INVIVO HEPATOPROTECTIVITY OF INTRAVENOUS VERAPAMIL DURING HYPOXIC HALOTHANE ANAESTHESIA IN DOGS

(Received October 5 1990)

N.Çiftci, M.D. **** / F.Y. Göğüş, M.D.* / S.Küllü, M.D.** / Ö. Aktan, M.D. ***

* *Associate Professor, Department of Anesthesiology and Reanimation, Faculty of Medicine, Marmara University, Istanbul, Turkey.*

** *Associate Professor, Department of Pathology, Faculty of Medicine, Marmara University, Istanbul, Turkey.*

*** *Associate Professor, Department of General Surgery, Faculty of Medicine, Marmara University, Istanbul, Turkey.*

**** *Research Assistant, Department of Anesthesiology and Reanimation, Faculty of Medicine, Marmara University, Istanbul, Turkey.*

SUMMARY

The hepatic protective effect of intravenous verapamil in dogs (n=8) during halothane anesthesia in hypoxic conditions was observed. The protective potential was defined by both histopathological evaluation and serum enzyme level measurements. Mean alkaline phosphatase and lactic dehydrogenase enzyme levels in the group administered with calcium channel blocker-verapamil were noted,although not statistically significant, to be lower than those of the control group.

Further prenchymal injury during histopathological evaluation using the light microscope were noted to be significantly less in the verapamil group administered when compared to the control group.

Key Words: Calcium channel blockers: verapamil, Liver: hepatoprotectivity, Anesthetics, inhalational: halothane, Hypoxia, Damage: hepatic.

INTRODUCTION

Although infrequent, since 1884 there have been case reports regarding general anesthesia induced liver toxicity (1). Halothane hepatitis has been accepted as a clinical entity which may differ in severity from a mild icterus or hepatic dysfunction to fatal liver insufficiency. Since 1963, when halothane became a routine anesthetic agent many cases of postoperative hepatitis or liver toxicity have been reported. Actiological factors including hypoxia, genetical factors, age, sex, obesity, successive administration and surgical procedure, may directly influence the incidence and severity of halothane hepatitis. Hypoxia which may occur due to decreased hepatic blood flow, central respiratory depression or intervention of ventilation perfusion rate by general anesthesia is thought to be the most important of all the causes of hepatic injury. On the other hand it has been shown by several experimental studies that calcium blockers provide significant protection for the related vital organs against hypoxia during ischemia due to surgical actiological factors (2-7).

In this present study, our aim is to determine the hepatoprotective effects of an intravenous calcium blocker, verapamil during halothane administration to dogs under hypoxic conditions.

MATERIAL AND METHODS

The study was performed in the Laboratory of Experimental Medicine at Marmara University. 16 dogs of both sexes varying in body weight between 17-20.5 kg were used. They were divided into two groups. An intravenous route was obtained using a 18 G angiocatheter and saline was infused prior to anesthesia induction by 15 mg/kg thiopental intravenously. Then endotracheal intubation was performed. The ventilation rate was maintained at approximately 30/pm. In all subjects the anesthesia was maintained at 30% oxygen, 70% nitrous oxide, 1% halothane using a bag, a Boyle anesthetic machine and an Abbott halothane vaporizer. Placing precordial electrodes on the anterior thoracic wall the heart was continuously monitored in all subjects. Laparatomic observation of the dogs was performed in a supine position under sterile conditions.

Protective effects of verapamil during halothane anesthesia on hepatic functions and the parenchyma

was observed in 16 dogs that been randomly divided into 2 groups (n=8).

Group I containing 8 dogs was the control group, having no agent administered during or following induction except thiopental.

Group II consisted of the remaining 8 dogs that were the subjects in the experiment. They were given verapamil 150 mikrogr/kg intravenously immediately after induction of anesthesia.

