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INFLUENCE OF NIFEDIPINE, NITRENDIPINE AND VERAPAMIL AT LOW CONCENTRATION ON ANTIPYRINE METABOLISM EXAMINED BY EXTRACORPOREAL RAT LIVER PERFUSION

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Influence of nifedipine, nitrendipine and verapamil at low concentration on antipyrine metabolism examined by extracorporeal rat liver perfusion. A. SZELĄG, J. MAGDALAN, M. RUTKOWSKA, W. DZIEWISZEK, M. TROCHA, M. RZEPKA, M. PIEŚNIEWSKA, L. FERENIEC. Pol. J. Pharmacol., 2003, 55, 203–208.

An increase in calcium ion concentration in the cytoplasm due to the influence of various toxic agents causes disturbances in the structure and function of hepatocytes, leading to their damage and even death. Calcium ions enter the cell mostly through calcium channels, therefore, it has been suggested that calcium channel inhibitors (CCI) could protect hepatocytes from the action of toxic substances.

The present study investigated the effect of the selected CCI (nifedipine, nitrendipine and verapamil) on liver function, measured by the efficiency of oxidation reaction, in this case by determination of the rate of antipyrine metabolism. The experiment was carried out using the method of extracorporeal liver perfusion (ELP).

None of the studied CCI applied at a concentration of 50 μmol/l increased the rate of antipyrine metabolism over the whole period of ELP. However, supplementation of perfusion fluid with nifedipine, nitrendipine or verapamil at a concentration of 20 μmol/l considerably improved metabolic liver efficiency during the second hour of perfusion, i.e. at the time, when large number of hepatocytes started to perish, which could indicate protective action of the tested CCI. However, the CCI-induced acceleration of antipyrine metabolism was not a result of their influence on calcium channels, since these drugs block calcium channels, when given at the concentrations as high as 100–400 μmol/l. Moreover, it seems that facilitation of antipyrine metabolism during ELP was not due to their action on microsomal enzymes because CCI were administered at very low concentrations, besides, they are metabolic inhibitors, and not inducers. The present experiment suggests that low concentrations of CCI can exert hepatoprotective effect. However, confirmation of this conclusion requires further studies using other experimental methods.

Key words: *nifedipine, nitrendipine, verapamil, antipyrine metabolism, extracorporeal liver perfusion*

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INTRODUCTION

Calcium ions play a role of universal second messenger, activating different intracellular biochemical processes in response to extracellular stimuli, such as catecholamines or membrane potential changes. Therefore, their physiological role is enormous not only in muscular tissue but practically in all cells, including nervous cells and even hepatocytes [5, 12].

In recent years, it has been more and more frequently suggested that excessive inflow of calcium ions to hepatocytes can disturb their function, leading to a decrease in metabolic liver efficiency and even to cell death. Thus, it seems that substances inhibiting calcium ion influx to cytoplasm could have protective effect, reducing hepatocyte damage by toxic agents or hypoxia [6].

Assessment of functional liver disturbances causes many difficulties. Measurement of the rate of metabolism of a probe substance, e.g. antipyrine is one of methods used for this purpose. Antipyrine is oxidized in hepatocytes, and the reaction is catalyzed by cytochrome P-450 isoenzymes. Due to hypoxia or toxic agents, metabolic efficiency of the liver, and, therefore, antipyrine oxidation rate are decelerated.

The aim of this study was to determine the effect of the selected calcium channel inhibitors (CCI: nifedipine, nitrendipine and verapamil) on liver function, measured by efficiency of oxidation reaction, in this case antipyrine metabolism rate.

MATERIALS and METHODS

Animals

The study was carried out on adult male Wistar rats (280–320 g) obtained from the Experimental Animal Farm at the Pharmaceutical Company Jelfa, Jelenia Góra, Poland. The rats were housed individually in chambers with a 12:12 h light-dark circle and temperature maintained at 21–23°C. Before the experiment animals had free access to standard food and water. All experiments were performed after at least two weeks of adaptation to this environment.

