BIOPHYSICS AND BIOCHEMISTRY

Dose- and Time-Dependent Effects of Menadione on Enzymes of Xenobiotic Metabolism in Rat Liver

Yu. A. Sidorova and A. Yu. Grishanova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 3, pp. 260-264, March, 2004
Original article submitted December 4, 2003

We studied the effect of synthetic vitamin K analogue menadione on enzymes of xenobiotic metabolism in rat liver (total content of cytochrome P450 and catalytic activities of CYP1A1/2, CYP2B1, CYP2C, NADPH-cytochrome P450 reductase, and glutathione S-transferase). Menadione induced phase I and II enzymes for metabolism of xenobiotics, drugs, and procarcinogens. The effect of menadione depended on its dose and duration of treatment.

Key Words: *menadione; vitamin K_3; CYP1A; CYP2B; CYP2C; NADPH-cytochrome P450 reductase; glutathione S-transferase*

Menadione (vitamin K_3) is a synthetic analogue of vitamin K possessing high physiological activity. Vitamin K is essential for γ-carboxylation of glutamine residues in plasma and bone proteins [1]. Ligands of the tyrosine kinase receptor are vitamin K-dependent proteins that stimulate replication and transformation of cells and prevent the induction of apoptosis [15]. Published data show that vitamin K modulates lipid and protein composition of biomembranes [1].

Enzymes of the monooxygenase system play an important role in biotransformation and synthesis of endogenous compounds. They are involved in metabolism and bioactivation of chemically different exogenous compounds (procarcinogens, drugs, and environmental pollutants). Various forms of cytochrome P450 (CYP) and NADPH-cytochrome P450 reductase are the key phase I enzymes for metabolism. Phase II enzymes include UDP-glucuronosyltransferase, glutathione S-transferase (GST), and N-acetyl-, sulfo-, and methyltransferase.

Much attention was given to the induction of monooxygenases with various xenobiotics, including drugs and food additives. However, the effects of vitamins K on these enzymes remain unknown. Vitamin K suppresses the monooxygenase system and decreases benzo(a) pyrene hydroxylase, aniline-p-hydroxylase, and aminopyrine-N-demethylase activities in liver microsomes from intact rats [3,7]. This vitamin reduces 7-ethoxycoumarin-O-dealkylase activity in recombinant cells of *Salmonella typhimurium* and *Saccharomyces cerevisiae* [6,9]. However, vitamin K increases benzo(a)pyrene hydroxylase activity in methylcholanthrene-induced microsomes of rat liver [10], cultured HEPG2 cells [4], and 17-day-old chicken embryos [5]. Moreover, vitamin K increases the content of specific CYP1A1 mRNA in cultured HEPG2 cells [4]. *In vivo* effects of vitamin K_3 on monooxygenase activities are poorly studied.

Here we studied the dependence of changes in activity of monooxygenases in rat liver (CYP1A1/2, CYP2B1, CYP2C, NADPH-cytochrome P450 reduc-

Laboratory for Biochemistry of Foreign Compounds, Institute of Molecular Biology and Biophysics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** sidorova@ngs.ru. Sidorova Yu. A.

0007-4888/04/1373-0231

tase, and GST) on the duration of treatment and dose of menadione.

MATERIALS AND METHODS

Experiments were performed on 54 male Wistar rats weighing 100-120 g. The animals fed a standard diet. They were deprived of food for 1 day before killing. Experimental rats perorally received 5% oil solution of menadione in doses of 30 (1, 4, 8, 12, and 17 days) and 1, 3, 7, 15, or 30 mg/kg (4 days). Oil was given to control animals. The cytosolic fraction and microsomes of the liver were isolated by differential centrifugation at 4°C. Protein content in microsomes and cytosol was measured by the method of Lowry. Bovine serum albumin served as the standard [13]. The total content of cytochrome P450 was estimated as described elsewhere [13]. The rate of O-dealkylation of highly specific substrates for CYP2B1, CYP1A1, CYP1A2, and CYP2C (7-pentoxy-, 7-ethoxy-, 7-methoxy-, and 7-benzoxyresorufins, respectively) in liver microsomes was determined fluorometrically by resorufin formation [2]. Activity of NADPH-cytochrome P450 reductase in liver microsomes was estimated by the rate of cytochrome *c* reduction [14]. Cytosolic GST activity was evaluated by the rate of 2,4-dinitrophenyl glutathione formation. 1-Chloro-2,4-dinitrobenzene and S-2,4-dinitrophenyl