Prior to induction, blood specimens from every subject in each group were obtained to determine the control levels of the serum enzymes, alkaline phosphatase and lactate dehydrogenase. Half an hour after laparatomy, liver biopsy was performed and the dogs were ventilated using a hypoxic gas mixture of 14% oxygen, 86% nitrous oxide, 1% halothane for 30 minutes at the same ventilation rate. The hypoxemia which occured during this period was confirmed by measuring PO2, PCO2 and pH levels of the arterial blood samples and using 'Nova Biomedical Stat Profile 3". At the end of the hypoxic period further blood samples were obtained in order to define any changes in enzyme levels and the lever biopsy was repeated to access any changes in the liver parencyhma. Liver biopsy material was fixed using 10% formaldehyde, sections of 8 mikron thickness were prepared and stained using hematoxylin- eosine and then they were observed using the light microscope. The histopathological evaluation of the liver parenchyma was performed in the light of the parameters in table I.

The data of each subject was compared with the data of other subjects in the same group. Further the result of each group were compared using The Student t test.

RESULTS

The serum enzymes, alkaline phosphatase and lactic dehydrogenase levels of the control group and the group administered with intravenous verapamil prior to and following hypoxic conditions are shown in table II and III respectively.

In the control group, all these enzyme levels were significantly higher than those of the verapamil administered group at the posthypoxic period ($p<0.01$). In the verapamil administered group, only SGOT and SGPT enzyme levels were statistically significantly higher at the posthypoxic period than control values ($p<0.01$).

Histopathological evaluation of the liver biopsies of the control group and the study group are shown in table IV and table V respectively.

The signs of cellular damage observed on the specimens which are taken during the posthypoxic period showed significant differences between the two groups. In the verapamil administered group the intensity of signs showing cellular damage such as fatty degeneration, intracellular oedema and macrovacuolisation were significantly lower than those of the control group (Figure 1,2.)

DISCUSSION

Although a perfused organ model is known to be a practical, cheaper and rapid way during the observation of hypoxic injury, our study was performed using an invivo model to be able to determine the serum enzyme levels.

Not only the hepatotoxicity of halothane but also the potential of hypoxia to enhance the hepatic injury in halothane anesthesia have been confirmed by several studies. In other words it has been known that the degree of perianesthetic hepatic injury caused by halothane administration is directly proportional to the degree of hypoxia (8). Since hypoxia alone may cause tissue injury in experimental models the inhalating oxygen concentration is recommended to be no lower than 10%.

Hepatic hypoxia in anesthesia is a potent stimulus for lysosomal derangment, leakage of cytoplasmic enzymes and finally proteolysis (9, 10). On the other hand hypoxia causes vasoconstriction and increases capillary permeability thus allowing extravasation into the extracellular space (11). There are some indications of the important role of intracellular calcium deposition in the aetiology of cellular damage during hypoxia regarding dysfunction of the calcium pump (11-17)

Decreased ATP level during hypoxia causes hypoactivity of the calcium pumps in cell membrane, endoplasmic and mitochondriae, thus causing maldistribution of calcium in the subcellular compartments (17,18). It has been known that increased intracellular calcium causes cell death, decreased metabolic product clearance and reticuloendothelial depression (11).

If the cell is traumatised by ischemia, hypoxia or toxic agents calcium influx causes cell death (15, 16).

TABLE I. The parameters of histopathological evaluation of the liver parenchyma.

Cell damage (cell inflation, cytoplasmic granularity,vaculisation)			+	++	+++
Fatty degeneration	Diffuse		+	++	+++
	Zonal	Peripheral	+	++	+++
		Midzonal	+	++	+++
		Centrizonal	+	++	+++
Necrosis	Diffuse		+	++	+++
	Focal		+	++	+++
	Single cell		+	++	+++
Kupffer cell hyperplasia			+	++	+++

Early studies on this subject using calcium blocker agents in the hope of prevention of cellular damage in such conditions showed encouraging correlation with the use of these agents and prevention of the tissues from either hypoxic, ischemic or toxic injuries (14). Thurman and King (13) and Thurman and Apel (19) in their studies on perfused rat liver concluded that nifedipine has a significant hepatoprotective effect in hypoxia and may be used in several clinical situations due to hypoxia.

