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Protective role of glutathione reductase in paraquat induced neurotoxicity

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ABSTRACT

Paraquat (PQ), a widely used herbicide is a well-known free radical producing agent. The mechanistic pathways of PQ neurotoxicity were examined by assessing oxidative/nitrosative stress markers. Focus was on the role of glutathione (GSH) cycle and to examine whether the pre-treatment with enzyme glutathione reductase (GR) could protect the vulnerable brain regions (VBRs) against harmful oxidative effect of PQ. The study was conducted on *Wistar* rats, randomly divided in five groups: intact-control group, ($n = 8$) and four experimental groups ($n = 24$). All tested compounds were administered intrastriatally (*i.s.*) in one single dose. The following parameters of oxidative status were measured in the striatum, hippocampus and cortex, at 30 min, 24 h and 7 days post treatment: superoxide anion radical ($O_2^{\cdot -}$), nitrate (NO_3^-), malondialdehyde (MDA), superoxide dismutase (SOD), total GSH (tGSH) and its oxidized, disulfide form (GSSG) and glutathione peroxidase (GPx).

Results obtained from the intact and the sham operated groups were not statistically different, confirming that invasive *i.s.* route of administration would not influence the reliability of results. Also, similar pattern of changes were observed between ipsi- and contra- lateral side of examined VBRs, indicating rapid spatial spreading of oxidative stress. Mortality of the animals (10%), within 24 h, along with symptoms of Parkinsonism, after awakening from anesthesia for 2–3 h, were observed in the PQ group, only. Increased levels of $O_2^{\cdot -}$, NO_3^- and MDA, increased ratio of GSSG/GSH and considerably high activity of GPx were measured at 30 min after the treatment. Cytotoxic effect of PQ was documented by drastic drop of all measured parameters and extremely high peak of the ratio GSSG/GSH at 24th hrs after the PQ *i.s.* injection. In the GR + PQ group, markedly low activity of GPx and low content of NO_3^- (in striatum and cortex) were measured during whole experiment, while increase value was observed only for $O_2^{\cdot -}$, at 7th days.

We concluded that oxidative/nitrosative stress and excitotoxicity are the most important events since the early stage of PQ induced neurotoxicity. Based on the ratio GSSG/GSH, the oxidation of GSH to GSSG is probably dominant way of GHS depletion and main reason for reduced antioxidative defense against PQ harmful oxidative effect. The GR pre-treatment resulted in the absence of Parkinson's disease-like symptoms and mortality of the rats. Additionally, oxidative/nitrosative stress did not developed, as well as almost diminished metabolism of the VBRs at 24th hours (as has been documented in the PQ group) did not occurred in the GR + PQ, suggesting a neuroprotective role for the GR in PQ induced neurotoxicity.

1. Introduction

Misuse of pesticides, such as paraquat (PQ), can lead to excessive contamination of the environment. Paraquat (1,1′-dimethyl-4,4′-bipyridinium), a fast-acting non-selective contact herbicide, is widely used to control broad-leaved weeds in growing corn, fruit trees, vegetables, and as a desiccant before harvesting and for destroying marijuana plants. Accidental and occupational exposures of agriculture workers are the primary hazards for human health. Occupational and professional exposure to PQ is addressed to inhalation or dermal route of administration and is considered as an eti-

Abbreviations: ATP, adenosine-triphosphate; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione (reduced form); GSSG, glutathione (oxidized form); HMPS, hexose monophosphate shunt; LPO, lipid peroxidation; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NAC, *N*-acetyl-cysteine; NO_3^-, nitrate; NS, nitrosative stress; $O_2^{\cdot -}$, superoxide anion radical; OS, oxidative stress; PQ, paraquat; SOD, superoxide dismutase; MDA, malondialdehyde; VBRs, vulnerable brain regions; *i.s.*, intrastriatally; *i.p.*, intraperitoneally.

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ological factor of Parkinson's disease [1,2]. Suicidal poisonings are referred to ingestion. Ingestion of concentrated solutions (12–20%) of PQ leads to early death due to organs failure, whereby the kidneys and lungs are main target organs. In the past few decades PQ has been a popular agent for intentional (suicidal) poisonings. Paraquat is extremely toxic to humans (LD_{50} 35 mg/kg) and animals (rats: LD_{50} is 110–150 mg/kg), by all routes of exposure. Since recently, usage of the PQ has been restricted [3–5]. There is no specific treatment for PQ poisoning, hence management of poisonings is to relieve symptoms and treat complications (supportive care) [6].

Red-ox metabolism is essential for PQ cytotoxic effect [7–10]. Several enzymes may be involved in PQ metabolism, such as: the cytochrome P-450 reductase, ferricytochrome oxidoreductase and the cytosolic enzyme nitric oxide synthase (NOS) [2,7,11]. In the presence of electron donors, PQ^{2+} (commercial product: a salt of a dication) readily undergoes the one-electron reduction to its radical form $PQ^{\cdot+}$. Unpaired electron of $PQ^{\cdot+}$ is stabilized by the conjugated double bond in the pyridine ring and quaternary nitrogen in another ring (Scheme 1). In aerobic conditions, $PQ^{\cdot+}$ conveys its unpaired electron to molecular O_2, generating superoxide anion radical ($O_2^{\cdot-}$), whilst $PQ^{\cdot+}$ becomes oxidized, to PQ^{2+}. This triggers a chain of free radicals reactions, which are essential for the PQ cytotoxic effect [10].