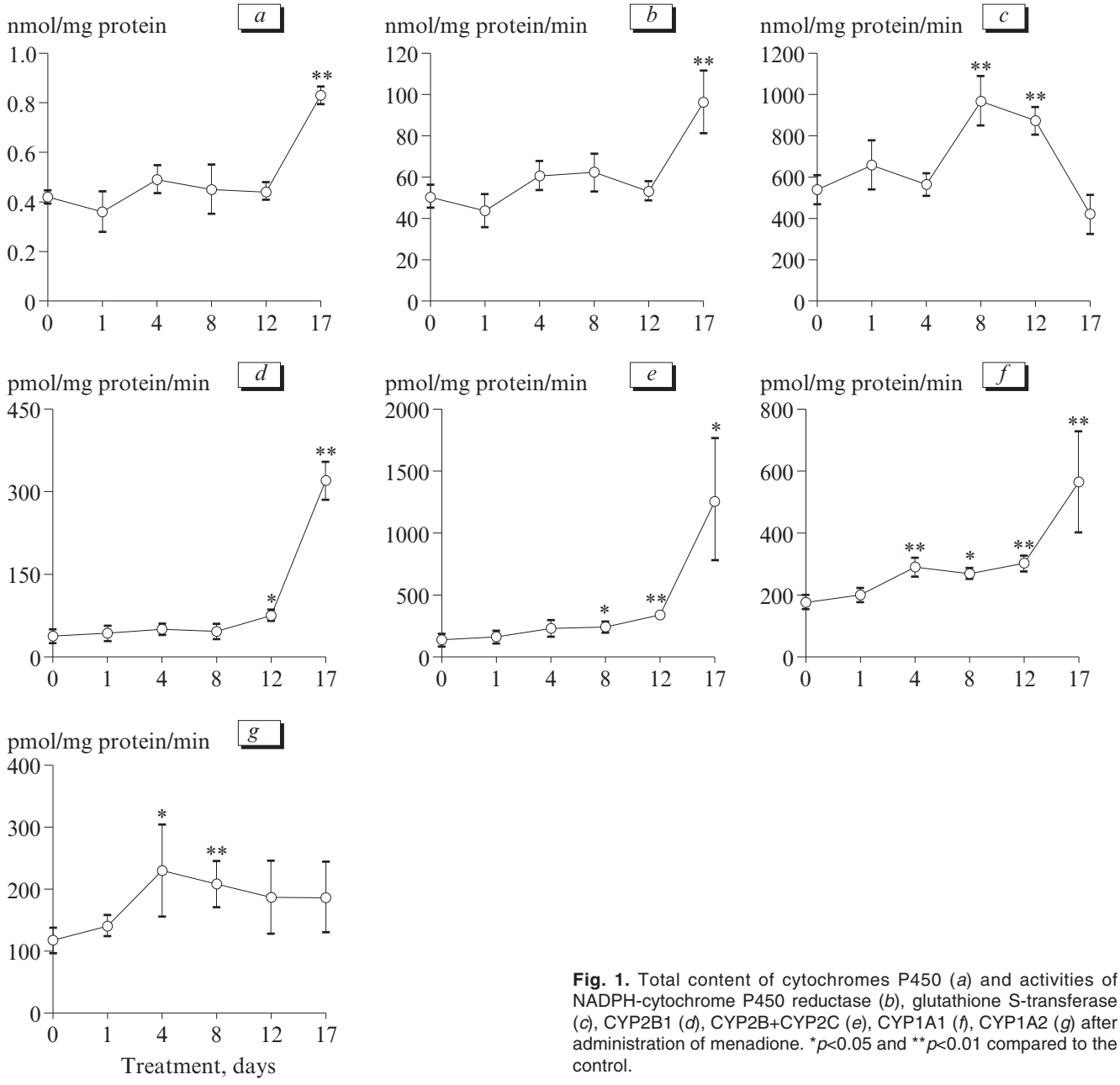

Fig. 1. Total content of cytochromes P450 (*a*) and activities of NADPH-cytochrome P450 reductase (*b*), glutathione S-transferase (*c*), CYP2B1 (*d*), CYP2B+CYP2C (*e*), CYP1A1 (*f*), CYP1A2 (*g*) after administration of menadione. *$p<0.05$ and **$p<0.01$ compared to the control.

glutathione served as glutathione acceptor and standard, respectively [8].

The results were analyzed by Students' *t* test and Mann—Whitney *U* test.

RESULTS

Total cytochrome P450 content in rat liver microsomes and NADPH-cytochrome P450 reductase activity increased by 2 times after administration of menadione for 17 days (Fig. 1, *a*, *b*). GST activity reached maximum on day 8, but decreased to the baseline level on day 17 (Fig. 1, *c*).

Activities of CYP2B1, CYP2C, and CYP1A1 underwent similar changes. Enzyme activities progressively increased and peaked on day 17 (Fig. 1, *d-f*). Activities of CYP2B1, CYP2B1+CYP2C, and CYP1A1 increased by 8.5, 9, and 3.2 times, respectively. CYP1A2 activity reached maximum after treatment for 4-8 days (2-fold above control), but did not differ from the baseline on day 17 (Fig. 1, *g*).

We studied the effects of daily treatment with menadione in various doses for 4 days on activity of enzymes for xenobiotic transformation in rat liver. In this period the content of cytochromes P450 was high, while activities of NADPH-cytochrome P450 reduc-

