PROTECTIVE EFFECTS OF TRIMETAZIDINE IN TRANSIENT SPINAL CORD ISCHEMIA

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The neuroprotective effect of trimetazidine (TMZ) was tested prospectively in a rabbit spinal cord ischemia model. Ischemia was induced by clamping the aorta just distal to the left renal artery and proximal to the aortic bifurcation for a duration of 20 minutes. Twenty five male New Zealand rabbits were randomized as follows: TMZ group (n=10) receiving 3 mg/kg trimetazidine intravenously before the occlusion of aorta, control group undergoing occlusion but receiving no pharmacological intervention (n=10), sham operation group (n=5) subjected to operative dissections without aortic occlusion. Physiological parameters and somatosensory evoked potentials (SEP) were monitored in the animals before and during ischemia and in the 1st, 15th, 60th minutes of reperfusion. Neurologic status was assessed 24 and 48 hours after the operation. Their spinal cord, abdominal aorta, and its branches were processed for histopathologic examination at 48th hours after the operation. At the end of the ischemic period, the average N1-P1 amplitude was reduced to 22% of the baseline in all ischemic animals. This was followed by a gradual return to 90±2% of the initial amplitude in TMZ group and 81±2% in control group ($p<0.05$) after 60 minutes of reperfusion. The average motor function score was significantly higher in TMZ group than control group (3.7±0.5 vs 3.1±0.6 at 24th and 3.5±0.7 vs 2.9±0.6 at 48th hours, $p<0.05$). Histologic observations were clearly correlated with neurologic findings. The results suggest that trimetazidine reduces spinal cord injury during thoracoabdominal aortic operations and may have therapeutic utility during high risk operations.

Key words: Trimetazidine, spinal cord ischemia, somatosensory evoked potentials.

Spinal cord ischemia is a devastating complication of thoracoabdominal aortic operations, with paraplegia occurring after as many as 31% of procedures (1). Temporary aortic occlusion may produce a critical reduction in spinal cord perfusion with the risk of irreversible ischemia injury. Reperfusion may also cause numerous further negative effects in case of severe and prolonged ischemia. The molecular and cellular mechanisms that underlie ischemia-reperfusion injury has a multifactorial etiology. During spinal cord ischemia, ischemic cascade starts and results in cellular metabolic dysfunction.

sciatic nerve was stimulated by a bipolar electrode with square wave pulses of 0.1 msec duration and approximately 7 mA intensity delivered at 3 Hz. The filter range was 20 Hz to 1 kHz. Sixty four responses were averaged and displayed. The latencies and amplitudes of SEP before and after the ischemic procedure were compared.

Postoperative care and assessment:

Femoral arterial and venous lines were removed at the 60th minute of reperfusion. When the animals ajakened from anesthesia, they were returned to their cağes. The Crede maneuver was used to empty the bladders of paraplegic animals at least twice daily.

Neurologic status was scored by assessment of thind limb neurologic function according to the Tarlov scale (9) [0: no movement; 1: sliht movement; 2: sits with assistance; 3: sits alone; 4: weak hop; 5: normal hop] at the 24th and 48th hours after ischemaia. Two observers (1 of whom was blinded to the experimental conditions) graded the neurologic status independently.

Histopatology:

After the last neurological examination at the 48th hour, the animals were anesthetized and killed with an intracardiac perfosion of 10% neutral formalin. The retroperitoneal region including the abdominal aorta and it branches where removed, extensively and fixed in %10 formalin solition. The spinal cord was then removed, immersed in the same fixative, and postfixed for abbout 14 days before being set in paraffin blocks for sectioning. Section of the thoracic and lumbar cord were stained with hematoxylin-eosin. The abdominal aorta and its branshes were examined for revealing possible thrombosis or embolic occlusion.

Statistical Analysis:

Statistical analysis of physiologic and hemodynamic parameters were performed by analysis of variance for repeated measures. All parametric data were expressed as mean ± standard deviation. Statistical analyses of the neurologic scores were done with the non-parametric Man-Whitney U test using the Bonferroni correction. Somatosensory evoked potential data are expressed as percent of baseline control value. Recovery of SEP amplitude in trimetazidine treated animals was compared with that of ischemia control animals using t-test and Bonferroni correction. A p value of less than 0.05 was considered significant

RESULTS

No differences in mean levels of blood glucose, arterial blood gases, pH, rectal temperature, heart rate, and mean arterial blood pressure were noted among groups. The femoral artery blood pressures were measured near zero in the animals subjected to ischemia during the ischemic period and became gradually normal within the first minute of beginning of reperfusion and corrected with intravenous sodium bicarbonate (1.7±0.4 mEq in TMZ group and 1.8±0.4 mEq in control group, statistically nonsignificant) and no correction was required in the sham group.