Chemicals and drugs

Nifedipine (Cordafen, tablets 10 mg, Polpharma, Poland), nitrendipine (Nitrendypina, tablets 10 mg, Anpharm, Poland), verapamil (Staveran, tablets 40 mg, Polpharma, Poland), antipyrine (cat. nr A-5882, Sigma Chemie, Germany) were used in the study.

Experimental procedure

The animals were anesthetized with intraperitoneal injection of 10% solution of urethane at a dose of 1.2 ml/100 g of body weight. The experiment was conducted using extracorporeal liver perfusion (ELP), according to the procedure described earlier [21] with our modification, which consisted in lowering the volume of perfusion fluid and adjusting it so that it approximated the volume of blood flowing through the liver *in vivo*. This modification enabled us to decrease the amount of the reagents and number of animals necessary to conduct an experiment.

Perfusion fluid was prepared by the addition of 27.5 ml of defibrinated blood with heparin to 27.5 ml of Ringer solution containing 1.65 mg of antipyrine and 44 mg of glucose.

Before the experiment started, the perfusion fluid was heated to 37°C, and then its 2.5 ml sample was collected to determine starting concentration of antipyrine. The remaining fluid was poured to the appropriate container of ELD apparatus and the experiment was initiated. After 10-min period of preliminary perfusion, another 2.5 ml portion of the fluid was sampled, in which initial antipyrine concentration at t_0 (beginning of perfusion, which lasted 120 min in total) was measured. Subsequent 2.5 ml samples were collected every 30 min. The samples were centrifuged at 3000 rpm at room temperature for 15 min. Supernatant was collected with Pasteur pipette. Antipyrine concentration (μg/ml) was determined in each sample according to the method of Brodie [21, 31], using Marcel S330 PRO spectrophotometer. Amount of the metabolized antipyrine was calculated according to the formulas computed by Paradowski, and the results were reported in nmols per g of liver parenchyma [21]. Average mass of a liver used in the experiment (± SEM) was 14.4 ± 0.55 g. The livers used in various groups were selected so that their masses did not differ statistically significantly.

The animals were assigned to the control group and test groups (A, a; B, b; C, c), 6 animals to each. Control livers were perfused with the fluid which did not contain any CCI. Perfusion fluid for test groups A, B and C was supplemented with one of

CCI (nifedipine, nitrendipine or verapamil, respectively) at a concentration of 50 μmol/l. Perfusion fluid for test groups a, b and c contained one of CCI (nifedipine, nitrendipine or verapamil, respectively) at a concentration of 20 μmol/l (Tab. 1–3).

Statistics

The data were evaluated by a one-way analysis of variance (ANOVA), followed, when appropriate, by individual comparison with the control using Student's *t*-test.

RESULTS

The changes in antipyrine metabolism rate in the initial period of ELP exerted the strongest effect on total amount of metabolically transformed antipyrine. Its largest quantity was metabolized within the first hour of perfusion. Within the second hour of the experiment, antipyrine metabolism significantly slowed down due to hepatocyte death.

None of the tested CCI used at a concentration of 50 μmol/l accelerated antipyrine metabolism

Table 1. Influence of nifedipine on mean amount of antipyrine metabolized by 1 g of the liver during extracorporeal liver perfusion

Time [min]		–10–0	0–30	30–60	60–90	90–120	0–60	60–120
Control	X (n = 6)	115.1	142.7	59.0	44.0	41.5	201.7	85.4
group	± SEM	9.2	7.7	3.5	2.3	3.8	11.05	5.5
	%	100	100	100	100	100	100	100
Group "A"	X (n = 6)	134.2	120.7	64.7	49.5	50.2	185.4	102.6
Nifedipine	± SEM	16.9	11.0	9.1	8.4	6.8	13.3	9.7
50 μmol/l	%	117	85	110	113	121	92	120
	p ≤	NS	NS	NS	NS	NS	NS	NS
Group "a"	X (n = 6)	171.9	111.4	74.3	62.8	51.2	184.1	114.0
Nifedipine	± SEM	12.5	8.4	8.7	6.1	5.9	8.4	9.0
20 μmol/l	%	149	78	126	143	123	91	133
	p ≤	0.005	0.02	NS	0.005	0.05	NS	0.02

