The effect of laparotomy on hydroxyl radicals, singlet oxygen and antioxidants measured by EPR method in the tails of rats

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Abstract

OBJECTIVES: The aim of the study was to demonstrate that direct measurement of hydroxyl radicals and singlet oxygen in the tail of living rats is possible. The basic level of hydroxyl radicals and singlet oxygen were measured and the effects of antioxidants on their levels were studied in the tail of living anaesthetized rats after acute postoperative pain. Laparotomy was performed as the source of acute abdominal pain. After closure of the abdominal cavity, the animals began to awaken within 30–60 minutes. They were left to recover for 2–3 hours; then they were reanesthetized and the effect of antioxidants was measured on the numbers of hydroxyl radicals and singlet oxygen via blood in the tail.

METHODS: The laparotomy was preformed under general anesthesia (Xylazin and Ketamin) using Wistar rats. After recovery and several hours of consciousness they were reanaesthetized and free radicals and singlet oxygen were measured. An antioxidant mixture (vitamins A, C, D and Selenium) was administered intramuscularly prior to the laparotomy. All measurements were done on the tail of anaesthetized animals. In this particular article, the effect of antioxidants is only reported for hydroxyl radicals.

RESULTS: After laparotomy, which represented both somatic and visceral pain, hydroxyl radicals and singlet oxygen were increased. Antioxidant application prior to laparotomy decreased the numbers of hydroxyl radicals.

CONCLUSION: Results are in agreement with our previous finding regarding the increase in hydroxyl free radicals and singlet oxygen following nociceptive stimulation, in this case a combination of both somatic and visceral pain. The administered antioxidants mitigated the increase. This is further confirmation that direct measurement of free radicals and singlet oxygen represents a very useful method for the biochemical evaluation of pain and nociception.

ORIGINAL ARTICLE

INTRODUCTION

Our group is committed to continuously monitoring the changes in free radicals during different types of nociceptive stimulation. We have tested changes in free radicals in both experimental animals [Pekarkova *et al* 2003; Rokyta *et al* 2003; Rokyta *et al* 2004; Rokyta *et al* 2008] and in humans [Kozák *et al* 2004; Křikava *et al* 2004; Rokyta *et al* 1996]. We measured the different free radicals products especially thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) and participating enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX). In recent years and, previously reported, we have made direct measurement of free radicals using electron paramagnetic resonance (EPR) methods in rats (blood and brain tissue) and in humans (blood only). In an earlier article, published in 2008 [Rokyta *et al* 2008], we described a new approach for measuring free radicals in the tail of living anaesthetized rats. The advantage of this method is the ability to measure the immediate effects of nociceptive stimulation. In previous publications we have used several different methods of nociceptive stimulation (mechanical, inflammatory and visceral); in this paper we use post-operative pain and visceral stimulation.

MATERIAL AND METHODS

Thirty-six adult, male (Wistar) rats (180–220 g) were used. They were housed according to the principles of good laboratory practice (alternation of 12 hours light and 12 hours darkness, food ad libitum and housed 8 animals per cage). All animal procedures were in strict accordance with the Declaration of Helsinki and the guidelines of the Ethics Committee of the International Association for the Study of Pain [Zimmermann 1983]. All experiments were approved by the Animal Care Committee of the 3rd Faculty of Medicine, Charles University, Prague, CZ. During experimental procedures, all efforts were made to minimize animal suffering.

The nociceptive stimulation, in this case laparotomy, was performed under general anesthesia (5% ketamine, at a dose of 90 mg/kg and 2% xylazine, at a dose of 15 mg/kg). Suturing of muscle and skin concluded the operation. The rats came out from under anesthesia 30–60 min. after the operation. An antioxidant mixture, consisting of vitamin A-β carotene 0.5 mg/kg, vitamin C 3.5 mg/kg, vitamin E (Trolox α – tocopherol, water soluble vit. E) 5 mg/kg and selenium in the form Na2SeO3. H2O, 0.25 μg/kg was applied intramuscularly over a period of several days prior to the surgical procedure.