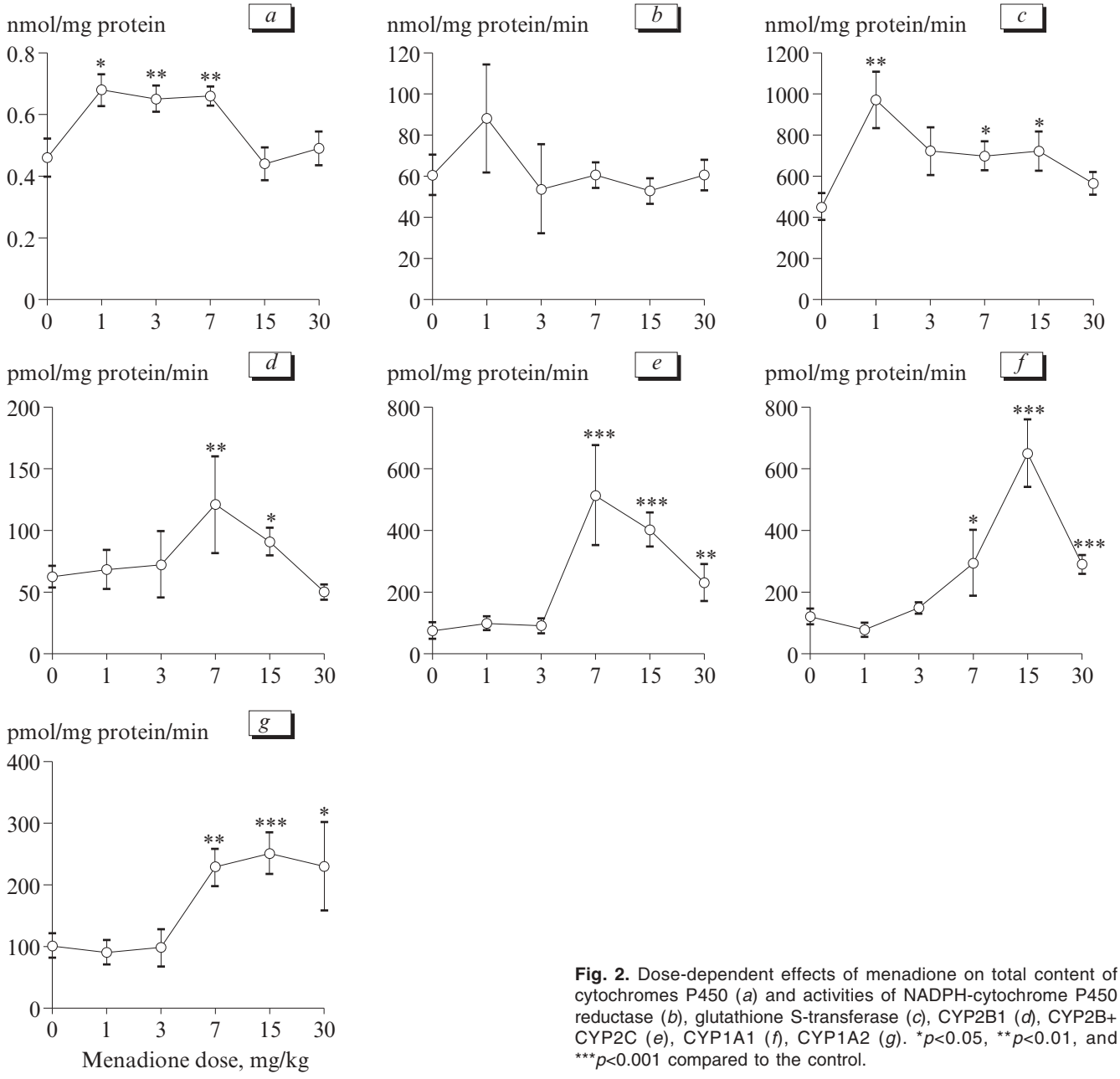

Fig. 2. Dose-dependent effects of menadione on total content of cytochromes P450 (*a*) and activities of NADPH-cytochrome P450 reductase (*b*), glutathione S-transferase (*c*), CYP2B1 (*d*), CYP2B+ CYP2C (*e*), CYP1A1 (*f*), CYP1A2 (*g*). **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to the control.

tase (rate-limiting phase I enzyme) and GST (phase II enzyme) remained unchanged.

Menadione in doses of 1, 3, and 7 mg/kg increased total cytochrome P450 content in rat liver microsomes by 1.5 times (Fig. 2, *a*). After administration of menadione in various doses NADPH-cytochrome P450 reductase activity in microsomes did not differ from the control (Fig. 2, *b*). GST activity increased in rats receiving menadione in doses of 1-15 mg/kg (especially 1 mg/kg, Fig. 2, *c*).

Menadione in a dose of 7 mg/kg increased activity of cytochromes P450 (Fig. 2, *d-g*). CYP2B1 and CYP2C activities increased most significantly after administration of 4 mg/kg menadione (by 2 and 7 times, respectively, Fig. 2, *d*, *e*). Activities of CYP1A1 and CYP1A2 reached maximum in animals receiving 15 mg/kg menadione (increase by 5.4 and 2.5 times, respectively, Fig. 2, *f*, *g*).

Our results show that menadione activates phase I and II enzymes of xenobiotic metabolism in the liver. This effect of menadione depended on its dose and duration of treatment.

The regulation of cytochrome P450 genes (*CYP*) is realized at various levels, including initiation of transcription and stabilization of mRNA and/or protein. NADPH-cytochrome P450 reductase can decrease activity of cytochromes P450. The general regulatory mechanism of *CYP1* and *CYP2* genes involves one of the following receptors: cytosolic receptor AhR (activation of *CYP1A1* and *CYP1A2*) and nuclear receptors CAR and PXR (activation of *CYP2B* and *CYP2C*). CYP1A2 is regulated by stabilization of mRNA.

