International Journal of Surgery 11 (2013) 354–359

Contents lists available at SciVerse ScienceDirect

International Journal of Surgery

journal homepage: www.theijs.com

Original research

Aprikalim a potassium adenosine triphosphate channel opener reduces neurologic injury in a rabbit model of spinal cord ischemia

Vasileios A. Lozos [a], Ioannis K. Toumpoulis [a,*], Georgios Agrogiannis [b], Evangelos J. Giamarellos-Bourboulis [c], Themistocles P. Chamogeorgakis [a], Ioannis K. Rizos [d], Efstratios S. Patsouris [b], Constantine E. Anagnostopoulos [a,e], Chris K. Rokkas [a]

[a] Department of Cardiothoracic Surgery, National and Kapodistrian University of Athens, Medical School, Attikon University Hospital Center, Rimini 1, Chaidari 12462, Athens, Greece
[b] Department of Pathology, National and Kapodistrian University of Athens, Medical School, Athens, Greece
[c] Department of Internal Medicine, National and Kapodistrian University of Athens, Medical School, Athens, Greece
[d] Department of Cardiology, National and Kapodistrian University of Athens, Medical School, Athens, Greece
[e] Department of Cardiothoracic Surgery, St. Luke's – Roosevelt Hospital Center at Columbia University College of Physicians and Surgeons, New York, NY, USA

ARTICLE INFO

Article history:
Received 26 August 2012
Received in revised form
30 January 2013
Accepted 23 February 2013
Available online 5 March 2013

Keywords:
Spinal cord ischemia
Aortic cross-clamping
Potassium channels
Aprikalim

ABSTRACT

Background: Potassium adenosine triphosphate (K_{ATP}) channel openers have been involved in the enhancement of ischemic tolerance in various tissues. The purpose of the present study is to evaluate the effects of aprikalim, a specific K_{ATP} channel opener, on spinal cord ischemic injury.
Methods: Fifty-four rabbits were randomly assigned to three groups: group 1 ($n = 18$, sham operation), group 2 ($n = 18$, 30 min of normothermic aortic cross-clamping) and group 3 ($n = 18$, aprikalim 100 μg/kg was administered 15 min before 30 min of normothermic aortic cross-clamping). Neurologic evaluation was performed according to the modified Tarlov scale. Six animals from each group were sacrificed at 24, 48 and 168 h postoperatively. The lumbar spinal cords were harvested and examined histologically. The motor neurons were counted and the histologic lesions were scored (0–3, 3: normal).
Results: Group 3 (aprikalim group) had better Tarlov scores compared to group 2 at all-time points ($P < 0.025$). The histologic changes were proportional to the Tarlov scores and group 3 had better functional outcome as compared to group 2 at 168 h (number of neurons: 21.2 ± 4.9 vs. 8.0 ± 2.7, $P < 0.001$ and histologic score: 1.67 ± 1.03 vs. 0.50 ± 0.55, $P = 0.03$). Although aprikalim exhibited improved effect on clinical and histologic neurologic outcome when compared to normothermic spinal cord ischemia, animals in group 3 had worse Tarlov score, reduced number of motor neurons and worse histologic score when compared to group 1 (sham operation) at 168 h ($P = 0.003$, $P = 0.001$ and $P = 0.019$ respectively).
Conclusion: Aprikalim reduces the severity of spinal cord ischemic injury in a rabbit model of spinal cord ischemia.

1. Introduction

Operations that require proximal aortic occlusion result in ischemia to the distal organs. The spinal cord is exquisitely vulnerable to ischemia. In contemporary published clinical series the incidence of paraplegia and/or paraparesis after operations in the thoracoabdominal aorta ranges from 5% to 14%,[1,2] while similar neurologic deficits can occur after endovascular interventions.[3] Therefore, it would be advantageous if pharmacologic agents were available that could increase the tolerance of the spinal cord to ischemia due to aortic occlusion.

Recent advances in molecular biology and pharmacology of potassium channels have enabled the investigation of potential therapeutic effects of potassium channel agonists.[4] There are experimental data showing that activation of potassium channels in neurons enhances protection against ischemia and reperfusion injury.[5–8] The purpose of the present study is to evaluate the effects of aprikalim, a specific potassium adenosine triphosphate (K_{ATP})

* Corresponding author. Tel.: +30 697 724 3942; fax: +30 210 724 0354.
E-mail address: toumpoul@otenet.gr (I.K. Toumpoulis).

