Effects of calcium channel blocking agents on tetracycline hepatotoxicity

Kalsiyum kanal blokörlerinin tetrasiklin hepatotoksisitesi üzerine etkileri

Dr. Güner ULAK[1], Dr. Ramazan ÇİÇEK[1], Dr. Atiye VURAL[2], Dr. Naime CANORUÇ[3], Dr. Mustafa ULAK[4]

Departments of Pharmacology[1], Pathology[2], Biochemistry[3], Faculty of Medicine, Dicle University, Diyarbakır and Pendik Goverment Hospital, Department of General Surgery[4], İstanbul, Turkey

ÖZET: *Kalsiyum kanal blokörü ilaçlar olan nifedipin, verapamil ve flunarizinin farelerde tetrasiklin hepatotoksisitesi üzerine etkileri araştırıldı. Fareler rastgele beş gruba ayrıldı. Birinci grup hayvanların içme suyuna 15 gün süreyle 300 mg/kg/gün tetrasiklin ilave edilirken diğerlerine 300 mg/kg/gün tetrasiklin + 50 mg/kg/gün nifedipin, verapamil ve flunarizin ilave edildi.*

Kan örneklerinde AST, ALT ve LDH değerleri ölçüldü, histopatolojik değerlendirme yapıldı. Bu çalışmanın sonuçlarına göre AST ve ALT değerlerini azaltmada nifedipin ve verapamil hemen hemen eşit etkinlik gösterirken flunarizin etki göstermedi. Nifedipin veya verapamil grubunda serum enzimlerindeki azalma hepatoselüler nekrozun azalması ile karakterize histopatolojik bulgularla desteklendi.

Anahtar kelimeler: **Tetrasiklin, kalsiyum kanal blokörleri, hepatotoksisite**

SUMMARY: *The effect of three calcium channel blocking agents-nifedipine, verapamil and flunarizine-on tetracycline-induced hepatotoxicity was studied in mice. Animals were randomly assigned to five groups. In the first group, 300 mg/kg/day tetracycline was added to drinking water, whereas others received 300 mg/kg/day tetracycline plus 50 mg/kg/day nifedipine, verapamil or flunarizine for 15 days. AST, ALT and LDH levels were measured and histopathological evaluation was carried out. The results of this study suggested that both nifedipine and verapamil were almost equally effective to reduce AST and ALT values, whereas flunarizine had no effect. A reduction in serum enzymes was supported by histopathological finding of reduced hepatocellular necrosis in nifedipine-or verapamil-treated mice.*

Key words: **Tetracycline, calcium channel blockers, hepatotoxicity**

MANY cellular processes such as DNA transcription and replication, cytoskeletal regulation, alteration in phospholipid and protein turnover, and regulation of enzyme-dependent pathways are closely related to changes in free cytosolic calcium. Deregulation of calcium homeostasis is an important factor in the development of hepatocellular injury (1). Beneficial effects of calcium channel blockers on the hepatotoxicities induced by various aetiological agents have been reported (2-4). They may protect against liver injury with a reduction in calcium concentration within the hepatocytes. One of the main problems encountered during antibiotic therapy is hepatotoxicity. Tetracycline, erythromycin estolate, rifampicin, isoniasid, paraaminosalicylic acid, sulfonamides and nitrofurantoin are the most leading hepatotoxic antibiotics (5,6). All tetracycline analogues cause necrosis in the liver and lead to fatty liver. Tetracycline hepatotoxicity is generally noticed in patients with renal failure, in pregnant patients and in patients exceeding the suggested dose of 2 g/day (6).

The present study was undertaken to investigate the action of calcium channel blocking agents (CCBA): nifedipine (Ni), verapamil (Ve) and flunarizine (Fl) on tetracycline-induced hepatotoxicity in mice, and thus to find a future role of CCBAs in the management of antibiotic hepatotoxicity.

MATERIALS and METHODS

The mice used in these experiments were male Balb/c mice (30-35 g) from Research Center of Medical Science, Dicle Univ., Diyarbakır, Turkey. The recommendations from the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals are applied in conformity with these principles. Three month aging animals were randomly assigned to five groups (n= 10) as follows and there was not any significant differ-

Geliş tarihi: 8.4.1995, Kabul tarihi: 25.12.1995

Table 1. *Effects of calcium channel blocking agents on tetracycline-induced serum transaminases and lactat dehydrogenase levels in mice (Values are means±SE, n= 10)*

	AST (U/L)	ALT (U/L)	LDH (U/L)
1. Control	251 ± 42	56 ± 8	1740 ± 163
2. Tetracycline	686 ± 129*	152 ± 22*	3059 ± 270*
3. Tetracycline + Nifedipine	309 ± 54**	83 ± 17**	2496 ± 263
4. Tetracycline + Verapamil	279 ± 40**	72 ± 8**	2168 ± 260**
5. Tetracycline + Flunarizine	442 ± 56*	127 ± 16*	3310 ± 243*

* Statistically different from that of control group ($p<0.05$).
** Statistically different from that of tetracycline-treated group ($p<0.05$).

