American Journal of Emergency Medicine 34 (2016) 1804–1807

Contents lists available at ScienceDirect

American Journal of Emergency Medicine

journal homepage: www.elsevier.com/locate/ajem

Original Contribution

The effect of ethyl pyruvate and *N*-acetylcysteine on ischemia-reperfusion injury in an experimental model of ischemic stroke

Suha Turkmen, MD [a,*], Ozgen Cekic Gonenc, MD [a], Yunus Karaca, MD [a], Ahmet Mentese, PhD [b], Selim Demir, PhD [b], Ercument Beyhun, MD [c], Aynur Sahin, MD [a], Abdulkadir Gunduz, MD [a], Esin Yulug, MD [d], Suleyman Turedi, MD [a]

[a] Department of Emergency Medicine, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey
[b] Department of Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey
[c] Department of Public Health, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey
[d] Department of Histology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

ARTICLE INFO

Article history:
Received 28 March 2016
Received in revised form 31 May 2016
Accepted 2 June 2016

ABSTRACT

Introduction: Reperfusion therapies play an important role in early-period treatment for patients presenting to the emergency department due to stroke. However, the ischemia-reperfusion injury that may occur with reperfusion must then be considered. The purpose of this study was to determine the effectiveness of *N*-acetylcysteine (NAC) and ethyl pyruvate in preventing ischemia-reperfusion injury.

Method: This study is a randomized, controlled experimental study. In group 1, rats' left main carotid arteries were clamped. Reperfusion was established by releasing the clamp after 1.5 hours. In group 2, the left main carotid artery was clamped, and 20 mg/kg intraperitoneal NAC was administered after 1 hour. The clamp was released 0.5 hour after NAC administration. In group 3, rats' left carotid arteries were clamped, and 50 mg/kg ethyl pyruvate was administered intraperitoneally after 1 hour. The clamp was released 0.5 hour after ethyl pyruvate administration. All tissue samples were collected 2.5 hours after reperfusion. Brain tissues were compared histopathologically.

Results: A higher percentage of degenerative neurons was determined in group 1 compared with groups 2 and 3 (P values: $P^a = .003$ and $P^c = .003$, respectively). A significant difference was also observed between groups 2 and 3 ($P^b = .003$), with the percentage of degenerative neurons being lower in the NAC group than in the ethyl pyruvate group.

Conclusion: The use of NAC and ethyl pyruvate reduces injury resulting from ischemia-reperfusion in an experimental animal model of acute ischemic stroke and subsequent reperfusion.

Introduction

Early diagnosis and treatment for patients presenting to the emergency department due to stroke have been shown to be capable of significantly reducing mortality and morbidity. Major successes have been achieved in early-period treatment with the development of thrombolytic agents and intra-arterial reperfusion techniques used in the treatment of ischemic stroke in particular [1]. However, another significant condition that must be considered in patients with ischemic stroke receiving these therapeutic techniques is injury resulting from ischemia-reperfusion. There has been insufficient research into ischemia-reperfusion injury that may occur in brain tissue with revascularization and into the effects of antioxidants on that injury.

Ethyl pyruvate, an agent with known neuroprotective effects, is an antioxidant and anti-inflammatory drug. Pyruvate has been reported to be capable of scavenging oxygen radicals and hydroxyl radicals [2]. *N*-acetylcysteine (NAC), another agent, has been reported to exhibit protective effects against tissue damage caused by free radicals [3].

The purpose of this experimental study was to investigate the effect of ethyl pyruvate and NAC, with their known antioxidant properties, on oxidative injury before reperfusion in an ischemic stroke model using histopathologic methods.

Method

Study design

This was a randomized, controlled, nonblinded interventional animal study, the protocol for which was approved by the Karadeniz Technical University Animal Care and Ethics Committee.

* Corresponding author. Tip Fakultesi Acil Tip AD, Karadeniz Teknik Universitesi, 61080 Trabzon, Turkey.
E-mail address: drsuhaturkmen@hotmail.com (S. Turkmen).

http://dx.doi.org/10.1016/j.ajem.2016.06.003
0735-6757/

Setting and selection of subjects

Twenty-one mature female Sprague-Dawley rats (10 weeks old, weighing 240-280 g) were used. The animals were kept in steel cages until the day of the study at a room temperature of 22°C and were given water and standard rat chow. Only water was provided for the last 12 hours before the study.

Intervention

The 21 rats were randomized into 3 groups of 7 individuals each.

Group I: control group ($n = 7$)

A midline neck incision was made. After superficial microdissection, the left common carotid artery was approached with deep dissection. The common carotid artery was accessed by visualizing the trachea and dissecting the paratracheal muscles. The left main carotid artery was dissected and clamped, and ischemia was established for 1.5 hours. Ischemia-reperfusion injury was subsequently induced by releasing the clamp, and tissue specimens were collected 2.5 hours later.

Group II: NAC group ($n = 7$)

Neck incision was performed in the rats in group 2. The left main carotid artery was dissected. The artery was clamped. After 1 hour, 20 mg/kg NAC was administered intraperitoneally. The clamp was released 0.5 hour after drug administration, and tissue specimens were collected 2.5 hours after reperfusion.