1743-9191/$ – see front matter
http://dx.doi.org/10.1016/j.ijsu.2013.02.025

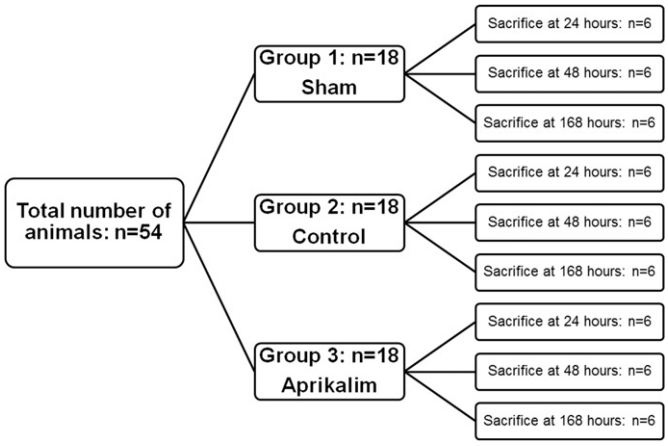

Fig. 1. The diagram shows the distribution of animals used in this study. Eighteen animals were used in each of the three groups and 6 animals from each group were sacrificed at 24, 48 and 168 h. Comparisons were performed among the three groups at 24, 48 and 168 h in terms of the Tarlov score, the number of motor neurons and the histologic score using 6 different animals from each group at the three time points. Therefore, these observations were not correlated in an attempt to avoid producing biased results.

channel opener, on spinal cord injury after aortic cross-clamping in a rabbit model of spinal cord ischemia after a follow-up period of 7 days in order to truly evaluate the neuroprotective efficacy of this pharmacologic agent.

2. Materials and methods

2.1. Animals

Fifty-four New Zealand rabbits of either sex were used in this study. All animals weighted between 3.0 and 3.5 kg and were randomly assigned to one of three groups. Group 1 ($n = 18$) underwent a sham operation, group 2 ($n = 18$) underwent aortic cross-clamping for 30 min, and in group 3 ($n = 18$) 100 μg/kg aprikalim (Sanofi-Aventis, Germany, GmbH) was administered intravenously 15 min before aortic cross-clamping for 30 min.

2.2. Experimental preparation

All animals were fasted for 12 h before the procedure. The rabbits were anesthetized with an intramuscular injection of xylazine (4 mg/kg) and ketamine hydrochloride (50 mg/kg). Gentamicin sulfate (1 mg/kg) was administered intramuscular. Animals were allowed to breathe spontaneously with a face-mask device in 100% oxygen. The arterial PaO_2 was maintained at greater than 100 mmHg, $PaCO_2$ maintained at 35–45 mmHg and pH at normal levels, as confirmed by means of arterial blood gas analysis. Tracheas were not intubated throughout experiments and anesthesia was maintained with intravenous xylazine (2.5 mg/kg) when necessary. A rectal probe was inserted to monitor body temperature, which was maintained at 39 °C (baseline value in our animals) using a heating pad. A marginal ear vein was cannulated for intravenous fluid and drug administration. The median ear artery and the right femoral artery were cannulated to monitor proximal and distal aortic blood pressure respectively. Electrocardiograms and pulse oximetry were continuously recorded.

Under sterile conditions, following intravenous administration of heparin (100 UI/kg), a midline laparotomy was made and the viscera reflected to the right. After opening the retroperitoneum the abdominal aorta was dissected distal to the left renal artery and proximal to the aortoiliac bifurcation, where Satinsky clamps were used to occlude the abdominal aorta. After 30 min of normothermic spinal cord ischemia Satinsky clamps were removed and all animals were fully resuscitated with intravenous fluids and phenylephrine hydrochloride to restore blood pressure. After 60 min of reperfusion, all animals were hemodynamically stable (mean arterial blood pressure >70 mmHg), without the need of fluid or drug administration. All catheters were removed and all wounds were closed. Finally, the animals were placed in their cages for postoperative care and follow-up.

2.3. Neurologic evaluation

Six animals from each group (Fig. 1) were evaluated by an independent observer at 24, 48 and 168 h after the end of the experiment according to the modified Tarlov[9] scoring system (0: atony, 1: slight movement, 2: sits with assistance, 3: sits alone, 4: weak hop, and 5: normal gait/hoping).