In another study by Michael et al. (20) it has been shown that nifedipine, nitrendipine and verapamil promptly relieve the physiological and pathological changes caused by hypoxia due to pulmonary vasoconstriction and chronic alveolar hypoxia in rats.

The study of Landon et al. (14) revealed that verapamil, nifedipine and nitrendipine decrease the hepatic injury centrlobular necrosis caused by carbontetrachloride.

In the present study disregarding the hepatoprotective effect the increase in serum enzyme levels in the hypoxic verapamil group is thought to be associated with systemic effects of hypoxia and effects of anesthesia or surgery since these enzymes are not specific to the liver. In contrast the control group (hypoxic group without verapamil) shows no increase in alkaline phosphatase and lactic dehydrogenase levels, of this group (hypoxic plus verapamil) almost confirms this hypothesis.

Important signs of acute hepatocellular damage observed under the light microscope due to hepatotoxic agents are fatty degeneration, intracellular oedema, hyperplasia of the Kupffer cells, micro and macrovacuolisation and necrosis. In the present study we observed some of these signs as well as in the prehypoxic period in both two groups. It is possible that there are reversible changes caused by anesthetic agents or preexisting lesions of nutritional origin (21). These signs of cellular damage observed on the specimens which are taken during the posthypoxic period showed significant differences in the two groups. In the verapamil administered group the intensity of signs showing cellular damage was significantly lower than those of the control group.

The results of the present study suggest that the hepatotoxic effects of hypoxic halothane anesthesia may be prevented by verapamil administration. On the other hand this conclusion must be confirmed by further ultrastructural studies.

TABLE II. The serum enzymes, alkaline phosphatase and lactic dehydrogenase levels of the control group

	SGOT (U/1)		SGPT (U/1)		Alkaline phosphatase (U/1)		Lactic dehydrogenase (IU/1)	
I. Group	**Before hypoxia**	**After hypoxia**	**Before hypoxia**	**After hypoxia**	**Before hypoxia**	**After hypoxia**	**Before hypoxia**	**After hypoxia**
1	39	110	76	117	79	265	230	352
2	31	32	33	30	100	79	68	62
3	40	40	33	23	127	110	164	149
4	40	163	33	145	97	140	152	195
5	34	12	28	12	101	99	180	99
6	14	33	23	44	67	43	52	149
7	25	61	32	50	125	170	176	195
8	41	70	28	50	89	140	115	172
	33.00±9.47	65.12±49.59*	35.75±2.64	58.87±47.04*	98.12±20.65	130.75±66.75*	142.12±60.03	171.62±82.06*

* $p<0.01$

TABLE III. The serum enzymes, alkaline phosphatase and lactic dehydrogenase levels of the group treated with intravenous verapamil

	SGOT (U/1)		SGPT (U/1)		Alkaline phosphatase (U/1)		Lactic dehydrogenase (IU/1)	
II. Group	**Before hypoxia**	**After hypoxia**	**Before hypoxia**	**After hypoxia**	**Before hypoxia**	**After hypoxia**	**Before hypoxia**	**After hypoxia**
1	37	42	23	28	169	175	245	255
2	30	46	20	50	33	63	349	284
3	41	65	32	50	135	128	205	195
4	37	31	29	33	130	100	76	68
5	36	91	53	67	166	80	170	125
6	40	152	26	99	215	240	123	145
7	39	95	61	76	202	145	130	169
8	36	87	82	123	189	153	329	185
	37.00±3.38	76.12±39.06*	40.75±22.16	65.75±32.69*	154.8±64.21	135.5±68.58	203.25±88.54	173.75±86.18

$p<0.01$

TABLE IV. The histopathological evaluation of the liver biopsies of the control group