Before, as well as after the operative procedure, EPR was measured under general anesthesia. Each rat, under total anesthesia (5% ketamine, at a dose of 90 mg/kg and 2% xylazine, at a dose of 15 mg/kg), was placed on a plastic table in an EPR resonator and part of its tail was placed into a silica glass tube within the resonator (Fig. 1).

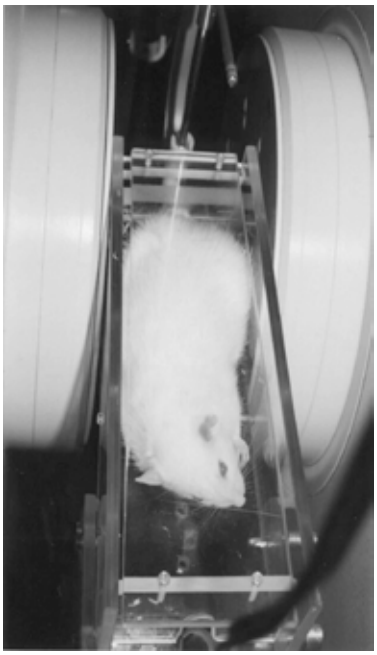

Fig. 1: Placement of anaesthetized rat in the EPR spectrometer

For statistical evaluation the paired T- test was used. The electron paramagnetic (spin) resonance spectroscopy (EPR-ESR) has been previously described in detail [Rokyta *et al* 2008; Gallez & Schwartz 2004; Weil *et al* 1994]. Spin trapping was done using DMPO (5-dimethyl pyrroline N oxide), PBN (phenyl-tert-butylnitrone) and TMP (2,2,6,6-tetramethylpiperidine). For illustration purposes, we demonstrate an example of an original recording of the EPR spectra of a rat before and after the operative procedure (Fig. 2, 3)

RESULTS

A statistically significant increased in the number of hydroxyl radicals was observed three to four hours following the procedure (Fig.4 and Table 1).

Additionally, the numbers of singlet oxygen (Fig. 5 and Table 1) was also increased after laparotomy.

In one group of rats, we administered a cocktail of antioxidants over five consecutive days. In the group that received the antioxidant cocktail, we observed lower levels of free radicals (Fig.6 and Table 1)

DISCUSSION

Oxidative stress arises from an imbalance between the formation of free radicals and anti-oxidative species. Free radicals can damage a variety of cell functions through lipoperoxidation, oxidation of proteins, destruction of DNA, and nitration of albumins. There are two possible explanations for out model of pain stimulation [Maoet al 1995]. Free radicals can be also generated in a variety of ways for example uric acid can be generated from xanthine oxidase and cell necrosis can create superoxide. The direct measurement of free radicals and singlet oxygen in the tail of rats is an "in vivo" measurement and demonstrates integral-body

