ANTIOXIDANT ENZYMES - POTENTIAL TARGETS IN INTRACEREBRAL HAEMORRHAGE

TOMA PAPACOCEA\textsuperscript{1}, IOAN BURAGA\textsuperscript{2}, RALUCA PAPACOCEA\textsuperscript{3*}, IOANA ANCA BADARAU\textsuperscript{3}, MAGDA BURAGA\textsuperscript{3}, CATALINA CIORNEI\textsuperscript{3}, GRAȚIELA MIHAI\textsuperscript{3}, IRINA STOIAN\textsuperscript{4}, ADAM DANIL\textsuperscript{1}

\textsuperscript{1}Emergency Hospital “St. Pantelimon”, Department of Neurosurgery, 340 Pantelimon, 21659 Bucharest, Romania
\textsuperscript{2}Clinical Hospital Colentina, Department of Neurology, 19-21 Ștefan cel Mare 020125 Bucharest, Romania
\textsuperscript{3}Physiology Department I, “Carol Davila” University of Medicine and Pharmacy, 8 Eroilor Sanitari, 050474, Bucharest Romania
\textsuperscript{4}Biochemistry Department, “Carol Davila” University of Medicine and Pharmacy, 8 Eroilor Sanitari, 050474, Bucharest Romania

*corresponding author: rpapacocea@gmail.com

Abstract

Intracerebral haemorrhage (ICH) is accompanied by a cascade of pathological events which involves the generation of oxygen and nitrogen reactive species. These very short lifetime molecules change the level of antioxidant mechanisms, including antioxidant enzymes. The aim of our study was to assess the superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (Gred), glutathione transferase (GTS) activities. The level of oxidative damage was underlined by malondialdehyde (MDA) as index of lipid peroxidation. We also assessed the total nitrite level.

The measurements were performed on cerebrospinal fluid (CSF) samples from 21 patients and 15 healthy volunteers.

We found significantly increased SOD and GPx and reduced Gred and GST activities in CSF of ICH patients. MDA and total nitrate levels were both increased.

Rezumat

Hemoragia intracerebrală e acompaniată de o cascadă de evenimente patologice care implică generaarea speciilor reactive de oxigen şi de azot. Aceste molecule cu viaţă foarte scurtă modifică nivelul mecanismelor de apărare inclusiv activitatea enzimelor antioxidante. În acest studiu au fost evaluate activităţile unor enzime: superoxid dismutazei (SOD), glutatión peroxidazei (GPx), glutatión reductazei (Gred), glutatión transferazei (GTS). Nivelul agresiunii oxidative a fost subliniat de creşterile semnificative ale nivelului malondialdehidei (MDA) ca indicator al peroxidării lipidice şi de concentraţia nitritului total.

Determinările au fost efectuate pe probe de lichid cefalorahidian provenind de la 21 de pacienţi cu hemoragie intracerebrală şi 15 voluntari sănătosi.

Au fost identificate creşteri semnificative ale activităţilor SOD şi GPx concomitent cu reduceri semnificative ale activităţilor Gred şi GST la pacienţii cu hemoragie intracerebrală. La aceiaşi pacienţi, nivelurile MDA şi nitritului au fost semnificativ crescute.
Keywords: antioxidant enzymes, intracerebral haemorrhage, oxidative stress

Introduction

Intracerebral haemorrhage (ICH) has a double incidence comparative to subarachnoid haemorrhage (SAH) and leads more often to death or major disability than cerebral infarction or SAH, according to American Heart Association American Stroke Association (AHA/ASA) Guidelines for the Management of Spontaneous Intracerebral Haemorrhage [1].

In patients with intracerebral haemorrhage (ICH) brain is highly sensitive to reactive oxygen and nitrogen species (ROS/RNS). Soon after the initial cause of the blood extravasation, a second wave of injuries is related to oxidative stress which produces damages of proteins, lipids, carbohydrates, DNA, RNA, resulting in neurotoxicity [2].

Among the most effective antioxidant interventions, enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are responsible for the neutralization of ROS by several mechanisms [3].

These enzymes are present in cerebrospinal fluid (CSF) and their assessment and modulation may allow a better protection in ICH patients.

Materials and Methods

Patients with ICH were admitted to the Department of Neurosurgery of the Cerebrovascular Diseases Institute Bucharest - University of Medicine and Pharmacy, “Carol Davila”, Bucharest, Romania, 48 h after the onset of symptoms. Before inclusion in the study, the written informed consent was obtained from each person or their relatives. The study protocol was approved by the local ethics committee.

All patients were the subject of complete physical and neurological examinations. ICH was confirmed by magnetic resonance imaging (MRI) or computerized tomography (CT) scan.

The study group included 21 patients both gender (10 male, 11 female) aged between 35-65 years old, with acute ICH. The control group consisted of 15 healthy subjects without any nervous diseases, to whom CSF was collected during lumbar punctures for spinal anaesthesia in other surgical interventions.

Exclusion criteria were: the presence of subarachnoid haemorrhage and/or intraventricular blood effusion confirmed by CT or MRI; post-traumatic brain injuries; haematological disorders; chronic diseases (lung, kidney); use of medications able to interfere in the oxidative equilibrium: (cortisol and derivatives) non-steroidal anti-inflammatory drugs, anticoagulants,
iron and derivatives, chemotherapy, vitamin E, ascorbic acid, other antioxidant substances.