2.4. Histologic study

Six animals from each group (Fig. 1) were randomly chosen and were sacrificed at 24, 48 and 168 h after the end of the experiment with an overdose injection of sodium pentobarbital (50 mg/kg), whereas lumbar spinal cords specimens were harvested immediately for histologic study by means of light microscopy. The lumbar spinal cords were fixed in 10% formalin solution for 120 h before being set in paraffin blocks for sectioning. Representative glass slices having 5-μm-thick sections were obtained from each animal at L_4-L_5 and stained with hematoxylin-and-eosin. Images of the stained sections were captured with a Nikon DS-2MW colour CCD digital camera mounted on a Nikon Eclipse 80i microscope (Nikon Co., Tokyo, Japan) under ×200 original magnification and stored as high quality JPG files. Images were then analyzed with Image-Pro Plus 5.1 software (Media Cybernetics, SilverSpring, MD). Size threshold settings of stained pixels were set manually prior to analysis in order to avoid counting inflammatory or glial cells and left unchanged throughout. Through the interactive message screen, cells that should not be included in the analysis were eliminated, concentrating the counting on motor neurons. The microscope slide-mounted tissue sections were coded, and the pathologist performing the computerized image analysis was blinded to the experimental data. In addition, a histologic score was created ranging from 0 to 3 (score 0: high grade of inflammation with high grade of interstitial edema and low viability of motor neurons, score 1: moderate grade of inflammation with moderate grade of interstitial edema and moderate viability of motor neurons, score 2: low grade of inflammation with low grade of interstitial edema and high viability of motor neurons, and score 3: no inflammation, no interstitial edema and very high viability of motor neurons).

2.5. Statistical analysis

Comparisons were performed among the three groups at 24, 48 and 168 h in terms of the Tarlov score, the number of motor neurons and the histologic score using 6 different animals from each group at the three time points. Therefore, these observations were not correlated in an attempt to avoid producing biased results. Data are presented as means ± SD and as median and interquartile range (IQR). Statistical evaluation was performed by means of One-way analysis of variance test with the post hoc Tukey honestly significant difference test for comparison of experimental variables between groups. The difference among groups in terms of the Tarlov scores and the histologic score was determined by means of nonparametric statistical analysis with the Kruskal–Wallis test with the post hoc Mann–Whitney *U* test for comparison between two groups, while significant level was corrected using Bonferroni method. *P* values <0.05 were considered significant as determined with IBM SPSS Statistics 20.0 software for all comparisons, while *P* values <0.025 were considered significant for post hoc Mann–Whitney *U* tests (the number of comparisons was 2 at each time point).

Table 1
Proximal and distal mean arterial blood pressure, rectal temperature and heart rate at baseline, during aortic cross-clamping and during reperfusion. Statistical analysis was by means of one-way analysis of variance.

	Baseline				Aortic cross-clamping				Reperfusion			
	Prox MAP	Distal MAP	Temp °C	Heart rate	Prox MAP	Distal MAP	Temp °C	Heart rate	Prox MAP	Distal MAP	Temp °C	Heart rate
Group 1 ($n = 18$) (sham operation)	79 ± 4	80 ± 4	39.0 ± 0.2	193 ± 17	79 ± 1	80 ± 2	38.9 ± 0.2	192 ± 17	79 ± 2	80 ± 3	39.0 ± 0.2	190 ± 15
Group 2 ($n = 18$) (30 min SCI)	78 ± 4	79 ± 3	39.0 ± 0.2	191 ± 15	80 ± 2	10 ± 1	39.0 ± 0.1	192 ± 16	78 ± 3	78 ± 3	39.0 ± 0.2	191 ± 19
Group 3 ($n = 18$) (30 min SCI + Aprikalim)	78 ± 3	79 ± 3	39.0 ± 0.2	184 ± 13	79 ± 3	10 ± 1	38.9 ± 0.2	189 ± 14	78 ± 3	78 ± 3	39.0 ± 0.2	187 ± 18
P-value	$P = 0.651$	$P = 0.484$	$P = 0.487$	$P = 0.198$	$P = 0.321$	$P < 0.001$	$P = 0.213$	$P = 0.770$	$P = 0.352$	$P = 0.318$	$P = 0.291$	$P = 0.754$

MAP, mean arterial blood pressure; SCI, spinal cord ischemia.