Reduction of Spinal Cord Injury with Pentobarbital and Hypothermia in a Rabbit Model

Ö. Tetik, F. İslamoğlu, T. Göncü, A. Çekirdekcioğlu, S. Büket

Department of Cardiovascular Surgery, Ataturk Medical Research Hospital, İzmir, Turkey,
Department of Cardiovascular Surgery, Ege University Medical Faculty, İzmir, Turkey,
Department of Cardiovascular Surgery, Yüksek İhtisas Hospital, Bursa, Turkey,
Department of Cardiovascular Surgery, Firat University Medical Faculty, Elazığ, Turkey and
Department of Cardiovascular Surgery, Ege University Medical Faculty, İzmir, Turkey

Objectives: to evaluate the effects of hypothermia and pentobarbital on spinal cord ischaemia induced in a rabbit model.

Materials and Methods: thirty-two rabbits, allocated into four equal groups, had the infrarenal aorta clamped distal to the left renal artery and above the iliac bifurcation for 40 min. Groups 3 and 4 had infusion of 15 mg/kg of pentobarbital intravenously for 5 min, 15 min before the cross-clamping. Groups 2 and 4 had infusion of 20 ml of Ringer’s lactate (LR) solution at 3°C for 3 min during aortic cross clamp into the isolated aortic segment. Group 1 was untreated and served as control. Postoperative functions of spinal cord were assessed.

Results: paraplegia occurred in all rabbits in Group 1, in one in each of Groups 2 and 3, whereas no paraplegia was observed in Group 4. In addition 2 and 3 animals of Groups 2 and 3, respectively revealed varying degree of neurological disturbances, whereas all animals of Group 4 had normal function. This difference between Groups 2, 3, and 4 vs Group 1 was significant (p < 0.002). So was the difference between Groups 2 and 4 (p = 0.03), whereas the difference between Groups 3 and 4 was not significant.

Conclusions: hypothermia and pentobarbital was more effective than hypothermia alone for prevention of spinal cord ischaemia in a rabbit model.

Key Words: Spinal cord protection; Paraplegia; Ischaemic injury; Pentobarbital.

Introduction

Ischaemic injury of spinal cord is a serious complication following operations on the thoracoabdominal aorta with varying rates from 6 to 40%.1–4 Although various protective methods have been evaluated both clinically and experimentally, postoperative spinal cord dysfunction has not been prevented completely yet by application of any of these techniques.1,2,3,4,8 Besides, dose dependent protective properties of these agents can cause systemic untoward effects, when they are used alone to obtain a desirable protection.4,6 Therefore, combined use of these techniques and agents to obtain most suitable doses for protection of spinal cord has been the focus of the most of the current studies.4,6

Barbiturates decrease oxygen consumption, reduce the usage of adenosine triphosphate (ATP) and phosphocreatine, limit the extent of edema, and diminish the development of infarction in the brain affected from ischaemic injury.4,8 Among the barbiturates, thiopental was the most investigated agent with controversial results.4,8,10 Furthermore, hypothermia augments the tolerance of spinal cord against ischaemia and lessens the postischaemic spinal cord injury.3,5 However, haemorrhagic, pulmonary and neural complications of generalised-deep hypothermia limit its usefulness.5 Local hypothermia is also protective as well as generalised hypothermia.3,5

The occlusion of the infrarenal aorta of the rabbits for longer than 20 min invariably causes paraplegia.8 In this study, we hypothesised that the combined use of local hypothermia and pentobarbital which is more potent and longer acting than thiopental, might show a significant protective effect on spinal cord ischaemia in a rabbit model.
Spinal Cord Protection with Pentobarbital

Materials and Methods

Thirty-two New Zealand white rabbits weighing 2.6 to 3.4 kg were used. In all steps of experiment, animal care and all procedures were performed in compliance with the “Guide for the Care and Use of Laboratory Animals” published by the “National Institutes of Health (NIH publications, No. 85-23, revised 1985)”. Rabbits were allocated into four groups of 8 animals each. All animals were premedicated with intramuscular ketamine hydrochloride 35 mg/kg and anaesthetised with 1.55 halothane in a mixture of 1:1 oxygen and nitrous oxide delivered by a nasal cone. Spontaneous breathing of all animals was maintained along the experiment, and arterial blood-gas analysis and pressure monitoring (Siemens SC 6000, Siemens Medical Systems, U.S.A.) were performed via 24-gauge catheter inserted into the suprarenal aorta. Rectal temperature was continuously monitored with a flexible probe (DeRoyal, Powell, TN, U.S.A.). An ear vein was also cannulated with a 24-gauge catheter and a single dose of Cefazolin (10 mg/kg) was administered preoperatively. Maintenance fluid of isotonic saline solution was infused intravenously at rate of 30 ml/h.

