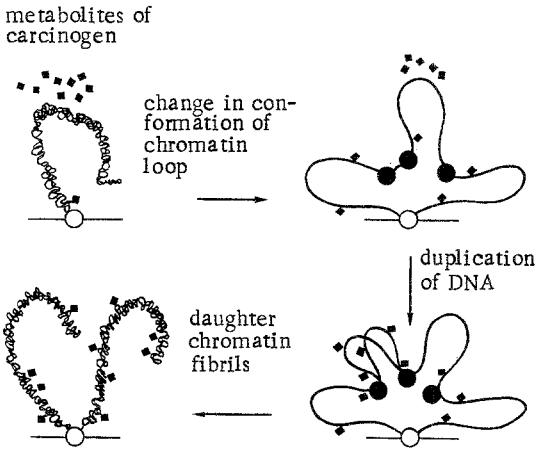

Fig. 3

Fig. 2. Relative concentration of benz(a)pyrene metabolites covalently bound with DNA fragments of different lengths. Ordinate, ratio of concentration of benz(a)pyrene metabolites (^{3}H) to that of total DNA (^{14}C).

Fig. 3. Potentiation of modifying action of benz(a)pyrene during DNA replication. Chromatin loop, interacting with matrix for DNA replication, changes its own conformation so that it becomes more accessible for metabolites of the carcinogen. Circles indicate conventionally permanent (empty) and functional (filled) sites of attachment of DNA to nuclear matrix.

If ^{3}H-benz(a)pyrene was added to the cells, the highest concentration of its metabolites also was determined in short fragments of newly synthesized DNA (Fig. 1a). The concentration of benz(a)pyrene metabolites in these fragments was 4-9 times higher than in total DNA (Fig. 2). Differences between replicating and nonreplicating DNA could evidently be even sharper, for some of the ^{3}H-label in mature DNA fragments (Fig. 1a) undoubtedly arises from short fragments of newly synthesized DNA as a result of displacement and fusion during maturation.

During the action of benz(a)pyrene on cells in which DNA is being synthesized, metabolites of the carcinogen thus cause maximal damage to the most immature form, namely replicating DNA.

However, not all the features distinguishing the process of DNA replication on the nuclear matrix have yet been studied. There is reason to support that temporary, functional connections may arise between replicating DNA and the nuclear matrix [10]. Models of DNA replication on the matrix, taking such functional connections into account, were suggested in [1, 10]. One such model (Fig. 3) illustrated how attachment of a compacted DNA loop to the nuclear matrix could lead to an increase in the number of lesions in the loop as a result of greater accessibility for benz(a)pyrene metabolites. In fact, if DNA replication takes place on the matrix, each gene in the proliferating tissue will be attached at the time of replication to the matrix. At that moment also it acquires increased accessibility for metabolites of the carcinogen. In other words, the probability of damage to any of the genes, including those whose damage is linked causally with transformation, may be much greater in dividing then in resting cells.

From the molecular biological point of view we can thus explain the phenomenon of increased effectiveness of action of carcinogens on proliferating cells. In fact, dividing cells are more sensitive to transformation by chemical carcinogens [9]. DNBA or 2-methyl-4-dimethylaminoazobenzene are not carcinogens for the adult rat liver, but they induce tumors of the liver after partial hepatectomy; N-nitroso-N-methylurea, if injected into a pregnant female, induces tumors in the offspring but not in the mother [11]. Since the frequency of initiation of transformation could be determined by the probability of injury to particular oncogenes, potentiation of the carcinogenic effect against the background of proliferation may be based on a mechanism leading to an increase in the probability of injury to these oncogenes during DNA replication. Attachment of the genes to the matrix for DNA replication is one such mechanism.

LITERATURE CITED

1. N. M. Mironov, V. V. Lobanenkov, and V. S. Shapot, Dokl. Akad. Nauk SSSR, 254, 1269 (1980).
2. N. M. Mironov, Biokhimiya, No. 3, 503 (1987).
3. R. Berezney and D. S. Coffey, Science, 189, 291 (1975).
4. P. Chambon, Cold Spring Harbor Symp. Quant. Biol., 42, 1209 (1978).
5. C. Cremisi, Microbiol. Rev., 43, 297 (1979).
6. P. L. Jack and P. Brookes, Nucl. Acids Res., 9, 5533 (1981).
7. V. V. Lobanenkov, M. Plumb, G. H. Goodwin, and P. L. Grover, Carcinogenesis, 7, 1689 (1986).
8. T. Maniatis, E. F. Fritsch, and J. Sambrook, Molecular Cloning, Cold Spring Harbor (1982).
9. H. Marquardt, A. Bendich, F. S. Phillips, and D. Hoffman, Chem. Biol. Interact., 3, 1 (1971).
10. S. J. McCready, D. A. Jackson, and P. R. Cook, Prog. Mutat. Res., 4, 113 (1982).
11. N. P. Napalkov, Transplacental Carcinogenesis, ed. by L. Tomatis and U. Mohr, Lyon (1973), pp. 1-13.
12. D. M. Pardoll, B. Vogelstein, and D. S. Coffey, Cell, 19, 527 (1980).

TIME COURSE OF FREQUENCIES OF SISTER CHROMATID EXCHANGE AND CHROMOSOMAL ABERRATIONS IN VIVO

S. V. Stukalov

UDC 612.6.052:575.224.23].014.46.66

KEY WORDS: thiotepa; rabbit; chromosomal aberrations; sister chromatid exchange.

During testing of the mutagenic activity of chemical substances in vitro, cell populations are studied during the period of mutagenic treatment and later under identical conditions. Under these circumstances, by the use of radioactive or BUdR labeling it is possible to monitor the rate of cell proliferation and the number of chromosomal aberrations (CA) and sister chromatid exchange (SCE) recorded in the cell [2, 5]. After exposure to mutagenic action in vivo the number and fraction of cells carrying SCE and CA diminishes with the course of time on account of selective death and proliferation of the cells, their redistribution in the tissues, and repair of injuries [6]. To determine the optimal times of taking blood samples after mutagenic action in vivo, and to choose the correct dose and interpret the results of cytogenetic analysis properly, it is essential to know the principles governing the time course of cells with cytogenetic lesions in vivo.

The aim of this investigation was to study changes in the frequency of SCE and CA with time after exposure to various doses of thiotepa (TP) in vivo.

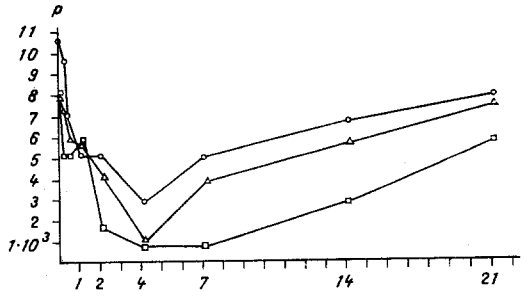

Fig. 1. Time course of number of nucleated cells (P, $10^3/mm^3$) in rabbits' blood. Here and in Figs. 2 and 3: circles — after injection of 2 mg/kg of TP; squares — 4 mg/kg, triangles — 6 mg/kg.

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 8, pp. 220-221, August, 1988. Original article submitted May 14, 1987.

 0007-4888/88/0008-1168$12.50