SEP recording showed a negative peak (N1) with an average latency of 5.3±0.2 msec, a positive peak (P1) with an average latency of 6.3±0.1 msec and a negative peak (N2) with average latency of 10.1±0.2 msec. N1-P1 amplitude was 5.2±0.4μV and P1-N2 amplitude was 7.4±0.3μV. The peaks were stable in sham group during the procedure. In the ischemic groups, N1 and P1 peaks progressively declined and with an average of 8.1±4 minute after aortic occlusion. At the end of the ischemic period, average N1-P1 amplitude decreased 22% pre-ischemic baseline level in all ischemic animals. The average amplitude of P1-N2 dropped to 15% of pre-ischemic baseline level in all ischemic animals. This was followed by a gradual return to 90±2% of the initial N1-P1 amplitude and 80±2% P1-N2 amplitude after the 60th minute of reperfusion in the TMZ group. However this recovery were 81±2% and 73±2%, respectively, of the initial amplitude in the control group. There was a corresponding statistical difference between the TMZ and the control group ($p>0.05$). The

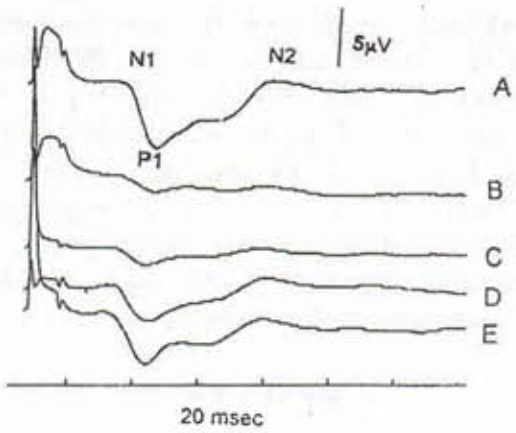

Figure 1. Typical SEP traces of an ischemic animal.
A: Pre-ischemic baseline SEP trace, and N1-P1 and P1-N2 amplitudes,
B: SEP traces during ischemia,
C: SEP trace at the 1st minute of reperfusion,
D: SEP trace at the 15th minute of reperfusion,
E: SEP trace at the 60th minute of reperfusion

peaks were stable in the sham operated group during the procedure. SEP recordings before and after the occlusion of the abdominal aorta and in the reperfusion period are illustrated in Figure 1.

Neurologic outcomes:

Sham operated animals showed full neurological recovery after the procedure. The evaluation of motor function score at the 24th and 48th hours of post-ischemic period in the TMZ and control groups is presented in Table 1. All ischemic animals exhibited neurologic deficiencies. The average motor score of the TMZ group was significantly ($p<0.05$) better than the control group 24 and 48 hours after the ischemia.

Histopathological findings:

Histopathologic examination of abdominal aorta and its branches were normal and revealed no thrombus formation in all animals. The histopathologic findings of spinal cord correlated with the neurologic outcomes. The animals with high motor score exhibited unaltered structure of gray matter or minimal necrosis (Figure 2). But the animals with low motor score had typical infarctions of variable size in the central gray matter (Figure 3).

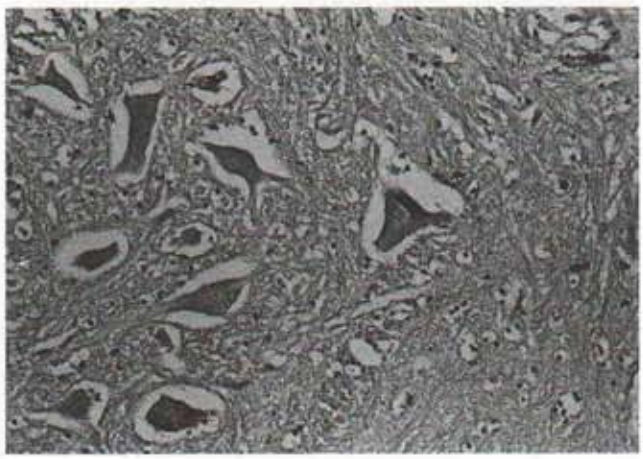

Figure 2. Photomicrograph of the cells in the anterior horn of spinal cord showing karyolysis and moderate changes in the cytoplasm (TMZ group, score 4 of motor neurological function, lumbosacral region, X200 hematoxylin-eosin).

Table 1. Tarlov scores of the TMZ and control groups at 24th and 48th hours after the ischemia.

Animal groups	n	Motor score						Average motor score
		0	1	2	3	4	5	
TMZ group								
24th h	10				3	7		3.7±0.5*
48th h	10			1	3	6		3.5±0.7*
Control group								
24th h	10			1	7	2		3.1±0.6
48th h	10			2	7	1		2.9±0.6

* Significantly different from control group ($p<0.05$) by Mann-Whitney U test.

