Biomedicine & Pharmacotherapy 87 (2017) 561–567

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Oxidative stress and mRNA expression of acetylcholinesterase in the leukocytes of ischemic patients

Mesut Işık[a], Şükrü Beydemir[b,*], Aslan Yılmaz[c], Muhammet Emin Naldan[d], Hatice Esra Aslan[e], İlhami Gülçin[e]

[a] Department of Pharmacy Services, Health Services Vocational School, Harran University, 63000, Şanlıurfa, Turkey
[b] Department of Biochemistry, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, Turkey
[c] Department of Neurology, Regional Training and Research Hospital, Erzurum, Turkey
[d] Department of Anesthesia, Regional Training and Research Hospital, Erzurum, Turkey
[e] Department of Chemistry, Faculty of Sciences, Atatürk University, 25240, Erzurum, Turkey

ARTICLE INFO

Article history:
Received 11 November 2016
Received in revised form 16 December 2016
Accepted 1 January 2017

Keywords:
Gene expression
Ischemic stroke
Oxidative stress
Free radical
Acetylcholinesterase

ABSTRACT

Background: Pathogenesis of ischemic brain injury is occurred by crucial metabolic reasons. For instance, oxidative stress from free radical generation is causing to the thrombotic cerebrovascular stroke. In this case, the measurement of the oxidative stress is very important for a better understanding of the stroke pathophysiology. Because, the oxidative stress in stroke is generally assumed as one of the mechanisms taking part in neuronal damage. Thus, oxidative stress has a vital role in the cholinergic system.
Methods: We performed on 18 adult patients with stroke and 24 healthy persons as control subjects. First, acetylcholinesterase (AChE) activity and oxidative status were assayed in plasma and subsequently, quantitative gene expression of acetylcholinesterase was determined in leukocytes of patients diagnosed with acute stage of ischemia.
Result: It was observed an increase in levels of the protein carbonyl content compared to the control ($p = 0.0011$, $p < 0.01$). The amount of the total thiol was lower than in the control groups of the ischemic patients ($p = 0.023$, $p < 0.05$). AChE and GST activities were significantly lower than control ($p < 0.01$) in acute ischemic patients ($p < 0.01$). mRNA expression of AChE was higher in the control groups than in the leukocytes of acute ischemic patients ($p < 0.05$).
Conclusion: These results suggest that oxidative status, AChE activity and its gene expression were changed significantly at the acute ischemic patients.

1. Introduction

Oxygen metabolism consists of important reaction series for living things. Particularly, the central nervous system (CNS) requires high amounts of oxygen. Adequate arterial blood flow which the oxygen is transferred with this way to the tissues is vital for the energy production system such as glucose metabolism [1]. On the other hand, if the blood flow is disrupted for even a short period of time, the result is cell damage or death. This disruption is collectively referred to as stroke [2]. Stroke contains to two main types from lack of blood flow and hemorrhagic from bleeding. The imbalance or severe reduction of blood flow in cerebral arteries causes ischemic stroke. Hypoperfusion resulting from imbalance or severe reduction of blood may result to neuronal death occuring within a few minutes. If the blood flow to the exposed tissue to the hypoperfusion is not restored, the tissue may be permanently damaged. On the other hand, brain tissue can be protected by reperfusion. However, this situation may cause to oxidative stress by reoxygenation. Thus, many nonenzymatic oxidation reactions may occur in the cells [3,4]. As a result of nonenzymatic oxidation reactions occur an increased generation of free radicals and other reactive species leading to oxidative stress. It is only one of diverse risk factors of pathophysiological mechanisms of ischemic and hemorrhagic stroke [5]. Both ischemic and hemorrhagic stroke are expressed to contain an increased production of free radicals and other chemical species by some reports. Already oxidative stress is defined as the disruption of the physiological balance between oxidant and antioxidant systems [6]. Thus, it is suggested that the basic mechanisms of brain damage can be caused by oxidative stress [6].

* Corresponding author.
E-mail addresses: sukrubeydemir@anadolu.edu.tr, beydemirs@gmail.com (Ş. Beydemir).

http://dx.doi.org/10.1016/j.biopha.2017.01.003
0753-3322/

It is well known that gene expression process is affected many parameters such as oxidative stress from some chemicals and other environmental factors in the cell. Increasing of the oxidant production causes the dramatic changes in the activities of various antioxidant defenses. These alterations influence the gene expression in different tissues of the living things [7,8]. On the other hand, free radical reactions and ROS are known to use in biological stimuli [9,10]. Particularly, the expression of a number of genes and signal transduction pathways is affected via ROS and some antioxidants [7,11].