	Cell inflation		Cytoplasmic granularity		Cytoplasmic vacuolisation		Fatty degeneration		Necrosis		Kupffer cell hyperplasia		Congestion	
	Hypoxia		Hypoxia		Hypoxia		Hypoxia		Hypoxia		Hypoxia		Hypoxia	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	-	-	++	++	-	-	+	++	-	-	+	++	+	+
2	-	-	+	++	-	-	-	-	-	-	+	+	+	+
3	+	+	+	+	-	-	-	-	-	+*	+	++	-	-
4	+	+	++	++	-	+	+	+	+*	+*	+	+	+	+
5	+	+	++	++	-	-	-	-	-	+*	+	+	-	-
6	+	+	++	++	++	+	+	+	-	-	+	+	+	+
7	+	+	++	++	-	-	+	++	-	-	+	++	+	+
8	-	-	+	+	-	+	+	+	-	-	+	+	-	-

*** Focal necrosis**

TABLE V. The histopathological evaluation of the liver biopsies of the group treated with intravenous verapamil

	Cell inflation		Cytoplasmic granularity		Cytoplasmic vacuolisation		Fatty degeneration		Necrosis		Kupffer cell hyperplasia		Congestion	
	Hypoxia		Hypoxia		Hypoxia		Hypoxia		Hypoxia		Hypoxia		Hypoxia	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	++	++	++	+	+	-	++	++	-	-	+	+	+	-
2	+	+	+	+	+	+	-	-	-	-	-	-	+	-
3	+	+	+	-	-	-	-	-	-	-	+	-	-	-
4	+	+	+	-	-	-	-	-	-	-	-	-	-	-
5	+	-	-	-	-	-	-	-	-	-	+	+	-	-
6	+++	++	+++	++	-	-	+	+	-	+	+	+	-	-
7	++	++	++	++	+*	+*	-	-	+	+	-	+		
8	++	++	++	+	-	-	+	+	+**	+**	+	-	+	+

*** Diffuse fatty degeneration**

**** Focal necrosis**

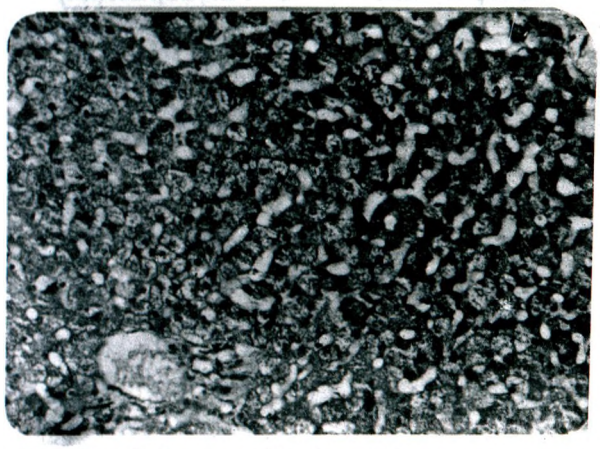

Fig. 1. Diffuse granulation (arrow), oedema and vacuolisation in the hepatocytes during posthypoxic period in the control group. (HE X200)

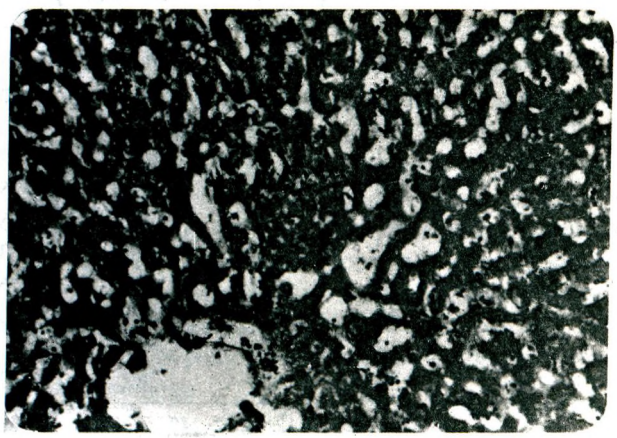

Fig. 2. Mild granulation and oedema in the hepatocytes during posthypoxic period in the verapamil treated group. (HE X200)