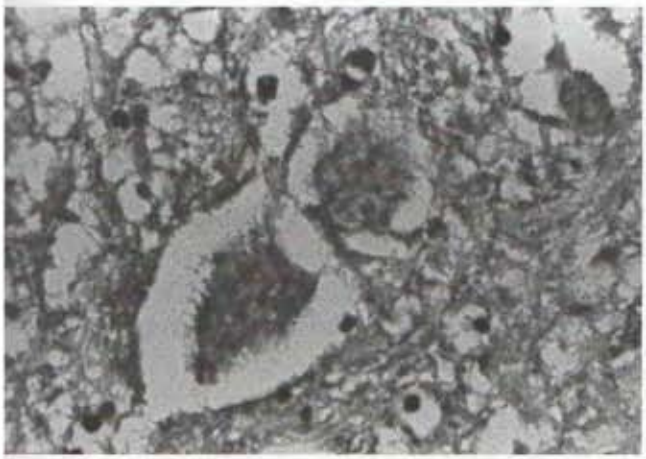

Figure 3. Neuronal death characterized by blurred cytoplasmic border, degranulation of Nissl bodies, karyoylsis, pericellular vacuolization (control group, score 2 of motor neurological function, lumbosacral segment, X400 hematoxylin-eosin).

DISCUSSION

Numerous surgical techniques and pharmacological interventions have been used to reduce the rate of perioperative paraplegia after operations on the thoracoabdominal aorta, but none can claim consistently proven efficacy (10). The data presented in this study demonstrated that reduction in neuronal damage has been achieved with pre-ischemic, intravenous administration of trimetazidine in a rabbit model of transient global ischemia. Animals that were subjected to 20 min of ischemia after receiving 3 mg/kg trimetazidine exhibited better neurologic outcomes. There are very little knowledge in the literature about neuroprotective effect of trimetazidine, although there is a great deal of studies about its cardioprotective effect. The efficacy of trimetazidine has recently been investigated on reducing organ damage in myocardium (5) but not in spinal cord ischemia.

In the presence of cellular ischemia, there is a deterioration of energy production, distortions of cellular homeostasis (acidosis and cytoplasmic sodium and calcium accumulation) and an overproduction of free radicals which have detrimental effects on cell structure and function. Trimetazidine antagonizes hypoxia induced reduction in myocardial ATP levels without diminishing phosphocreatine levels (11), and accelerates the reconstitution of energy pools during reperfusion perhaps by inhibiting mitochondrial ATPase (12). Under normal physiological conditions, trimetazidine does not affect the plasma membrane ionic channels or ionic pumps in the cardiac cells. But under ischemic conditions it reduces myocardial intracellular acidosis, and indirectly limits the accumulation of Na^+ and Ca^{2+} (13, 14).

Another mechanism is that trimetazidine significantly reduces H_2O_2 production (13,15) and finally it decreases adhesiveness and aggregation of platelets which may be due to a blockade of the process preceding the cyclooxygenase pathway and related to the free-radical scavenging property of this compound (16, 17).

According to Harpey et al. (15), there is no major difference in pharmacokinetic parameters between single or repeated doses. So, we decided to administer trimetazidine as single bolus treatment. Trimetazidine at intravenous doses from 0.25 to 3.0 mg/kg, do not modify hemodynamic parameters in the systemic or coronary circulation (13,15). We also had similar hemodynamic findings on the systemic circulation with a dose of 3 mg/kg. Although oral administration of trimetazidine is widely used in clinical practice, there are some literature on the effectiveness of parenteral route and bolus dose (10,18-21).

The present study was similar to the clinical setting of thoracoabdominal aortic surgery leading to spinal cord ischemia. There was no observed significant difference in blood pressure and blood glucose levels among all comparable groups. This model might increase the tolerance of spinal cord to ischemia before elective surgery.

The rabbit spinal cord is a reliable model for systematically and rapidly observing the protective effects of investigated agents on ischemia and reperfusion injury. However, ligation of the rabbit's abdominal aorta may not produce complete ischemia in the spinal cord. There are considerable individual variations in residual collateral blood flow, and in most animals, about 2% of normal blood flow remains after 20 minutes of ligation (9). In the present study, we used a

second clamp at the distal abdominal aorta for occluding the iliac collateral circulation and monitored the spinal cord ischemia with SEP. Monitoring of SEP during aortic occlusion has been used for identification and exclusion of animals which have sufficient collateral blood flow to permit the spinal cord to survive long periods of aortic occlusion (22). Previous studies have shown that a decrease of 15-20% in amplitude can be taken as a criteria of significant SEP change (23). In the present study, despite minimal individual variations, SEP amplitudes decreased to about 22% of their pre-ischemic baseline values in most of the animals at 20th min of ischemia, and monitoring of SEP in this model has permitted observation of similar collateral blood flow properties in the studied groups of animals.

In conclusion, this study indicated that trimetazidine potentially ameliorated reperfusion injury in a rabbit model of transient spinal cord ischemia, and animals treated with trimetazidine showed better neurologic outcome. In case of neurologic deficit caused by aortic dissection, this drug may be used until reperfusion is able to be established. In addition, trimetazidine can be used in the prevention of spinal cord injury during thoracoabdominal aortic operations.

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