Bull. Environ. Contam. Toxicol. (1986) 37:55–61

Environmental Contamination and Toxicology

Effect of the Benzene Fraction of Petroleum on Thymidine Kinase Activity and DNA Content in Rat Liver and Kidney

M. Bong-Mastek, T. Laskowska-Klita, and T. Szymczyk

Department of Biochemistry, Warsaw Medical School, Banacha 1, 02-097 Warsaw, Poland

The first step in the incorporation of thymidine into DNA is phosphorylation of the nucleoside catalysed by thymidine kinase (EC 2.7.1.21) . The activity of this enzyme is affected by various conditions, especially those related to the proliferative capacity of the cell.
This paper describes the effect of the low boiling benzene fraction of petroleum on thymidine kinase in relation to DNA level in liver and kidney of rat.

MATERIALS AND METHODS

White male Wistar rats weighing 160-200 g were used for experiments. Animals were fed with LSM standard mixture (Bacutil,Warsaw) and had water ad libitum. Animals were intoxicated with the benzene fraction of petroleum from Masovian Petrochemical Works in Płock.

Characteristics of the fraction as given by the Analytical Laboratory of Masovian Petrochemical Works is as follows: boiling range: 98 - 163°C, chemical composition: nonaromatics (paraffin, cycloparafins) - 92,59%, benzene - 0.31%, toluene - 1.96%, ethylbenzene - 0.93%, para-xylene - 0.41%, meta-xylene - 1.27%, orthoxylene - 0.75%, kumene - 0.24%, C_9 - aromatics - 1.54%.
Animals were given an intraperitoneal injection of benzene in a single dose of 3.09 ml per kg of body weight (LD_{25}). Control animals were injected with the same volume of 0.9% NaCl.
Animals were exposed to vapors of benzene at concentration of 33.75 g/m^3 in an inhalation chamber (Rusiecki et al. 1977) for 6 hrs daily, 6 times a week for a period ranging from 3 to 90 days.
Rats were decapitated without anaesthesia and liver and kidney were isolated.

For separation of nucleic acids by the method of Henrichs et al. (1964) tissues were homogenized with 2

volumes of redistilled water. DNA was determined by the method of Burton (1968) with calf thymus DNA as a standard and expressed in µg/g of tissue.
For thymidine kinase activity determination, the isolated tissues were homogenized with 4 volumes of 0.02 M Tris HCl buffer, pH 7.5 containing 0.1 M KCl. Homogenates were centrifuged at 16000 x g for 30 min and in supernatant the activity of the kinase was determined by the method of Klemperer and Haynes (1968) in the modifications of Machovich and Greengard (1972) and Munch-Petersen and Tyrsted (1977). Activity of the enzyme was expressed in pmoles of [^{14}C]dTMP formed/g of tissue under the condition of assay.
Molecular forms of thymidine kinase were separated by the method of Okuda et al. (1972). Isolated tissues were homogenized with 4 volumes of 0.1 M Tris HCl buffer, pH 8.0 and centrifuged at 8000xg for 30 min. The supernatant fraction was applied to Sephadex G-25 column (2.5 x 25 cm) equilibrated with 5 mM Tris HCl buffer, pH 8.0. The same buffer was used for elution of the enzyme. Active fractions of the eluate were pooled and applied (5 ml) to DEAE-cellulose column (1.5x10cm) equilibrated with 5 mM Tris HCl buffer pH 8.0. The protein was eluted with 50 ml portions of 10,100,150 and 500 mM Tris HCl buffer (pH 8.0). For determination of thymidine kinase activity 5 ml samples of the eluate were used.

RESULTS AND DISCUSSION

The activity of thymidine kinase in liver was distinctly increased as early as six hours after intraperitoneal injection of the benzene fraction of petroleum, and after 12 hours it was 2.5 times higher than the control value (Table 1) .
This increase preceded the increase in DNA content (Table 2) . On the second and third day after the injection the level of DNA reached the highest values, whereas the activity of thymidine kinase dropped to about 50% of the control value. These changes in the enzyme activity were transient, and within one month after the injection the activity in liver of the treated animals was again the same as in control rats.
In kidney the extent and character of changes in thymidine kinase activity after injection of the benzene fraction were similar to those observed in liver. The initial rapid increase in enzyme activity preceded the increase in DNA content (Table 1,2) .
When the benzene fraction was administered by inhalation, the effects were similar to those observed after intoxication by intraperitoneal injection. On the third day of exposure of the rat to benzene vapors the

Table 1. Intraperitoneal injection of the benzene fraction of petroleum and thymidine kinase activity in rat liver and kidney.

