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Effects of nimodipine and pentoxyfylline in prevention of hepatic ischemic damage in rats at normal and hypothermic conditions

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ABSTRACT

Hepatic ischemia should be considered in serious liver injury, liver tumor resection and liver transplantation. There are other conditions that decrease hepatic blood flow and cause hepatic ischemia, such as hemorrhagic shock, sepsis, hepatic artery ligation, trauma, and certain vascular lesions. In this study, effects of nimodipine (a calcium channel blocker) and pentoxyfylline (a derivative of methylxanthine) on duration and degree of hepatic ischemia in rats at normothermic and hypothermic conditions are investigated.

This study was performed on 6 groups of Wistar Albino type rats, each group consisting of 7 rats. Groups were separated into normothermic (A) and hypothermic (B) conditions AI—Control group, AII—Nimodipine group and AIII—Pentoxyfylline group, B IV—Control group, BV—Nimodipine group and BVI—Pentoxyfylline group respectively. After hepatic pedicle occlusion lasting 45 min, blood samples were drawn from the rats for evaluation of alanine aminotransferase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH) values. Moreover, hepatic biopsies were taken to assess pathological changes under electron microscopy. These changes were evaluated through a grading system.

As a result; it has been shown that both nimodipine and pentoxyfylline delayed effects of hepatic ischemia in a statistically significant manner in comparison with the control group and these effects were found to be more significant in hypothermic conditions.

1. Introduction

Liver injuries, liver tumor resection, and liver transplantation are responsible for hepatic ischemia. Also there are some other conditions that decrease hepatic blood flow and cause hepatic ischemia such as hemorrhagic shock, sepsis, hepatic artery ligation, trauma and some vascular lesions. Current surgical practice gives us the opportunity to perform partial liver resection, which is a reliable method for the treatment of benign and malignant liver diseases. The mortality of liver resection decreased below 5% through innovations in the surgical field and a better understanding of liver anatomy and physiology (Schlee and Altendorf-Hofmann, 2000; Schwartz, 1999).

Several recent studies have been carried out to find ways to delay hepatic ischemia and avoid severe damage to liver tissue (Mc Curry et al., 1993; Hamamoto et al., 1993; Walsh et al., 1990). In this study pentoxyfylline and nimodipine were used in normothermic and hypothermic conditions to prevent hepatic injury and delay the ischemic period. Calcium plays a major role in inflammatory reactions and the generation of free oxygen radicals (Packer and Glazer, 1990; Cheeseman and Slater, 1993; Halliwell, 1989). Therefore a calcium channel blocker nimodipine was used to control inflammation and generate of free oxygen radicals. The other agent used—pentoxyfylline—is a derivative of methylxanthine which also decreases the hazardous effects mentioned above by regulating microvascular circulation. Pentoxyfylline also increases the flexibility of erythrocytes and leucocytes while inhibiting platelet aggregation and neutrophil infiltration (Vadiel et al., 1989; Waxman et al., 1991). The effects of ischemia on hepatic tissue were evaluated under an electron microscope. The experiments were performed under normo and hypothermic conditions. During this study, the hepatic artery and the vena porta were occluded for 45 min via a surgical instrument (Pringle manoeuvre). The differences in electron microscopic examinations of the control group, the nimodipine group and the pentoxyfylline group were evaluated. Blood samples drawn preoperatively and after the hepatic pedicle occlusion were tested for alanine aminotransferase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH) levels.

Previous studies have shown that Pentoxyfylline reduced TNF—alpha and alanine aminotransferase (ALT) levels in patients with Non-Alcoholic Steatohepatosis (Satapathy et al., 2007). Peng et al. reported that pentoxyfylline treatment reduced hepatic injury and improved survival after normothermic ischemia and reperfusion (Peng et al., 1995).

In this study, effects of nimodipine, as a calcium channel blocker, and pentoxyfylline, as a derivative of methylxanthine, which can be

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Table 1
AST, ALT and LDH levels in hypothermic and normal conditions

Rat	Normothermic/normal			Hypothermic/normal			Normothermic/ischemic			Hypothermic/ischemic		
	AST	ALT	LDH	AST	ALT	LDH	AST	ALT	LDH	AST	ALT	LDH
1	190	89	500	180	85	470	750	650	6000	640	440	4000
2	195	85	510	190	85	460	760	640	5500	630	450	4100
3	198	87	520	190	90	470	770	660	6200	650	460	3700
4	193	93	480	185	90	460	740	640	5700	670	430	4200
5	195	95	510	190	90	450	760	650	6800	680	470	3500
6	199	89	505	190	85	470	750	660	6300	690	440	3000
7	201	88	490	195	85	480	780	680	5900	700	450	2800
Mean	195.9	89.4	502	188.6	87.1	465.7	758.6	654.3	6057	665.7	448.6	3614.3
P	AST 0.0079, ALT 0.1916, LDH<0.0001						AST, ALT and LDH<0.0001					

considered as a regulator of circulation, on duration and degree of hepatic ischemia in rats at normothermic and hypothermic conditions are investigated.