Brain tissue is particularly vulnerable to oxidative injury induced by PQ, documented with manifestation of Parkinsonism like symptoms (development of extrapyramidal behavior) [12]. Some parts of the brain [including: pyramidal neurons of CA1 and CA3 sectors of the hippocampus, a small pyramidal neurons and third layer of the cerebral cortex and striatum (*Nucleus caudatus* and *putamen*)] are more sensitive to oxidative/nitrosative stress [13]. Given systemically PQ produces hyper-excitability, convulsions, hyperkinesis and incoordination in the early stages whilst intracerebral administration leads to neurodegenerative, morphological and behavioral changes in the central nervous (CNS) system, in a dose-dependent manner [14–19].

Glutathione (GSH) acts as a neuromodulator, neurotransmitter and neurohormone in the CNS. Its antioxidative role is recognized in several reactions including glutathione peroxidase (GPx) catalyzed reduction of lipid hydro peroxides and conjugation with thiol, nitroso and metal ions [20–28].

According to literature, several approaches have been undertaken to retain GSH at the level to achieve its antioxidative effect. One of them is systemic administration of L-cysteine and/or *N*-acetyl-cysteine (NAC) which are substrates for the biosynthesis of GSH. L-cysteine passes across biological membranes and serves as a good source of SH groups, whereas GSH is ineffective when given orally because of its poor absorption from the digestive tract and/or poor ability to permeate through the membrane. However, therapeutic use of cysteine is limited by its excitotoxicity since it interacts with glutamate causing acidification and neurotoxicity [29–31].

During oxidative stress, GSH becomes oxidized to glutathione disulfide (GSSG). Recycling of glutathione (reduction of GSSG back to GSH) by glutathione reductase (GR) is particularly important to maintain GSH concentrations at levels necessary to achieve its antioxidative role. Leading by the fact that GR does not pass the blood–brain barrier, we decided to administer GR intrastriataly (*i.s.*). In this toxicological-experimental mechanistic study we examined whether neuroprotection of vulnerable brain regions (VBRs) (striatum, hippocampus and cortex) against harmful oxidative injury induced by *i.s.* administered PQ would be achieved by the pre-treatment with GR intrastriatally (*i.s.*) applied. A positive outcome would have confirmed that the oxidative/nitrosative damage of VBRs was the consequence of the reduced antioxidant defense, primarily due to GSH oxidation to GSSG, thus minimizing the significance of the other GSH depletion pathways in this particular case of PQ neurotoxicity.

2. Materials and methods

2.1. Animals

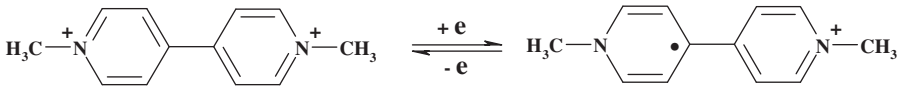
The experimental animals were treated according to Guidelines for Animal Study, No. 282-12/2002 (Ethics Committee of the Military Medical Academy, Belgrade, Serbia and Montenegro). The experiments were performed on adult male *Wistar* rats weighing approximately 220 g, randomly divided into two control groups (the intact group $n = 8$, and the sham-operated, $n = 24$) and three experimental groups ($n = 24$, each) which were further subdivided into three subgroups ($n = 8$) according to the time of sacrificing. The rats were housed in cages under standardized housing conditions (ambient temperature of 23 ± 2 °C, relative humidity of 55 ± 3% and a light/dark cycle of 13/11 h) and had free access to standard laboratory pellet food and tap water. All the experiments were performed after 7 days period of adaptation to laboratory conditions, and were carried out between 9 a.m. and 1 p.m.

2.2. Experimental design

Rats were anesthetized by sodium pentobarbital (45 mg/kg per body weight) given intraperitoneally (*i.p.*). In this study we used yeast GR, according to: (a) literature evidence of its homology with GR sequences of rats and humans, substrate specificity, kinetics characteristic and substrate affinity; and (b) our positive previous experience in different animal models (unpublished data) [32–35]. Testing substances were administrated as single doses, *i.s.* in final volumes of 10 μL, which is insufficient to burden nerve tissue. For this purposes we used Hamilton syringe, which was accurately coordinated by using a stereotaxic instrument for small laboratory animals (coordinates: 8.4 mm behind the bregma, 2.6 mm left from the midline suture and 4.8 mm ventral from dura) [36]. The experiment was accomplished with the following (five) experimental groups, which received different testing substances: the intact group (not treated, $n = 8$), the sham-operated rats (10 μL of saline), $n = 24$; the GR group(GR, 15.63 U/10 μL), $n = 24$; the PQ group (2.5 μg PQ/10 μL, *i.e.* 0.01 μM/10 μL) $n = 24$; and the GR + PQ group (GR, 15.63 U/5 μL, immediately before PQ administration, 0.01 μM/5 μL), $n = 24$. The animals were sacrificed by decapitation at 30 min, 24 h and 7 days after the treatments. Biochemical parameters of OS were measured in the ipsi- and contra- lateral side of the VBRs. To exclude the possibility that mechanical injury caused OS in the VBRs, we compared OS parameters between the sham-operated and the intact groups.

2.3. Reagents

All chemicals were of analytical grade. The following compounds were used in this study: Paraquat–Galokson® (200 g/L)



Scheme 1. One-electron reduction of PQ^{2+} results in the formation of its stabile radical form ($PQ^{\cdot+}$) Stability is achieved by the conjugated double bond in the pyridine ring and quaternary nitrogen in another ring.