Surgical technique

After the placement of each rabbit in the supine position, abdominal skin was shaved. Following the sterilisation of surgical site with 10% povidone–iodine solution and the covering with sterile drapes, a midline laparotomy incision about 10 cm in length was performed. The abdominal aorta was exposed from just below the left renal artery to the iliac bifurcation. The aorta was encircled with a silk ligature immediately below the renal arteries and proximal to bifurcation to provide secure occlusion. A 2/0 silk suture was also passed around the posterior mesenteric artery. Heparin 150 IU/kg was given intravenously before the cross-clamping and was not reversed. One clamp was positioned just below the renal arteries, another was placed above the bifurcation, and the loss of pulsation in this isolated segment was checked. Rabbits were subjected to 40 min of cross-clamping after which all control animals are rendered paraplegic.5

In Group 1 (control group), cross-clamping of infra-renal aorta was performed and no solution was infused. In Groups 2 and 4, a 24-gauge vascular catheter was inserted into the isolated aortic segment and various solutions were delivered through an infusion set which had been described in detail in our previous study.5 In Group 2, 20 ml of Ringer’s lactate (LR) solution at 3°C was infused for 3 min. In Group 3, 15 mg/kg of pentobarbital was administered intravenously for 5 min, 15 min before the cross-clamping. In Group 4, besides the infusion of 20 ml of LR solution at 3°C for 3 min into the isolated aortic segment, 15 mg/kg of pentobarbital was infused intravenously for 5 min, 15 min before the aortic occlusion (Fig. 1). After the cessation of cross-clamping and the removal of the catheter, the puncture site in the aorta was repaired with a 7/0 polypropylene suture. Abdominal wall was closed with a standard technique. After the postoperative recovery from anaesthesia, animals were returned to cages.

Clinical evaluation

Neurologic status of animals was assessed blindly at 24 and 48 h postoperation by two neurologists using Tarlov’s criteria which classify limb motor function as indicator of the degree of spinal cord injury.5,6 A score of 0–4 was assigned to each animal, as follows: grade 0, spastic paraplegia with no movement of the hind limbs; grade I, spastic paraplegia with slight movement of the hind limbs; grade II, good movement of the hind limbs, but unable to stand; grade III, able to stand, but unable to walk normally; grade IV, normal walking and complete recovery.

Histopathological evaluation

After the last neurological examination at 48th hour, animals were sacrificed, and their spinal cords were
harvested for histological evaluation. Specimens of lumbosacral spinal cords were prepared and fixed by a solution containing 2.5% glutaraldehyde phosphate. After the staining with cresyl-violet, samples were examined and photomicrographs were taken using an Olympus BH2 light microscope. The infarctions of the spinal cord were not scored. Sections were studied only for detection of ischemic injury to evaluate the compatibility of the histological findings with neurologic status. The histological evaluation was performed by only one histopathologist who was blinded from the experimental conditions.

Statistical analysis

Statistical analyses were performed by SPSS/PC+ (ver 10.0) computer program. The median and range values of all data were calculated and indicated. For the changes in neurologic status, Mann–Whitney U-test and Kruskal–Wallis analysis of variance (one-way ANOVA) were used. Wilcoxon’s test was used in the comparison of arterial pressures. The probability \((p)\) less than 0.05 was considered significant.