The data suggest that induction of CYP1A1, CYP2B1, and CYP2C in the liver of rats receiving menadione was related to activation of gene transcription by the receptor-mediated mechanism. We observed similar intrafamily and different interfamily changes in activity of cytochromes P450-1 and P450-2. The data suggest that menadione interacts with various receptors and induces these cytochromes via different pathways of signal transduction. Activities of CYP1A1 and CYP2 sharply increased after administration of menadione for 17 days, which was probably related to activation of NADPH-cytochrome P450 reductase in monooxygenase-catalyzed reactions (simultaneous events). The mechanism for negative regulation of CYP1A2 can be induced in this period, which is followed by a decrease in activity of CYP1A2. Therefore, the induction of this enzyme is insignificant. The increase in activity of reductase (membrane-bound protein) can be associated with the influence of menadione on physicochemical characteristics and lipid composition of the membrane [11].

It should be emphasized that in our experiments the doses of vitamin K_3 far exceeded human daily requirement for vitamin K (100 μg) [11]. However, therapeutic doses of this vitamin used for long-term treatment are comparable with the concentration inducing cytochromes P450 1A and 2B. Cytochromes P450 1A play an important role in biotransformation of environmental pollutants (polycyclic aromatic carbohydrates and arylamines) and catalyze the formation of ultimate carcinogens. Cytochromes P450-2 metabolize several procarcinogens, various drugs, and endogenous compounds [16]. Vitamin K_3 activates these enzymes and modulates the rate of metabolism and half-life of xenobiotics and drugs in the organism.

This work was supported by the Russian Foundation for Basic Research (grant No. 02-04-48639).

REFERENCES

1. A. A. Sokol'nikov and V. M. Kodentsova, *Vopr. Med. Khim*., **45**, No. 6, 453-461 (1999).
2. M. D. Burke, S. Thompson, and R. T. Mayer, *Biochem. Pharmacol*., **34**, 3337-3346 (1985).
3. J. Z. Byczkowski and T. Gessner, *Int. J. Biochem*., **19**, No. 12, 1973-1979 (1987).
4. Y. T. Chen and J. H. Ding, *Biochem. Biophys. Res. Commun*., **143**, No. 3, 863-871 (1987).
5. S. C. Dogra and L. G. Israels, *Int. J. Biochem*., **19**, No. 5, 471-473 (1987).
6. R. Edenharder, A. Worf-Wandelburg, M. Decker, and K. L. Platt, *Mutat. Res.*, **144**, No. 1, 235-248 (1999).
7. M. Floreani and F. Carpenedo, *Toxicol. Appl. Pharmacol*., **105**, No. 2, 333-339 (1990).
8. W. H. Habig, M. J. Pabst, and W. B. Jakoby, *J. Biol. Chem*., **249**, No. 22, 7130-7139 (1974).
9. K. Inouye, T. Mae, S. Kondo, and H. Ohkawa, *Biochem. Biophys. Res. Commun.*, **262**, No. 2, 565-569 (1999).
10. M. V. Izotov, V. M. Shcherbakov, V. M. Devichenskii, *et al*., *Biokhimiya*, **52**, No. 7, 1072-1079 (1987).
11. N. I. Krinsky, *Mol. Aspects Med*., **24**, No. 6, 317-324 (2003).
12. O. U. Lowry, N. I. Rosebrough, A. L. Farr, and R. I. Randall, *J. Biol. Chem*., **153**, 265-275 (1951).
13. T. Omura and R. Sato, *Ibid*., **239**, 2370-2385 (1964).
14. A. H. Phillips and R. G. Langdon, *Ibid*., **237**, 2652-2660 (1962).
15. S. P. Saxena, E. D. Israels, and L. G. Israels, *Apoptosis*, **6**, 57-68 (2001).