REFERENCES

1. *Defalque RJ. The first delayed chloroform poisoning. Anest Analg 1968; 47: 374-375.*
2. *Hock CE, Su JY, Lefer AM. Salutary effects of nitrendipine, a new calcium entry blocker, in hemorrhagic shock. J Pharmacol 1984; 97:37-46.*
3. *Cefer AM, Carrow BA. Salutary actions of nimedipine in traumatic shock. Life Sci 1981; 29: 1347-53.*
4. *Higgins AJ, Blackburn KJ. Perevention of reperfusion damage in working rat hearts by calcium antagonists and calmoduline antagonists. J Moll Cell Cardiol 1984; 16: 427-38.*
5. *Crottogini AJ., Depaoli JR, Barra JG, Fischer EC, Chatruc MR, Pichel RH, Delafuente L. The effect of the new calcium antaqonist nisoldipine (BAY.k-5552) on myocardial infarct size limitation in conscious dogs. Am Heart J 1985; 110: 753-60.*
6. *Gelmers HJ. Effect of nimodipine on the clinical course of patients with acute ischemic stroke. In: Betz E, Deck K, Hoffmeister F, eds. Nimodipine: Pharmacological and Clinical Properties. F.K. Stuttgart: Schattauer Verlag 1985; 467-71.*
7. *Peck RC, Lefer AM. Protective effect of nifedipine in the hypoxic perfused cat liver. Agents Actions 1981; 11: 421-24.*
8. *George E, Mc Lain MD, Sipes IG, Brown BR. An animal model of halotan hepatotoxicity roles of enzyme induction and hypoxia. Anesthesiology 1979; 51: 321-26.*
9. *Neely AN, Montimore GE. Localization of products of endogenous proteolysis in lysosomes of perfused rat liver. Biochem Biophys Res Comm 1974; 59: 680-87.*
10. *Carlson RP, Lefer AM. Hepatic cell integrity in hypodynamic states. Am J Physiol 1976; 231: 1408-14.*
11. *Lefer AM, Stahl GL. Mechanism of hepatoprotective effect of nitrendipine in the isole perfused cat liver. J of Cardiovas Phar 1987; 9 (Suppl.4): 66-70.*
12. *Lemasters JJ, Stemkowski CJ, Sungchol SI, Thurman RG. Cell surface changes and enzyme release during hypoxia and reoxygenatin in the isolated perfused rat liver. J of Cell Bio 1983; 97: 778-786.*
13. *Thurman RG, King JN, Lemasters JJ. Nitrendipine protects the liver against hypoxi ainduced damage at submicromolar concentrations in the perfused rat liver. J Cardiovas Phar 1987: 71-76.*
14. *Landon EJ, Naukam RJ, Sastry VR. Invivo effects of nitrendipine on hepatotoxic cell injury in rats. J Cardiovas Phar 1987: 77-84.*
15. *Schanne F, Kane AB, Young EE, Farber JL. Calcium dependence of toxic cell death a final com-*

mon pathway. Science 1979; 206: 700-2.
16. *Farris MW, Reed DJ. Mechanism of chemical induced toxicity II. Role of extracellular calcium. Toxicol Appl Pharmacol 1985; 79: 296: 306.*
17. *Schanne AX, Kane AB, Young EE, Farber JL. Calcium dependence of toxic cell death: A final pathway. Science 1979; 206: 700-2.*
18. *Chien KR, Pfau R, Farber JL. Ischemic myocardial cell injury. Am J Pathol 1979; 97: 502-22.*
19. *Thurman RG, Apel ED, Lemasters JJ. Protective effect of nitrendipine against hypoxic injury in perfused livers from ethanol treated rats. J Cardiovas Phar 1988; 1113-116.*
20. *Michael JR, Kennedy TP, Buescher P, Farrukh I, Rock PC, Gurtner G, Monteand SM, Hutchins GM. The effect of treatment with nitrentipine and other calcium channel blockers on the hysiologic and pathologic changes caused by hypoxia in rats. J Cardiovas Phar 1987; 61-65.*
21. *Cotran RS, Kumar V, Robbins SL. Pathologic Basis of Disease. W.B. Saunders Company. 4th Ed. 1989; 1-38.*