X – mean amount of the metabolized antipyrine expressed as nmol/g of liver parenchyma; n – number of animals per group; p – in comparison with the control group

Table 2. Influence of nitrendipine on mean amount of antipyrine metabolized by 1 g of the liver during extracorporeal liver perfusion

Time [min]		–10–0	0–30	30–60	60–90	90–120	0–60	60–120
Control	X (n = 6)	115.1	142.7	59.0	44.0	41.5	201.7	85.4
group	± SEM	9.2	7.7	3.5	2.3	3.8	11.05	5.5
	%	100	100	100	100	100	100	100
Group "B"	X (n = 6)	172.3	70.5	57.2	49.0	30.8	127.7	79.9
Nitrendipine	± SEM	18.3	8.5	6.3	8.0	4.7	9.3	8.0
50 μmol/l	%	148.7	49.6	97.1	112.0	74.0	63.9	93.8
	p ≤	0.01	0.001	NS	NS	NS	0.001	NS
Group "b"	X (n = 6)	233.2	90.5	130.9	99.0	66.2	221.4	165.3
Nitrendipine	± SEM	28.9	14.7	15.0	15.3	8.6	23.3	20.0
20 μmol/l	%	201.1	63.6	222.3	226.0	159.0	110.8	193.8
	p ≤	0.001	0.001	0.001	0.001	0.001	NS	0.001

X – mean amount of the metabolized antipyrine expressed as nmol/g of liver parenchyma; n – number of animals per group; p – in comparison with the control group

Table 3. Influence of verapamil on mean amount of antipyrine metabolized by 1 g of the liver during extracorporeal liver perfusion

Time [min]		–10–0	0–30	30–60	60–90	90–120	0–60	60–120
Control	X (n = 6)	115.1	142.7	59.0	44.0	41.5	201.7	85.4
group	± SEM	9.2	7.7	3.5	2.3	3.8	11.05	5.5
	%	100	100	100	100	100	100	100
Group "C"	X (n = 6)	188.7	115.4	63.6	39.9	40.8	178.9	79.4
Verapamil	± SEM	17.2	13.1	10.5	11.7	12.1	14.9	12.4
50 µmol/l	%	162.8	81.1	107.9	91.2	98	89.6	93.1
	p ≤	0.005	0.05	NS	NS	NS	NS	NS
Group "c"	X (n = 6)	251.7	137.7	145.6	75.7	54.9	278.2	130.6
Verapamil	± SEM	26.5	24.1	29.6	16.8	12.0	48.7	26.2
20 µmol/l	%	217.1	96.8	247.3	172.9	132	139.3	153.2
	p ≤	0.001	NS	0.005	0.05	NS	NS	0.05

X – mean amount of the metabolized antipyrine expressed as nmol/g of liver parenchyma; n – number of animals per group; p – in comparison with the control group

over the whole ELP period. However, supplementation of perfusion fluid with nifedipine, nitrendipine or verapamil at a concentration of 20 µmol/l considerably improved the metabolic efficacy of the liver. Facilitation of antipyrine metabolism in comparison with the control group was observed only within the second hour of ELP, i.e. in the period when large number of hepatocytes began to die.

DISCUSSION

An increase in cytoplasmic concentration of calcium ions causes disturbances in the structure and function of hepatocytes, leading to their damage and even death [16, 19, 32]. Many chemical compounds, including therapeutic agents, injure hepatocytes by raising the concentration of free calcium ions [15, 20]. These ions enter the cells mostly through calcium channels, therefore, it has been suggested that CCI could protect hepatocytes from the effects of toxic substances [10]. It was proved that certain drugs, belonging to this group exerted protective action on the isolated rat hepatocytes exposed to ethanol. Verapamil and nifedipine at a concentration of 25 µmol/l and diltiazem at 50 µmol/l inhibited elevated release of hepatic enzymes (AlaAT, AspAT, LDH), when administered 30 min before ethanol treatment. These drugs suppressed also the accelerated lipid peroxidation (verapamil and nifedipine at 50 µmol/l and diltiazem at 25 µmol/l) [6].