ence among their mean weights ($p>0.05$). Before begining of the experiments we determined that there was approximately 50 ml daily water consumption for each group. In addition we assumed that each mouse would drink approximately equal amount of water. However, at the end of the experiment, it would be accepted that there was not any difference among the water consumption of the mice, although this amount would be different from day to day. Therefore, the investigated drugs were added to these determined amounts of water every day. In the first group, the optimal hepatotoxic dose of 300 mg/kg/day tetracycline (Doxycycline HCl, 4020334, Fako) (7) was added to drinking water, wehereas the others received 300 mg/kg/day tetracycline plus 50 mg/kg/day nifedipine (DIF, DI), verapamil (Knoll AG, HHW) or flunarizine (Eczacıbaşı, 155/79) for 15 days. The last group received only tap water. During this period, all mice were fed with standart mouse food. After 15 days, intracardiac blood was taken from all of the mice under light ether anaesthesia and serum transaminase (AST, ALT), and lactat dehydrogenase (LDH) levels were measured by Abbott spectrum 2 auto analyser. For histopathologic estimates of cell damage caused by tetracycline, lobes of liver were fixed in 10% formalin, sectioned and stained with hematoxylin and eosin. Light microscopic examinations with 40x, 100x, 200x and 400x magnification were evaluated.

Student's t-test and one way variance analysis were used to determine any statistical significance.

RESULTS

Tetracycline given at 300 mg/kg/day for 15 days resulted in liver injury in mice as judged by up to three, three and two fold elevations in liver enzymes AST, ALT and LDH, respectively in plasma (Table 1). These findings point to the presence of hepatic damage in these mice.

Ni or Ve administered orally with tetracycline significantly ($p<0.05$ and $p<0.02$, respectively) lowered AST levels. No significant difference was found among the AST values of Ni, Ve and control ($p>0.05$). Fl failed to reduce tetracycline-induced increment in AST levels ($p> 0.05$), and there was a significant difference between Fl and control ($p<0.02$) or Ve ($p<0.05$).

Ni and Ve reduced tetracycline-induced increment of ALT levels significantly ($p<0.05$ and $p<0.01$), whereas Fl failed to lower it ($p>0.05$). Therefore ALT levels in Fl treated group was higher than those in control values ($p<0.005$). No significant difference was found among the control, Ni and Ve groups ($p>0.05$). Significant difference was found between ALT levels of Fl and Ve treated groups ($p<0.01$).

Tetracycline-elevated LDH levels were lowered by Ve ($p<0.05$), but Ni or Fl failed to show this effect ($p>0.05$). Significant difference was found between LDH values of Fl and Ni or Ve groups ($p<0.05$).

Histopathological structures of the liver were also studied in each group, using light microscopy. On microscopic evaluation of these groups, normal liver tissue samples were found in control group. Hydropic degeneration of hepatocytes, nuclear pleomorfism, microvesicular lipid accumulation, advanced kupffer cell activation, evident dilatation and congestion in sinusoides, few fecal necrotic lesions, mononuclear cell infiltration in portal tract, congestion and dilatation of hepatic artery and vein were determined in tetracycline-treated groups. Slight hydropic degeneration of hepatocytes, slight nuclear pleomorfism without fatty changes, slight kupffer cell activation, dilatation and congestion in sinusoides, oedema in

portal tract, slight hepatic artery and vein dilatation and congestion was observed in Ni and Fl treated groups. In Ve treated group normal hepatocytes, very slight kupffer cell activation, normal sinusoides, slight dilatation and congestion of hepatic artery and vein were observed.

DISCUSSION

Raised intracellular calcium level is the common pathway in hepatocellular damage by ischemia or toxic agents (8,9). CCBAs have been shown to be active on especially L-type voltage-operated calcium channels, and thus reduce calcium influx. They exert their cytoprotective effects through several mechanisms such as blockade of L-type voltage-operated calcium channels (10,11) and thus inhibition of oxygen radicals formation, reduction of oxidative stress (12), antagonism at inflammatory mediator receptor sites (13,14) and interaction at other intracellular sites.

Uncontrolled cellular calcium influx is a critical factor in the pathogenesis of hepatic ischemic injury (15). Studies relating to the liver sugest that CCBAs may limit hepatocellular damage especially those arising from toxic agents (2,8). A diversity of toxic agents are known to alter tissue Ca^{++} content significantly (16,17). As a result, cytosolic Ca^{++} concentration become elevated in liver cells. Raised intracellular calcium levels have been shown to potentiate cell damage by free oxygen radicals (12,15), decrease mitochondrial ATP synthesis, activate Ca^{++} AT Pase, and as a result of depletion of energy reserves, the cell dies (2). Administration of tetracycline to the rat is associated with large increases in the calcium content of rat liver, and thus potentiate lipid peroxidation products which in turn elevate the reactive oxygen particles (18).

Elevation in liver enzymes point to the presence of hepatic damage (9). Toxic doses of tetracycline have been shown to elevate AST, ALT and alkaline phosphatase in rats and mice (7,18). In our study, 300 mg/kg/day tetracycline given for 15 days increased AST, ALT and LDH values up to three, three and two folds, respectively. Ni and Ve diminished biochemical markers of liver injury significantly, and this was supported by histopathological findings of reduced hepatocellular necrosis.