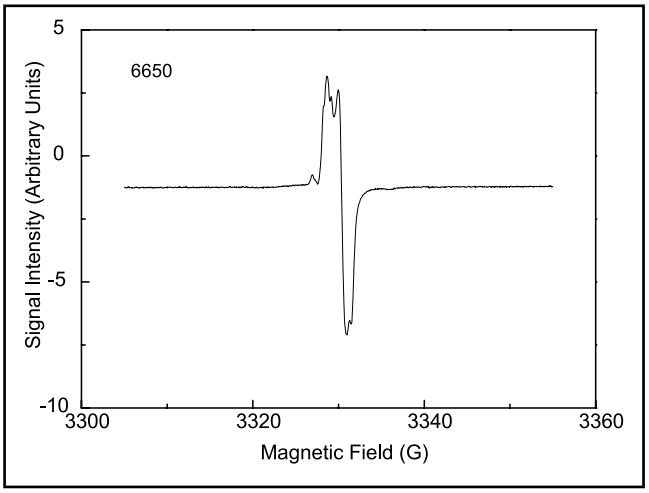

Fig. 2: Hydroxyl radicals before laparotomy

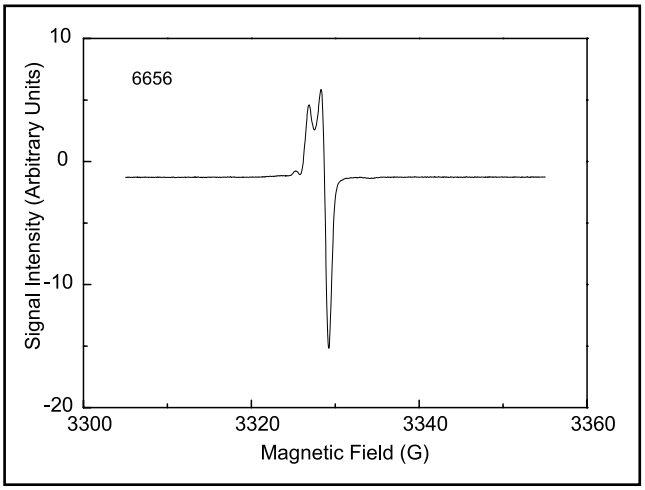

Fig. 3 Hydroxyl radicals after laparotomy

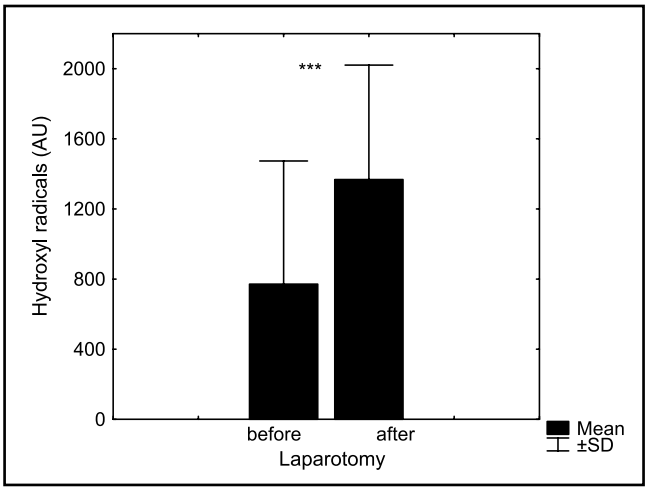

Fig. 4. Hydroxyl radicals before and after laparotomy

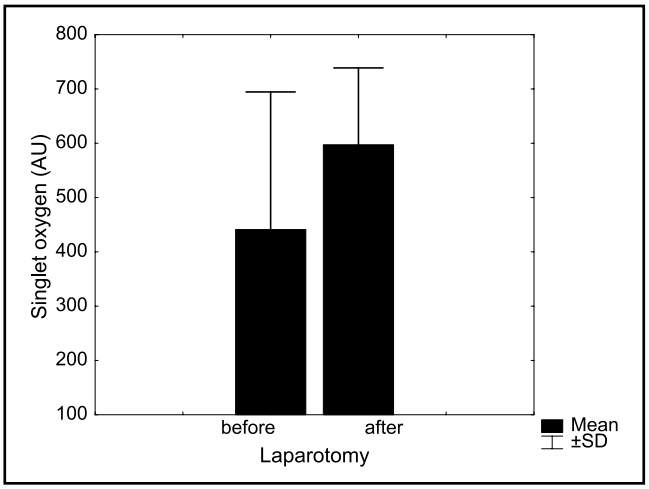

Fig. 5. Singlet oxygen before and after laparotomy

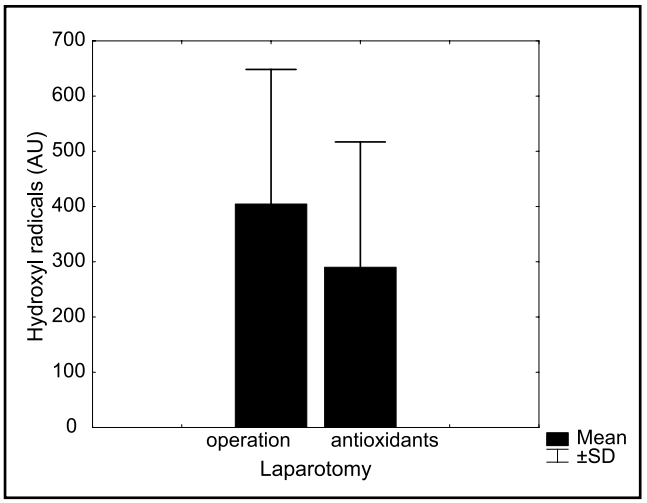

Fig. 6. Hydroxyl radicals after laparotomy and after antioxidant treatment

Table 1.