Antipyrine is a substrate for oxidizing enzymes belonging to cytochrome P-450 complex [8] and it is commonly used as an indicator of metabolic activity of the liver. Facilitation of antipyrine metabolism in the presence of CCI can be a sign of their protective influence on hepatocytes, since in the present experiment, nifedipine, nitrendipine and verapamil added to the perfusion fluid at a concentration of 20 µmol/l increased antipyrine metabolism rate in the second hour of ELP, thus, in the period when most of hepatocytes start to die. The mechanism of cytoprotective action of these drugs has not been fully understood so far. There are calcium channels in hepatocyte plasma membrane, in mitochondria and microsomes. It was established that nifedipine and verapamil inhibited calcium transport across hepatocyte membranes, by influencing vasopressin-activated channels, dissimilar to potential-operated Ca^{2+} channels, present in so-called excitable cells [10]. Moreover, it was demonstrated that in rat hepatocytes, verapamil, nifedipine or diltiazem inhibited calcium inflow through receptor-gated channels, when their concentrations were as high as 100–400 µmol/l [29, 33]. These concentrations are 5–20 times higher than that which accelerated antipyrine metabolism (20 µmol/l) and 2–16 times higher than those showing protective effect on hepatocytes exposed to toxic levels of ethanol (25 and 50 µmol/l) [6]. Therefore, it can be expected that cytoprotective CCI effect is a result of complex mechanisms, and most probably it does

not depend on calcium channel blockade. It has been suggested that it can be due to such actions as stabilization of plasma membrane [13, 17], protection of structure and function of mitochondria [14] and lysosomes [28], and to their antioxidant effect [4].

Both antipyrine and CCI are metabolized by liver microsomal enzymes, including cytochrome P-450 isoenzymes, e.g. CYP 3A group. Interaction of these compounds at the level of liver metabolism also cannot be excluded. It is known that verapamil inhibits oxidative metabolism of other drugs. Metabolites of this drug (e.g. norverapamil) probably have inhibitory effect as well. *In vitro* studies indicated that verapamil and norverapamil suppressed cytochrome P-450-dependent 4- and 3-hydroxylation of antipyrine. Verapamil administered *in vivo* also significantly prolonged biological half-life of antipyrine in a dose- and time-dependent manner [1, 3, 25]. In our experiment, verapamil used at a concentration of 50 μmol/l did not significantly affect antipyrine metabolism, while its concentration of 20 μmol/l elevated metabolism of this drug in comparison with the results obtained with the control group. However, it was probably not caused by the interaction at the level of microsomal enzymes. It does not seem quite probable that verapamil used at such low concentrations could influence liver microsomal enzymes, besides, only its inhibitory effect was described and not stimulating action on cytochrome P-450.

Nifedipine, a dihydropyridine derivative, possesses considerably weaker potential to inhibit oxidative metabolism in comparison with verapamil. In *in vitro* studies, it suppressed metabolism of many drugs, e.g. propranolol, tolbutamide, theophylline, cyclosporin and midazolam [9, 18, 22, 23, 30]. On the other hand, no nifedipine effect on antipyrine clearance or metabolism of other drugs was observed *in vivo* [2, 7]. Causes underlying such differences between *in vitro* and *in vivo* studies have not been elucidated as yet. To date, no significant interactions of nifedipine with other drugs have been noted, and especially its effect on oxidizing enzymes of cytochrome P-450 complex has not been demonstrated [11, 24, 26, 27, 32]. In the light of the aforementioned data, there is no basis to assume that acceleration of antipyrine metabolism by low concentrations of nifedipine and nitrendipine is connected with their effect on microsomal enzymes.

The drugs belonging to the group of dihydropyridine derivatives could theoretically increase liver blood flow by dilating blood vessels and in such a way facilitate antipyrine metabolism. However, it was not very probable in our experiment, since the drugs were applied at very low concentrations, i.e. such which do not significantly affect calcium channels in vessel wall.