A derivative of 1,4-dihydropridine Ni and phenylalkylamine Ve are selective CCBAs, so they are more spesific, inhibiting calcium current with little effect on sodium current. Fl, a non selective CCBA is less selective and has significant effects on sodium currents before calcium current block is complete (10). In our study, it failed to lower the elevation in liver enzymes induced by tetracycline; however, it has been shown to be protective against calcium-mediated cell death in neuronal cultures by inhibition of sodium channels (19).

Since biochemical observations were supported by histopathological findings of reduced hepatocellular necrosis in Ni and Ve treated mice, we suggest that selective CCBA Ni and Ve may have utility in the treatment of tetracycline-induced liver injury, and the effect of Ve to reduce LDH values and to effectively attenuate hepatocellular necrosis may favour the potential value of this drug in the management of tetracycline hepatotoxicity.

REFERENCES

1. Lauterberg BH. Early disturbance of calcium translocation across the plasma membrane in toxic liver injury. Hepatology 1987; 7:1179-1183.
2. Deakin CD, Fagan EA, Williams R. Cytoprotective effects of calcium channel blockers. J Hepatol 1991; 12:251-255.
3. Garay GL, Annesley P, Burnette M. Prevention of experimental liver injury in rats by nicardipine (N), a calcium entry blocker. Gastroenterology 1984; 86:1319(Abst).
4. Ulak M, Ulak G, Çiçek R, Nergiz Y, Yenice N. The protective effect of diltiazem against carbon tetrachloride and thioacetamide induced liver injury. Gastroenteroloji 1994; 5:139-143.
5. Sorensen EMB and Acosta D. Erythromycin estolate-induced toxicity in cultured rat hepatocytes. Toxicology Letters 1985; 27:73-82.
6. Standiford HC. Tetracyclines and chloramphenicol. Principles and Practice of Infectious Diseases (Eds. GL Mandell, RG Douglas, JE Bennett) Wiley Medical Publications. New York 1990 pp:284-295.
7. Felek S, Kılıç SS, Özercan MR, Ocak S, Yekeler H, Akbulut A, İlhan N. Prevention of tetracycline hepatotoxicity by Nicardipine. Tr J of Medical Sciences 1994; 22:25-29.
8. Landon EJ, Naukam RJ, Sastry BVR. Effects of calcium channel blocking agents on calcium and centrilobular necrosis in the liver of rats treated with hepatotoxic agents. Biochem Pharmacol 1986; 35:697-705.
9. Smejkalova J, Simek J, Rouchal J Dvorackova J. The time course of biochemical and histological changes following carbon tetrachloride-induced liver damage in rats of both sexes. Physiol Bohemoslov 1985; 34:494-501.
10. Janis RA, Triggle DJ. Drugs acting on calcium channels. In: Calcium channels: Their properties, functions, regulation and clinical revelance (Eds. L Hurwitz, LD Partridge, JK Leach). Florida, CRC Pres, 1991 pp:195-249.
11. Mauger JP, Claret M. Calcium channels in hepatocytes. J Hepatol 1988; 7:278-232.
12. Malis C, Banvetre JM. Mechanism of calcium potentiation of oxygen free radical injury to renal mitocondria. J Biol Chem 1988; 261:14201-14208.

13. Mayhan C, Joyner WI. The effect of altering the external calcium concentration and a calcium channel blocker, verapamil, on microvascular leaky sites and dextran clearance in hamster cheek pouch. Microvasc Res 1984; 28:159-179.

14. Weichman BM, Muccitelli RM, Tucker SS, Wasserman MA. Effects of calcium antagonists on leukotriene D4 induced contractions of the guinea pig trachea and lung parenchyma. J Pharmacol Exp Ther 1988; 225:310-315.

15. Stein HJ, Oosthuizen MM, Hinder RA. Effect of verapamil on hepatic ischaemia-reperfusion injury in normal and glutathione depleted rats. Gastroenterology 1989; 96:663 (Abstr.).

16. Farbar JL. Reactions of the liver to injury. In: Toxic injury of the liver, Part A (Eds. E Farber, MM Fischer). New York, Marcel Dekker Inc., 1980, pp:215-241.

17. Gallagher CH, Gupta CN, Judah JD, Rees KR. Biochemical changes in liver in acute thioacetamide intoxication. J Pathol Bacteriol 1956; 72:193-201.

18. Skakun NP, Oleinik AN, Shman'ko VV, Koval'chuk NA. Koval'chuk SF. Effect of ethyl alcohol on bile production and lipid peroxidation in tetracycline-induced liver lesions. Antibiot Med Biotechnol 1985; 30:115-118.

19. Pauwels PJ, Van Assouw HP, Leysen JE, Janssen PAJ. Ca2+ mediated neuronal death in rat brain neuronal cultures by veratridine: Protection by flunarizine. Mol Pharmacol 1989; 36:525.