Besides all of these, the antioxidant activity of plasma and erythrocytes may have a crucial role for protection mechanism against oxidative stress-related neurological defect [12]. Antioxidants are considered as a parameter of the protection mechanisms against stroke and dementia, which is a major public health issue for particularly elderly people. Especially, vascular dementia (VaD) is one of the largest causes of dementia in the elderly [13]. A common clinical syndrome of mental decline, VaD leads the ischemic or hemorrhagic cerebrovascular disease (CVD). It also results the hypoperfusive ischemic cerebral injury caused by cardiovascular and circulatory disorders [14]. Some literatures show that cholinergic system and VaD is closely associated. [15]. It has been reported that the VaD cases in human includes deficits of cholinergic markers [15,16]. Because, cholinergic mechanisms have important role in the modulation of regional cerebral blood flow [17]. The activities of some enzymes can also effective on the cholinergic system. In particular, the acetylcholinesterase (AChE) enzyme activity is also crucial for cholinergic function [18]. The activity of the enzyme exists in many tissues including erythrocytes, platelets and lymphocytes [19]. Erythrocyte AChE has a similar structure and mechanistic property to brain synapse AChE [20,21]. Thus, the assay of erythrocyte AChE activity may suggest on cholinergic function.

In here, we aimed to determine the protein oxidative profile using protein carbonyl assay; antioxidant defense using glutathione S-transferases (GST) and total thiol levels in this study. Besides, we evaluated the activity and expression mRNA of AChE in ischemic patients of acute stages.

2. Materials and methods

This study included 18 adult patients (8 males and 10 females, mean age 72.34) and 24 (11 male and 13 female, mean age 70.06) healthy adults. The patients in the study were acute ischemic stroke patients within the first 48 h of thromboembolic ischemic episode. Neither patients nor controls had a previous history of a cerebrovascular event, cerebral hemorrhage, hemorrhagic infarct or transient ischemic attack. Those with a history of an infectious or inflammatory disease, cancer, autoimmune disorder, hematological disorder, renal or hepatic disease or use of immune-suppressant, anticoagulant or anti-inflammatory drugs in the previous two months were excluded. Antiagregan (acetylsalicylic acid and/or clopidogrel) treatment was applied to the patients. The Clinical Findings of patients were quantified using the stroke scale defined by the American National Institutes of Health (NIHSS). This scale scans the consciousness, view, visual field, facial paralysis, weakness of arm and leg, ataxia, sens, tongue, dysarthria and all neurological findings, including neglect. The higher the score in NIHSS that can be up to 42 points, the greater the clinical picture. While NIHSS 0–6 score of 16 patients became mild to moderate, two patients had moderate to severe deficits with 6–22 score. It was used the serum samples which have already taken in this study. Aliquots of this serum were kept frozen at −20 °C until assayed. The enzyme activities of GST, AChE, amount of protein carbonyl, acetylcholine (ACh) and total thiol in 24 healthy adults and 18 adult patients diagnosed with acute stage of ischemia were measured by spectrophotometric methods. Quantitative gene expression of AChE mRNA in leukocyte was detected by Real time-PCR.

2.1. Sampling and RNA extraction

Peripheral blood samples (2.5 mL in EDTA) were collected. Leukocytes were isolated by the osmotic lysis method and the resulting cell pellets were stored at −80 °C until RNA extraction. RNA was extracted with the QIAamp RNA Blood Mini Kit provided by QIAGEN (Hilden, Germany), according to the manufacturer's protocol. Each RNA sample was eluted with RNase-free water. RNA concentration was determined by measuring the absorbance at 280 nm and stored at −80 °C.

2.2. Reverse transcription polymerase chain reaction (RT-PCR)

All process of the RT-PCR was performed according to the Superscript III First Strand Synthesis System for RT-PCR (Invitrogen). 10 μL of the reaction mix (A1) included RNA, random primer and dNTPs. The incubation was done for 5 min at 65 °C and then placed on ice for one min. Also, 10 μL of cDNA synthesis mix (A2) were added on the A1 solution and incubated for 10 min at 25 °C. The reaction mix was incubated at 50 °C for 50 min and the incubation followed by 5 min at 85 °C. The mix was taken on ice-bath. Subsequently, 1 μL of RNase H to each tube was added and incubated the tubes for 20 min at 37 °C. Thus, cDNA was synthesized.

2.3. Real-time PCR

Gene specific primers and probes were designed (Table 1). They were blasted to confirm their species and gene specificity. As a template, the 2 μL of the synthesized cDNA was used for real-time PCR. The template was added to the reaction mixture (TaqMan FastStart Probe Master Mix, Roche) for multiplex real-time PCR. Quantitive gene expression profile was obtained by using Qiagen Rotor-Gene Q. The experiment comprises the steps of the reaction mixture incubated 2 min at 50 °C for the initial step, 10 min at 95 °C for deactivation and subsequently 45 cycles of 10 s at 95 °C for

Table 1
Primers and Taqman probe sequence for the human genes characterized in cerebrovascular patients and controls.

Genes	Accession	Primers/probes	Primers and probes[a]	Product Size
GAPDH	M33197.1	Forward primer	GGTTCTCCTTCGTGCCTGT	113
		Reverse primer	AGCCCTCATCCTTCACCAC	
		Taqman probe	GCCCTCATCAACGCGGGAGA	
AChE	M55040.1	Forward primer	GACACCCACTCCTCCACCT	120
		Reverse primer	TCCACCACCCTGTTGCTG	
		Taqman probe	TGACGCTGGGGCTGGCATTG	

[a] The Taqman probe has a reporter fluorescent dye, FAM (6-carboxyfluorescein) at the 5′ end and fluorescence dye quencher, TAMRA (6-carboxytetramethyl-rhodamine) at the 3′ end.