Confirmation of protective CCI action on hepatocytes requires further studies using diversified experimental methods. If CCI indeed exhibit hepatoprotective effect, these drugs could be used in the treatment of liver diseases and transplantology, besides their typical use in the treatment of cardiovascular diseases. It is known that the survival time of organs for transplantation, including liver, is short. Addition of CCI to storage fluids (e.g. Collins, University of Wisconsin) could prolong this time, which would allow for transport of organs to more remote destinations, thereby increasing donor population.

REFERENCES

1. Abernethy D.R., Egan J.M., Dickinson T.H., Carrum G.: Substrate-selective inhibition of verapamil and diltiazem: differential disposition of antipyrine and theophylline in humans. J. Pharmacol. Exp. Ther., 1988, 244, 994–999.
2. Adebayo G.I., Mabadeje A.F.: Effect of nifedipine on antipyrine and theophylline disposition. Biopharm. Drug Dispos., 1990, 11, 157–164.
3. Bach D., Blevins R., Kerner N., Rubenfire M., Edwards D.J.: The effect of verapamil on antipyrine pharmacokinetics and metabolism in man. Brit. J. Clin. Pharmacol., 1986, 21, 655–659.
4. Bauer L.A., Stenwall M., Horn J.R., Davis R., Opheim K., Greene L.: Changes in antipyrine and indocyanine green kinetics during nifedipine, verapamil, and diltiazem therapy. Clin. Pharmacol. Ther., 1986, 40, 239–242.
5. Beridge M.: Calcium: a universal second messenger. Triangle, 1985, 24, 70–90.
6. Cobreros A., Sainz L., Lasheras B., Cenarrzabeitia E.: Hepatotoxicity of ethanol: protective effect of calcium channel blockers in isolated hepatocytes. Liver, 1997, 17, 76–82.
7. Dickinson T.H., Egan J.M., Abernethy D.R.: Effects of nifedipine on hepatic drug oxidation. Pharmacology, 1988, 36, 405–410.
8. Engel G., Hofmann U., Heidemann H., Cosme J., Eichelbaum M.: Antipyrine as a probe for human oxidative drug metabolism: identification of the cytochrome P-450 enzymes catalyzing 4-hydroxyantipyrine, 3-hydroxy-methylantipyrine and norantipyrine formation. Clin. Pharmacol. Ther., 1996, 59, 613–623.