Results

There was no statistically significant difference in systemic temperature and proximal aortic pressures between groups during cross-clamping. Because we did not use heating pads, rectal temperature decreased slightly without any hypothermia after induction of anaesthesia and during aortic clamping in all animals. There was not also any difference at 24 and 48 h controls of all animals with respect to neurologic status. At 48 h, the neurologic status of Groups 2, 3, and 4 was significantly superior to that of Group 1. All rabbits in Group 1 had spastic paraplegia. No paraplegia was observed and all animals had completely normal neurologic findings in Group 4. In each of Groups 2 and 3, there was one animal with spastic paraplegia. In Group 2 and Group 3, 4 and 5 animals had normal neurologic status, respectively. Neurologic disturbances in varying degrees were observed in the other animals of both groups. The neurologic status of Group 2 and 3 was significantly superior to that of Group 1 \((p = 0.002)\). Group 4 also had significantly better neurologic findings than Group 1 \((p < 0.0001)\). No significant neurologic difference was observed between the Group 2 and Group 3 \((p = 0.728)\). The neurologic status was significantly better in Group 4 than that in Group 2 \((p = 0.027)\), but the difference was not significant between Group 4 and Group 3 \((p = 0.064)\) (Table 1).

In the light microscopic evaluations, the histological sections of lower lumbar and sacral spinal cords of paraplegic animals demonstrated the swelling of the ependyma cells that surrounds the central canal, pyknotic nucleus in neurons, loss of Nissl substance, chromatolysis (Fig. 2a), oedema surrounding the pericarion, pyknotic nucleus and the swelling of glial and axonal extensions of neurons (Fig. 2b). The cords of animals with no motor function deficit in Groups 2, 3, and 4 showed completely normal spinal cord histology (absence of oedema, chromatolysis, and pyknotic cells with normal appearance of all intrastoplasmic organelles) (Fig. 2c).

Discussion

Several previous studies have suggested that hypothermia and barbiturates may help to ameliorate spinal cord ischaemia and reperfusion injury.\(^4\)–\(^8\)–\(^15\) The mechanisms involved are complex and incompletely understood.\(^14\)–\(^25\) Our study also indicates that regional hypothermia reduced substantially hazards of spinal cord injury. However, a complete protection could not be achieved by using hypothermia alone. Therefore, in the present study, pentobarbital which is longer active than thiopental was selected as a potential adjuvant protective agent.

Pretreatment with intravenous administration of 15 mg/kg pentobarbital minimised considerably spinal cord damage. There was only one animal with paraplegia, and motor functions in 5 of 8 animals recovered completely without any neurologic deficit. The neurologic status of animals subjected to pentobarbital alone was significantly superior than that of ones in control group. However, the difference was not statistically significant in comparison to hypothermia group.

Hetzler and Krekow showed in a rat model that hypothermia augmented the suppressive effects of
pentobarbital on visual-evoked potentials.\textsuperscript{26} Similar potentiation of the effects of pentobarbital by generalised hypothermia was also observed in a rabbit model by Kazama and colleagues.\textsuperscript{4} These authors suggested that the inhibition of aminoacid release by hypothermia and suppression of excitatory amino acids by pentobarbital might exert a combined protective effect on spinal cord.\textsuperscript{4} Results of present study are also in a full agreement with recent studies indicating the augmented protection of spinal cord, and the combination of pentobarbital with hypothermia provided a complete recovery of motor function and spinal cord protection without paraplegia or another neurologic deficit in all animals. When the results of our two prior studies\textsuperscript{5,6} using same experimental model (our first and second trials with local hypothermia, methylprednisolone, vitamins E, C and local hypothermia, L-carnitine, respectively) and the present study are compared with each other, our first trial containing methylprednisolone and the present combination seem somewhat more protective than the L-carnitine combination, because of the superiority of neurologic status of the animals included in these studies.

In conclusion, the combined use of hypothermia and pentobarbital seems as a promising method for spinal cord protection, although further studies are still needed to indicate correctly its mechanism of action, dosage, timing, and possible undesired effects.

References

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Fig. 2. Photomicrographs of lower lumbar and sacral spinal cords of paraplegic animals that showed in (a) the swelling of the ependyma cells that surrounds the central canal, pyknotic nucleus in neurons, loss of Nissl substance, chromatolysis (cresyl-violet, original magnification ×40) and in (b) oedema surrounding the pericarion, pyknotic nucleus and the swelling of glial and axonal extensions of neurons (cresyl-violet, original magnification ×100). (c) Normal microscopic appearance of somato-motor neuronal cells in histological section of lower lumbar spinal cord in a healthy rabbit with no motor deficit (cresyl-violet, original magnification ×40).


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