2. Materials and methods

This study was performed on 6 groups of Wistar Albino type rats, each group consisting of 7 rats. Groups were separated into two main groups normothermic and hypothermic with control, nimodipine and pentoxyfylline subgroups; I—normothermic Control group, II—normothermic Nimodipine group and III—normothermic Pentoxyfylline group, IV—hypothermic Control group, V—hypothermic Nimodipine group and VI—hypothermic Pentoxyfylline group. Preoperative blood samples for AST, ALT, and LDH levels were determined. After 12 h of starvation, median laparotomy was performed under ether anesthesia to all rats. Before the operation, 0.5 ml NaCl was administered to the control group intraperitoneally (i.p.). 2.5 mg/kg nimodipine+0.5 ml NaCl (i.p.) and 40 mg/kg pentoxyfylline+0.5 ml NaCl (i.p.) were administered to the nimodipine and pentoxyfylline groups respectively. After the hepatic pedicle occlusion of 45 min, blood samples were drawn from the rats for evaluation of ALT, AST and LDH values in arbitrary units. In the second stage of the study same procedures were performed in hypothermic conditions. Hypothermia was achieved by placing ice cubes into the abdomen, especially around the liver. Hepatic biopsies were taken to observe tissue changes under electron microscopy. These changes were evaluated by grading them individually. Statistical evaluations were done with ANOVA.

3. Results

Mean values for blood test results in normothermic and hypothermic conditions were calculated. Mean blood values for normothermic conditions were as follows: AST 195.9±3.8 U/l (mean±S.D.), ALT 89.4±3.5 U/l, LDH 502±13.5 U/l. Mean values for hypothermic conditions were calculated as AST 188.6±4.8 U/l (mean±S.D.), ALT 87.1±2.7 U/l, and LDH 465.7±9.8 U/l. Statistical comparison of these values were performed with ANOVA post hoc analysis ($P=0.0079$ for AST, $P=0.1916$ for ALT and $P<0.0001$ for LDH). Only the aspartate transaminase and lactate dehydrogenase levels showed statistically significant difference.

When blood samples were taken in normothermic and ischemic conditions mean enzyme values were significantly higher (AST 758.6±13.5 U/l, ALT 654.3±14 U/l, LDH 6057±427.6 U/l) when compared to enzyme levels in hypothermic ischemic conditions (AST 665.7±26.4 U/l, ALT 448.6±13.5 U/l, LDH 3614.3±546 U/l). We obtained $P<0.0001$ for AST, $P<0.0001$ for ALT and $P<0.0001$ for LDH according to ANOVA. The comparison of these values showed statistically significant differences between normothermic ischemic and hypothermic ischemic conditions (Table 1).

When pentoxyfylline was administered in normothermic ischemic conditions, enzyme levels were lower than previous conditions: AST 371.4±19.5 U/l, ALT 322.9±40.3 U/l, LDH 3714.3±195.2 U/l. In the hypothermic pentoxyfylline group these levels were even lower: AST 295.7±15.1 U/l (mean±S.D.), ALT 268.6±34.4 U/l, LDH 2728.6±189 U/l (Table 2). The comparison of these results showed statistically significant differences with ANOVA (AST $P<0.0001$, ALT $P<0.0189$, LDH $P<0.0001$).

The following enzyme levels were reported in blood samples with nimodipine administration in normothermic conditions: AST 431.4±33.3 U/l, ALT 365.7±13.5 U/l, LDH 3971.4±269 U/l. The values in hypothermic nimodipine group were AST 365.7±33.1 U/l, ALT 261.4±13.5 U/l, LDH 2957.1±171.8 U/l. The comparison of these results showed statistically significant differences with ANOVA (AST $P=0.0030$, ALT $P<0.0001$, LDH $P<0.0001$). These results are depicted in Table 2.

The histological evaluations and comparisons of the six groups were carried out. No electron microscopic degenerative changes were observed in normothermic or hypothermic conditions, but in normothermic and ischemic conditions, loss of crista and necrosis in mitochondria, mixed pattern and necrosis in GER, indistinct appearance and loss of sinusoids in sinusoidal area were observed. In hypothermic and ischemic conditions there were changes in shape and loss of crista in mitochondria, mixed and irregular pattern in GER, and dilatation and indistinct appearance in sinusoids. When pentoxyfylline was administered in normothermic conditions, changes in the shape of mitochondria, irregular pattern in GER, dilation of sinusoids were found whereas in pentoxyfylline administration in hypothermic

Table 2
AST, ALT and LDH levels in hypothermic and normal conditions, with application of nimodipine and pentoxyfylline

Rat	Normothermic/normal			Hypothermic/normal			Normothermic/ischemic			Hypothermic/ischemic		
	AST	ALT	LDH	AST	ALT	LDH	AST	ALT	LDH	AST	ALT	LDH
1	400	300	3900	300	270	2700	450	400	4100	300	250	2900
2	350	310	3800	290	280	2500	490	390	4200	350	260	3000
3	390	320	3700	310	290	2700	400	380	4100	370	270	3100
4	380	350	3500	280	250	2800	450	370	4000	380	260	2800
5	370	400	4000	320	200	2900	400	350	3700	390	280	2700
6	360	300	3500	280	300	3000	420	340	4200	400	270	3000
7	350	280	3600	290	290	2500	410	330	3500	370	240	3200
Mean	371.4	322.9	3714.3	295.7	268.6	2728.6	431.4	365.7	3971.4	365.7	261.4	2957.1
P	AST<0.0001, ALT=0.0189, LDH<0.0001						AST=0.0030, ALT and LDH<0.0001					