Time after intoxication (days)	Activity of thymidine kinase			
	liver		kidney	
	pmole[^{14}C]dTMP/g of tissue	% control	pmole[^{14}C]dTMP/g of tissue	% control
0 (20)	1210± 81.6	100	340±20.8	100
6hrs (8)	x1815±112.8	150	x535±21.3	157
12hrs (8)	x3074±230.0	254	x909±30.3	267
1 (9)	x 878± 29.1	72	x155± 6.3	46
2 (8)	x 467± 25.2	39	x170± 7.2	50
3 (8)	x 777± 28.0	64	x298±19.8	88
7 (8)	x1001± 64.5	83	x415±22.6	122
30 (12)	x1173± 72.0	97	x362±18.9	107

Experimental conditions as described in Methods.
Results are mean values ± mean standard deviation.
Number of animals is given in parentheses.
x $p \leq 0.05$ (Student's "t" test)

Table 2. Intraperitoneal injection of the benzene fraction of petroleum and DNA content in rat liver and kidney.

Time after intoxication (days)	DNA content			
	liver		kidney	
	µg/g of tissue	% control	µg/g of tissue	% control
0 (13)	1.54±0.08	100	1.42±0.05	100
6hrs (8)	1.66±0.08	107	1.49±0.02	105
12hrs (8)	x1.74±0.10	113	x1.78±0.04	125
1 (8)	x2.01±0.07	131	x1.97±0.06	139
2 (8)	x2.05±0.07	134	x1.93±0.03	135
3 (8)	x1.99±0.08	130	x1.77±0.03	124
7 (8)	x1.91±0.08	124	x1.74±0.04	122
30 (8)	1.69±0.02	110	1.44±0.12	102

Experimental conditions as described in Methods.
Results are mean values ± mean standard deviation.
Number of animals is given in parentheses.
x $p \leq 0.05$ (Student's "t" test)

activity of thymidine kinase in liver was increased by a half but after a week it dropped to 68% of the control value (Table 3). On more prolonged intoxication the enzyme activity rose again but still remained lower

than in control animals. These difference disappeared only after 3 months of inhalations. In kidney, the activity of thymidine kinase increased almost twofold on the third day of intoxication but after a week it returned to the control value but on further intoxication remained slightly lowered (Table 3).
On exposure of the animals to benzene by inhalation, similarly as in the case of intraperitoneal intoxication, the changes in enzyme activity preceded the changes in DNA content (Table 4).

Table 3. Inhalation of the benzene fraction of petroleum and the activity of thymidine kinase in rat liver and kidney.

Time of intoxication (days)		Activity of thymidine kinase			
		liver		kidney	
		pmole [^{14}C]dTMP/g of tissue	% control	pmole [^{14}C]dTMP/g of tissue	% control
0	(52)	1152±74.9	100	318±19.4	100
3	(16)	x1860±89.6	161	x598±20.8	188
5	(14)	x 784±36.5	68	328±23.6	103
14	(14)	x1020±63.8	89	310±18.8	97
30	(8)	x1024±60.8	89	x294±14.3	92
90	(8)	1124±62.8	98	x308±15.6	95

Experimental conditions as described in Methods.
Results are mean values ± mean standard deviation.
Number of animals is given in parentheses.
x $p \leq 0.05$ (Student's "t" test)

Table 4. Inhalation of the benzene fraction of petroleum and DNA content in rat liver and kidney.