9. Hansten P.D., Horn J.R.: Drug interactions and updates quarterly. Vancouver, WA: Lea & Febiger, Applied Therapeutics Inc., 1993.
10. Hughes B.P., Milton S.E., Barrit G.J., Aul D.A.M.: Studies with verapamil and nifedipine provide evidence for the presence in the liver cell plasma membrane of two types of Ca^{2+} inflow transporter which are dissimilar to potential-operated Ca^{2+} channels. Biochem. Pharmacol., 1986, 35, 3045–3052.
11. Hung B.A., Self T.H., Lalonde R.L., Bottorf M.B.: Calcium channel blockers as inhibitors of drug metabolism. Chest, 1989, 96, 393–399.
12. Kostowski W., Gomułka W.: Mechanism of Drug Action on Regulatory Cellular Processes (Polish). 1st edn. Ed. Kostowski W., PZWL, Warszawa, 1998, 110–142.
13. Kurita K., Tanabe G., Aikou T., Shimazu H.: Inhibition of the increase of intrahepatic Ca^{2+} by diltiazem in rats with liver ischemia. J. Hepatol., 1994, 21, 567–571.
14. Landon E.J., Jaiswal R.K., Naukam R.J., Sastry B.V.R.: Effects of calcium channel blocking agents on membrane microviscosity and calcium in the liver of the carbon tetrachloride-treated rat. Biochem. Pharmacol., 1984, 33, 3553–3560.
15. Landon E.J., Naukam R.J., Sastry B.V.R., Effects of calcium channel blocking agents on calcium and centrilobular necrosis in the liver of rats treated with hepatotoxic agents. Biochem. Pharmacol., 1985, 35, 697–705.
16. Lemasters J.J., Di Giuseppi J., Nieminen A.L., Herman B.: Blebbing free Ca^{2+} and mitochondrial membrane potential preceding cell death in hepatocytes. Nature, 1987, 325, 78–81.
17. Michael A.D., Whiting R.L.: Cellular action of nicardipine. Amer. J. Cardiol., 1989, 64, 3H–7H.
18. Miners J.O., Smith K.J., Robson R.A., McManus M.E., Veronese M.E., Birkett D.J.: Tolbutamide hydroxylation by human liver microsomes: kinetic characterization and relationship to other cytochrome P-450 dependent xenobiotic oxidations. Biochem. Pharmacol., 1988, 37, 1137–1144.
19. Nicotera P., Bellomo G., Orrenius S.: Calcium-mediated mechanisms in chemically induced cell death. Annu. Rev. Pharmacol. Toxicol., 1992, 32, 449–470.
20. Nicotera P., Hartzell P., Davis G., Orrenius S.: The formation of plasma blebs in hepatocytes exposed to agents that increase cytosolic Ca^{2+} is mediated by the activation of a non-lysosomal proteolytic system. FEBS Lett., 1986, 209, 139–144.
21. Paradowski L.: Applicability of the model of extracorporeal rat liver perfusion to the studies into effect of drugs on mixed function monooxygenase system with cytochrome P-450 participation, using cimetidine as an example. Thesis Qualifying for Assistant Professorship, Medical University of Wrocław, Wrocław, 1990, vol. 29/1990.
22. Pasani F., Elliott H.L., Meredith P.A., McSharry D.R., Reid J.L.: Combined alpha adrenoceptor antagonism and calcium channel blockade in normal subjects. Clin. Pharmacol. Ther., 1984, 36, 716–723.
23. Pichard L., Fabre I., Fabre G.: Cyclosporine A – drug interactions: screening for inducers and inhibitors of cytochrome P-450 (cyclosporine A oxidase) in primary cultures of human hepatocytes and in liver microsomes. Drug Metabol. Dispos., 1990, 18, 595–606.
24. Rosenthal T., Ezra D.: Calcium antagonists: drug interactions of clinical significance. Drug Safety, 1995, 13, 157–187.
25. Rumiantsev D.O., Piotrovskii V.K., Riabokon O.S., Slastnikova I.D., Kokurina E.V., Metelitsa V.I.: The effect of oral verapamil therapy on antipyrine clearance. Brit. J. Clin. Pharmacol., 1986, 22, 606–609.
26. Schlanz K.D., Myre S.A., Bottorff M.B.: Pharmacokinetic interactions with calcium channel antagonists (Part I). Clin. Pharmacokinet., 1991, 21, 344–356.
27. Schlanz K.D., Myre S.A., Bottorff M.B.: Pharmacokinetic interactions with calcium channel antagonists (Part II). Clin. Pharmacokinet., 1991, 21, 448–460.
28. Stein H.J., Oosthuizen M.M., Hinder R.A.: Effect of verapamil on hepatic ischaemic-reperfusion injury in normal and glutathione-depleted rats. Gastroenterology, 1989, 96, A663.
29. Striggow F., Bohnensack R.: Verapamil and diltiazem inhibit receptor-operated calcium channels and intracellular calcium oscillations in rat hepatocytes. FEBS Lett., 1993, 318, 341–344.
30. Stringer K.A., Mallet J., Clarke M., Lindenfeld J.A.: The effect of three different oral doses of verapamil on the disposition of theophylline. Eur. J. Clin. Pharmacol., 1992, 43, 35–38.
31. Szeląg A., Paradowski L.: Influence of chenodeoxycholic acid on antipyrine metabolism during extracorporeal liver dialysis in rats (Polish). Gastroenterol. Pol., 1994, 1, 215–220.
32. Thomas C.E., Reed D.J.: Current status of calcium in hepatocellular injury. Hepatology, 1989, 10, 375–384.
33. Wu J., Danielsson A., Lindstrom P., Karlsson K., Sehlin J.: Protective effects of calcium channel blockers on acute bromobenzene toxicity to isolated rat hepatocytes. Inhibition of phenylephrine-induced calcium oscillations. Scand. J. Gastroenterol., 1995, 30, 590–600.

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