All CSF samples – 5mL each were collected in sterile, chemically inert (Eppendorf) recipients and frozen at –80°C. Each sample was examined in duplicate for the determination of GPx, SOD, glutathione transferase (GTS), glutathione reductase (Gred) activities, nitrit-nitrate and MDA level.

GPx stimulates glutathione formation and was spectrophotometrically assayed, based on the reduction of glutathione in the presence of Glutathione reductase in excess. The concomitant nicotinamide (NADPH) reduction was monitored for 3 minutes (340 nm) [4].

For the SOD assay it was used a spectrophotometric method based on the ability of the enzyme to inhibit pyrogallol autoxidation in the presence of EDTA. Readings were performed at 420 nm every 30 seconds [5].

SOD activity was expressed as U/mL where one unit of SOD is the amount of enzyme which inhibits pyrogallol autoxidation to 50%.

Glutathione reductase (Gred) catalyzes the reduction of oxidized glutathione (GSSG) in the presence of reduced NADPH.

Gred activity was determined in vitro in the presence of flavin adenine dinucleotide (FAD) and GSSG, by measuring the absorbance at 340 nm for 3 minutes.

Glutathione transferase activity was determined spectrophotometrically at 340 nm by measuring the transfer of CDNB (4-chloro 1,2 dinitrobenzene) on GSH for 3 minutes. One unit of the enzymatic activity of GST is the quantity of enzyme that catalysis the formation of a one mol S- 2, 4 -dinitrophenyl glutathione/minute. The enzymatic activity was expressed as IU/ mg protein.

Malondialdehyde was measured using thiobarbituric acid method to evaluate lipid peroxidation [6]. MDA levels were expressed as µmol/mL. Total proteins were determined using Markwell method [7].

Nitrit-nitrate level was determined using Griess reactive [8].

Resulted data were expressed as means ± standard deviation (SD) and the differences between group means were tested by Student’s t-test, where p < 0.05 was considered statistically significant.

Results and Discussion

Glutathione peroxidase was significantly reduced (p < 0.05) in ICH group 132.6 ± 58.7 U/mL compared to control 284.14 ± 44.8 U/mL (Figure 1). SOD was significantly reduced (p < 0.001) in the ICH group 2.76 ± 0.41U/mL compared to the control group 1.73 ± 1.05 U/mL (Figure 2).
Gred activity was significantly increased (p < 0.001) in the ICH group 349.67 ± 67.6 ng/min/mL compared to control 259.05 ± 58.15 ng/min/mL (Figure 3). GTS was significantly increased (p < 0.01) in ICH group 144.18 ± 19.05 ng/min/mg protein compared to control 84.7 ± 22.86 ng/min/ mg protein (Figure 4).
MDA level was significantly increased ($p < 0.001$) in ICH patients $5.55 \pm 3.15 \text{µmol/mL}$ versus control $15.47 \pm 2.56 \text{µmol/mL}$ (Figure 5).
Nitrite nitrate levels were significantly increased ($p < 0.001$) in ICH patients $15.9 \pm 2.54$ ng/mL compared to control $2.06 \pm 0.4$ ng/mL (Figure 6).

![Figure 6.](image)

Total nitrite level in ICH patients versus control group

Determination of the antioxidant enzymes activities in cerebrospinal fluid is an indirect method for the evaluation of the brain oxidative stress [9, 10].

SOD rapidly neutralizes free radicals resulted from the installation of an intracerebral hematoma and is overexpressed in the first 6 hours [11].

However, after that, SOD level is reflected by conflicting results in different studies. Some authors found maintenance of this enzyme at high values even after 24 hours, while others in contrast showed a reduction [12, 13].

Our data showed a reduced SOD activity, caused by superoxide anion excess [14].

Enzyme inactivation through distortion of protein structure or inactivation of the active site could be exerted by the increased concentration of lipid peroxidation products such as MDA [15].

GPx was also reduced in patients compared to healthy subjects; existing data showed that after brain injury, astrocytes release high amounts of ascorbate which is converted in dehydroascorbate (DHHA) in the presence of ROS. DHHA showed an inhibitory effect on GPx [16].

It was also showed that GPx is inactivated in contact with lipid peroxidation products (MDA and hydroxynonenal- HNE) [17].

Significant decreases in the activity of the main antioxidant enzymes SOD and GPx in the ICH group are primarily due to their active site inactivation. This mechanism has been documented experimentally for both SOD and GPx and was highlighted both on cultures of neurons and astrocytes. This significant decrease may be a marker of intense cerebral oxidative stress [18].
Gred significantly increased in ICH group compared to control. Due to oxidative stress, the reduction of intracellular GSH takes place and GSH reduction has a stimulatory effect on the activity Gred. Gred is also exposed to inactivation through oxidation of the two susceptible sulfhydryl groups. However, GST activity significantly increased in ICH group; in the presence of oxidative aggression GST activity doubled, this increase may a long-term adaptive response of the cerebral tissue [19].

The nitrite level was significantly increased in all ICH patients (100 %) versus control. This parameter reflects the generation of NO, secondary to N methyl D aspartate (NMDA) receptors activation [20]. Once synthesised, NO generates ONOO\(^{-}\), a much aggressive compound for both neurons and astrocytes [21, 22].

Conclusions

In ICH patients, the first line antioxidant enzymes activity decrease (SOD and GPx) is accompanied by a significant increase of Gred and GST. This process takes place on high oxidative aggression evoked by MDA and nitrite levels increase.

As long as oxidative stress worsens the clinical outcome, we propose the use of certain antioxidant enzymes as markers of the oxidative stress intensity in ICH patients.

References


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