Time of intoxication (days)		DNA content			
		liver		kidney	
		µg/g of tissue	% control	µg/g of tissue	% control
0	(52)	1.64±0.07	100	1.55±0.05	100
3	(10)	x1.49±0.05	91	x1.42±0.04	91
5	(16)	x1.36±0.04	83	x1.78±0.04	115
14	(14)	x1.76±0.02	108	1.57±0.07	101
30	(8)	x1.94±0.06	119	1.58±0.04	102
90	(8)	x1.73±0.07	108	x1.35±0.04	87

Experimental conditions as described in Methods.
Results are mean values ± mean standard deviation.
Number of animals is given in parentheses.
x $p \leq 0.05$ (Student's "t" test)

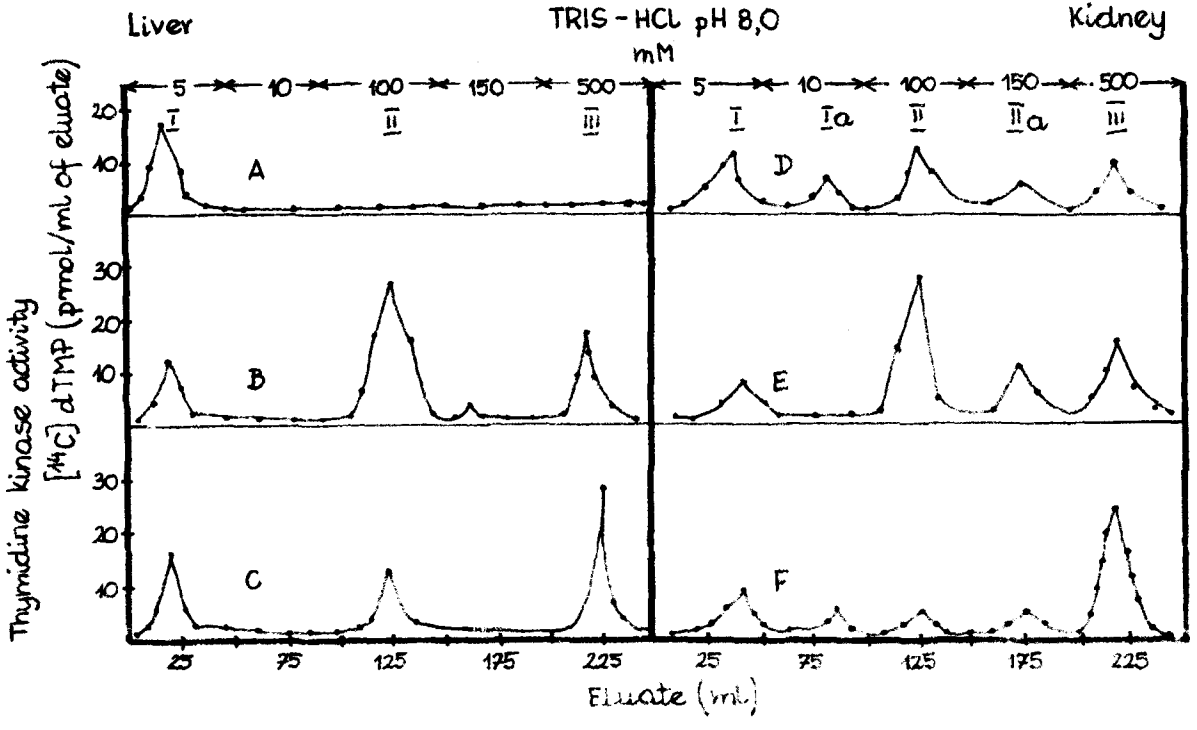

Figure 1. Molecular forms of thymidine kinase from liver and kidney of rats intoxicated with the benzene fraction of petroleum. Chromatograms of cytosol: from (A), (D) - control rat; (B), (E) - 12 hours after intraperitoneal injection; (C), (F) - after 3 days of inhalations. For conditions of chromatographic separation of molecular forms and determination of enzyme activity, see **Methods**.

In normal rat liver, cytosolic thymidine kinase appears as a single molecular form which shows low affinity to DEAE-cellulose at pH 8 and is eluted from the column with Tris HCl buffer of low concentration (Fig. 1A) . After intoxication with the benzene fraction, either by intraperitoneal injection or by inhalation, additional molecular forms of the kinase(II and III) were found in liver; these forms showed higher affinity to DEAE-cellulose than form I, and were eluted from the column with 100 and 500 mM Tris HCl buffer, respectively (Fig. 1B,C) . In normal rat kidney several forms of the enzyme were present (I, Ia, II, IIa, III) on ion-exchange chromatography they were eluted with increasing concentrations of the buffer (Fig. 1D) . The increased activity of the enzyme observed after intraperitoneal injection of the benzene fraction was due to the increased activity of forms II and III of thymidine kinase (Fig.1E) . On intoxication by inhalation, only form III of the enzyme showed increased activity (Fig. 1F) .