Group III: ethyl pyruvate group ($n = 7$)

Neck incision was performed on the rats in group 3. The left main carotid artery was clamped. After 1 hour, 50 mg/kg ethyl pyruvate was administered intraperitoneally. The clamp was released 0.5 hour after drug administration, and tissue specimens were collected 2.5 hours after reperfusion.

Histopathologic analyses

Brain tissue was removed from all rats at the end of the study. Brains were divided into two halves from the middle region in such a way as to include all layers. Tissue samples were taken and kept in 10% neutral formalin for 48 hours. Routine histologic tissue procedures were performed for histopathologic study. Specimens were dehydrated in a graded alcohol series. They were subsequently made transparent with xylene and embedded in paraffin blocks. Sections 5 μm in thickness were taken from the paraffin blocks using a fully automatic microtome (Leica RM 2255, Tokyo, Japan). These sections were then stained with hematoxylin-eosin and cressyl violet for detailed evaluation of general histologic morphology. The preparates were evaluated by an experienced histologist (E.Y.), blinded to the different groups, under a light microscope (Olympus BX-51; Olympus Optical Co, Tokyo, Japan). All layers of the brain tissue cortex were reviewed in terms of general histologic structure under small magnification (×100). Histologic scoring in terms of neuronal changes in the cortex region in both hemispheres was also performed [4]. Accordingly;

Grade 1 was defined as mildly shrunken neurons with or without cytoplasmic vacuolation;

grade 2, as moderately shrunken neurons (eosinophilic cytoplasm) and increased nuclear basophilia/or vacuolated cytoplasm and a vesicular nucleus; and *grade 3*, as severely shrunken neurons (eosinophilic cytoplasm) with pyknotic nucleus.

One hundred pyramidal cells in all layers in the cortex region were counted under a light microscope at ×200 magnification on Analysis 5 Research software (Olympus Soft Imaging Solutions, Münster, Germany). The percentage of degenerative pyramidal neurons was calculated. Cells with shrinking in the body, loss of Nissl bodies in cytoplasm and eosinophilia, and a dark colored, shrunken nucleus were regarded as degenerative [5].

Statistical analysis was performed on SPSS (Statistical Package for Social Sciences for Windows v.13.0, Chicago, IL) software. Categoric variables were calculated as median, percentage, and quartile values. The Bonferroni-corrected Mann-Whitney *U* test was used in histologic analysis. *P* values less than .016 after correction were regarded as significant.

Results

Because one of the rats in group 1 died during the experiment, this animal was excluded from the analysis. Brain tissues from 20 rats, 6 from group 1, 7 from group 2, and 7 from group 3, were therefore finally assessed in terms of ischemia-reperfusion injury.

Histopathologic evaluation of brain tissue revealed widespread degenerative pyramidal neurons with pyknotic nuclei and eosinophilic cytoplasm in all layers of the cortex in the rats from group 1. Degenerative neurons were also observed in parts of the cortex close to the white matter junction. A few neurons with normal morphology were observed (Fig. 1A). Almost normal brain cortex and pyramidal neuron histologic morphology was observed in rats from group 2. A few degenerative neurons were observed (Fig. 1B). A normal cortex and pyramidal neurons were observed in rats from group 3. Occasional degenerative neurons were determined in the upper layers of the cortex (Fig. 1C).

Brain tissues from groups 1, 2, and 3 were subjected to histopathologic examination, and atrophic neuron percentages were calculated. In terms of injury percentages, these exhibited heterogeneous, normal distribution. Percentages of degenerative neurons calculated in brain tissue are shown in Table 1. A statistically significant difference was observed between the groups in terms of percentages of degenerative neurons. The Bonferroni-corrected Mann-Whitney *U* test was used to determine the groups that differed significantly ($P < .016$). The percentage of degenerative neurons was significantly lower in groups 2 and 3, in which agents materials were used, compared with that in group 1. A significant difference was also determined between groups 2 and 3 ($P = .003$), with the percentage being significantly lower in the NAC group compared with the ethyl pyruvate group. The percentage of degenerative neurons was higher in group 1 than in groups 2 and 3, and the difference was statistically significant ($^{a}P = .003$ and $^{c}P = .003$, respectively).

At histologic grading, grade 3 degeneration was observed in 5 of the 7 rats in group 1, exposed to ischemia-reperfusion injury alone, and grade 2 degeneration in 1. One rat died during the procedure. Grade 1 degeneration was observed in 6 of the 7 rats in group 2 and grade 2 degeneration in 1. Grade 1 degeneration was determined in 4 of the 7 rats in the ethyl pyruvate group, and grade 2 degeneration in 3 (Fig. 2).

No grade 3 degeneration was observed in any rat in either of the groups receiving NAC or ethyl pyruvate. Comparison revealed a better histologic grade in the NAC group compared with the ethyl pyruvate group, and NAC was observed to have greater neuroprotective efficacy.

Discussion

Studies concerning the use of existing neuroprotective agents have involved their use together with standard treatments applied in patients with acute ischemic stroke or before hospital. Rather than preventing reperfusion injury, the use of neuroprotective agents is intended to protect brain tissue against direct ischemia or to prevent infarction in the still-living ischemic penumbra. Several neuroprotective therapies are safe and at least as effective in ischemic stroke as in hemorrhagic stroke. The ideal neuroprotective therapy must be initiated as early as possible in ischemic stroke, including in the prehospital