	Mean ± SD	N	T test	p
Hydroxyl radical				
Before operation	771 ± 702	21	-4.41	0.0003
After operation	1368 ± 653			
Singlet oxygen				
Before operation	457 ± 259	11	-2.42	0.036
After operation	597 ± 141			
Hydroxyl radical after operation and after antioxidants				
After operation	405 ± 244	11	-2.50	0.031
After antioxidants	293 ± 238			

oxidative stress caused by pain. Free radicals that enter the blood can be detected in the tail. The positive effect of antioxidants reduces the quantity of free radicals and consequently reduces free radical concentrations in tissues. In this and previous experiments, we have shown increased formation of free radicals in the cerebral cortex in experimental rats [Pekarkova *et al* 2003] and also increased numbers of free radicals in the blood of human patients [Kirk & White 2006; Křikava *et al* 2004; Karbownik-Lewinska *et al* 2007].

How can changes in numbers of singlet oxygen be explained [Stief 2003; Sies 1989]? Stress is known to affect synaptic plasticity, dendritic morphology, and induce neurotoxic damage in humans probably through generation of free radicals. Restraint stress induces a decrease in the level of glutathione (GSH) [Brucknerová *et al* 2006]. Vitamin E has been found to be most effective in restoring inherent anti-oxidant systems, no additive effect has been observed using combined vitamin treatments [Zaidi & Banu 2004]. Oxidative stress is an important pathophysiological mechanism of many neurological diseases. Reactive oxygen and nitrogen species have been cited as molecules involved in nociceptive processes [Cini *et al* 1994; Kim *et al* 2004].

Recent investigations suggest that oxidative stress markers are useful in the evaluation of some types of abdominal pathology. We hypothesized that the severity of abdominal pain is correlated with oxidative stress and can be quantified using total antioxidant capacity (TAC) and malondialdehyde (MDA). Patients with acute abdominal pain had lower levels of TAC and higher levels of MDA than normal controls. The above mentioned study found a correlation between oxidative stress and disease severity, relative to abdominal pain in patients. This suggests that TAC might be useful as a guide for patient assessment in the emergency department [Chi *et al* 2002]. The pathophysiology of chronic and persistent pain is a side-effect of pro-oxidant substances. Reactive oxygen species (ROS) have been implicated in contributing to and prolonging chronic pain. Recent pre-clinical reports suggest that antioxidants are effective analgesics in neuropathic and inflammatory pain models.

In studies with PBN; 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxy (TEMPOL), and N-acetyl-L-cysteine (NAC) applied 5 min before intraplantar formalin injection the nociceptive responses were indicated by licking or biting the affected hind limb and were quantified for 30 min after formalin injection. Each drug was shown to be effective in attenuating two or more phases (acute, quiescent, and tonic) of the formalin response [Hacimuftuoglu *et al* 2006].

TEMPOL produced an 83% reduction in the nociceptive response during the tonic phase, but no significant attenuation of the acute phase response. Tert-BuOH coadministration reversed TEMPOL-induced analgesia in the tonic intraplantar formalin response reduction. The data suggest that pro-oxidant species may be important mediators of tissue injury-induced pain in rodents.

The relationship between protein oxidation and the functional state of organisms, particularly aging, hyperoxia and hypoxia, and heat shock, as well as with different pathologies has been analyzed. The final part of the article is devoted to possible ways of protecting proteins against oxidation in vivo [Lushchak 2007]. Vitamin E offers protection against oxidative stress and is an efficient scavenger of singlet oxygen [Dad *et al* 2006].

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