In normal adult rats the activity of hepatic thymidine kinase is low. High activity of the enzyme has been observed in foetal liver (Machovich and Greengard 1972; Taylor et al. 1972; Ibsen and Fishman 1979) and in the liver regenerating after partial hepatectomy (Okuda et al. 1972; Taylor et al. 1972) . An increase in the kinase activity by a factor of several hundreds was observed both in hepatomas and in other neoplasms unrelated to liver tissue such as HeLa cells, SV_{40} virus-transformed fibroblasts as well as in the case of cancer of bladder, digestive tract or placenta (Stafford and Jones 1972; Ibsen and Fishman 1979) .

In liver of normal adult rats, thymidine kinase is located in mitochondria, whereas in foetal and regenerating liver and in neoplasms the observed high activity of the enzyme is related to cytoplasm (Hashimoto et al. 1969; Okuda et al. 1972; Ibsen and Fishman 1979). Okuda et al. (1972) demonstrated heterogeneity of cytoplasmic thymidine kinase and separated its particular molecular forms by ion-exchange chromatography. Under physiological conditions, form I is the predominating one, whereas under conditions of enhanced DNA synthesis the highly increased enzyme activity is due to forms II and III.

On intraperitoneal intoxication of rats with the benzene fraction of petroleum and also on intoxication by inhation the high activity of the enzyme due to increase of forms II and (or) III was obserwed. Thus, it seems that hydrocarbons of the benzene fraction of petroleum are able to evoke expression of genes of those forms of thymidine kinase which appear under conditions of increased cell proliferation. Several authors (Machovich and

Greengard 1972; Weber 1972; Hopkins et al. 1973) have reported that, in the course of the division cycle of the eukaryotic cell, the level of cytosolic thymidine kinase undergoes distinct changes. The enzyme activity increases throughout the interphase and reaches the highest values at the S phase, i.e. during synthesis of DNA.
In our experiments, both after intraperitoneal injection and after inhalation of the benzene fraction of petroleum, the increase in DNA content in rat liver and kidney was proceded by the increase in thymidine kinase activity.

Acknowledgement. This work was done within the contract between the Warsaw Medical School and the Masovian Oil Distillery and Petrochemical Works in Płock.

REFERENCES

Burton KJ (1968) Determination of DNA concentration with diphenylamine. In: Grossman L, Moldave K ed Methods in Enzymology. Academic Press, New York, p163
Hashimoto T, Shiosaka T, Toide H, Okuda H, Fujii S (1969) Thymidine kinases from normal and tumor tissues. Gann 60:41-47
Hopkins HA, Campbell HA, Barbiroli B, Potter UR (1973) Thymidine kinase and deoxyribonucleic acid metabolism in growing and regenerating livers from rats on controlled feeding schedules. Biochem J 136:955-966
Ibsen KH, Fishman WH (1979) Developmental gene expression in cancer. Biochem Biophys Acta 560:243-280
Klemperer HG, Haynes GR (1968) Thymidine kinase in rat liver during development. Biochem J 108:541-546
Machovich R, Greengard O (1972) Thymidine kinase in rat tissues during growth and differentiation. Biochim Biophys Acta 286:375-381
Munch-Petersen B, Tyrsted G (1977) Induction of thymidine kinases in phytohaemagglutinin-stimulated human lymphocytes. Biochim Biophys Acta 478:364-375
Okuda H, Arima T, Hashimoto T, Fujii S (1972) Multiple forms of deoxythymidine kinase in various tissues. Cancer Res 32:791-794
Rusiecki W, Podleśny J, Zawada M (1977) Inhalation chamber for poisoning animals. Med Dyd Wych 9:183-184
Stafford MA, Jones OW (1972) The presence of "fetal" thymidine kinase in human tumors. Biochim Biophys Acta 277:439-442
Taylor AT, Stafford MA, Jones OW (1972) Properties of thymidine kinase partially purified from human fetal and adult tissue. J Biol Chem 247:1930-1935
Weber G (1972) Molecular correlation concept: ordered pattern of gene expression in neoplasia. Gann Monograph Cancer Res 13:47-77
Received May 30, 1985; accepted August 21, 1985.