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Plasma antioxidant status in patients undergoing long-term opioid treatment

Ogurčáková Daniela[1,2], Kočan Ladislav[3], Šimonová Jana[4], Martuliak Igor[5], Sabol František[6], Vašková Janka[2*]

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Authors' Affiliation:
[1]Pain management clinic Algmed, Rastislavova 45, 04001 Košice, Slovak Republic
[2]Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Trieda SNP 1, 040 11 Košice, Slovak Republic
[3]Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Ondavská 8, 040 11 Košice, Slovak Republic
[4]1st Clinic of Anaesthesiology and Intensive Care Medicine, Louis Pasteur University Hospital, SNP 1, 040 11 Košice, Slovak Republic
[5]Department of Algeziology, F.D. Roosevelt Teaching Hospital with Policlinic Banská Bystrica and Slovak Medical University in Banská Bystrica, nám. Ludvika Svobodu 8, 97401 Banská Bystrica, Slovak Republic
[6]Department of Cardiac Surgery, East Slovak Institute of Cardiovascular Disease, Ondavská 8, 040 11 Košice, Slovak Republic

***Corresponding author**
Janka Vašková, assoc. prof. Dr. PhD.
Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice,
Tr. SNP 1, 04066 Košice, Slovak Republic
Email: janka.vaskova@upjs.sk

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ABSTRACT

Background: Opioid treatment is now an integral part of pharmacotherapy for severe chronic malignant and non-malignant pain. Currently, there is a sufficient selection of opioids to allow individualized pain treatment. Several experimental studies have confirmed the effect of opioids on oxidative stress. The aim of this work is to determine the presence of redox changes occurring as a result of long-term opioid use in patients with chronic pain. *Results:* Six months of opioid use for severe pain was evaluated in 37 patients. Patients formed three groups depending opioid treatment (oxycodone, fentanyl and tapentadol) and were compared with 42 healthy probands. Compared to control, activities of superoxide dismutase were decreased, while those of glutathione peroxidase and glutathione reductase were significantly increased in all groups. Together with lowered levels of reduced glutathione, this indicated conditions of oxidative stress. There were no differences between treatment groups. *Conclusion:* It is necessary to know the risks of side effects and provide patients with possible solutions. At this stage and with this number of subjects, we can conclude that neither the form of administration nor the type of opioid has any effect on reducing oxidative stress from opioid metabolism in the treatment of severe pain.

Keywords: antioxidant enzymes, pain, glutathione, opioids, oxidative stress

1. INTRODUCTION

Chronic pain and non-malignant pain is a widespread serious public health problem (Breivik et al., 2006; Ripamonti, 2012). There are no clear epidemiological studies available in the European Union; however, approximately 50% of adults suffer from one or more chronic pain with an incidence of moderate to severe pain in the European population at 20%. 70% of patients are in active age, 11% of patients are untreated, and 50% do not have enough pain treatment. The prevalence is higher in women and the number of patient's increases with age, reaching 80% in geriatric patients. The economic costs, namely indirect costs due to incapacity for work, are not negligible. The most common chronic pains such as back pain, osteoarthritis, headache, and neuralgia are often considered to be a normal part of life. The treatment of chronic pain is the elimination of pain and restoration of all

functions (physical, mental, social); in the most optimal case, this includes the possibility of returning to work (Breivik et al., 2006; 2013).

The choice and management of analgesic treatment is based on patient data regarding the intensity and nature of the pain and on the specific clinical condition. It does not determine the origin of the pain (malignant, non-malignant), but in chronic pain: the procedure follows a "bottom-up" approach (step up). In intense acute pain, parenteral administration of an analgesic, possibly also an opioid (in cases of angina pectoris, heart attack, renal and gallbladder colic) is appropriate; otherwise non-invasive administration of analgesics (*per os*, transdermal, *per rectum*) is preferred. Regarding time, analgesics with a rapid onset of action are the most advantageous in acute pain; in chronic pain, analgesics are administered "on an hourly basis" and thus the development of pain is prevented.

Opioid analgesics are essential for pain management (Gilson et al., 2011) due to efficiency and safety treatment under competent physicians. The biopsychosocial status in non-terminal patients with chronic pain should be considered by physician to set up a treatment plan with patient motivation to reach functional goals (Von Korff et al., 2011; Kalso et al., 2004). Morphine is considered to be the gold standard of opioid treatment, with the properties of other opioids derived from it. Many side effects of opioid treatment are under thorough investigation. Recent findings suggest that long-term opioid treatment may contribute to oxidative stress, which is a serious pathological problem due to its key role in the pathogenesis of many diseases (Cacciapuoti, 2016).

Recent studies have also revealed new roles for oxidative stress or reduced antioxidant activities relevant to mitochondria functions behind the development of a migrains (Ferroni et al., 2018). Clinical trials focusing on the redox state of patients taking opioids for chronic pain are still lacking. Due to unclear evidence of oxidative stress intensity in chronic pain patients dosed with opioids for severe pain we have started a prospective multicentre observational study. The aim of the study was to monitor the clinical condition of patients, as well as changes in the activities of antioxidant enzymes after starting opioid treatment for severe pain and during their use.

2. MATERIALS AND METHODS

The study was carried out in co-operation of three pain treatment centres, Pain management clinic Algmed in Košice, Department of Algesiology, F.D. Roosevelt Hospital in Banská Bystrica, and the East Slovak Institute of Cardiovascular Diseases in Košice. The Ethic Committee of Faculty of Medicine Pavol Jozef Šafárik University in Košice no. 1N/2017, Ethic Committee of Slovak Medical University in Banská Bystrica 28/11/2016, and Ethic Committee of East Slovak Institute of Cardiovascular Diseases in Košice no. 1/2019/VUSCH/EK approved the study. The study was registered in the International Database U.S. National Institutes of Health ClinicalTrials.gov under the number NCT03105232. Patients recruitment started august 2020 and was completed December 2021.

The course of the research was explained to potential study participants and, after signing informed consent, followed up with a pain specialist. Screening of parameters was carried out, namely: consumption of analgesics, pain type examination (nociceptive vs. neuropathic - filling out the questionnaires for Pain Detect, DN4, and numeric pain scale), and demographic data (weight, height, age). The inclusion criteria of patients were: non-malignant pain, no previous opioid treatment for visceral, neuropathic, or nociceptive, pain and age over 18 years. Patients with oncological conditions were excluded. Enrolled participants were divided into 4 groups: group 1 - control group (C), healthy individuals (42); group 2 (O) - patients with chronic pain using morphine, hydromorphone, oxycodone, or buprenorphine (14); group 3 (F) - patients with chronic pain receiving transdermal fentanyl patch (12); group 4 (T) - patients with chronic pain using tapentadol (11). The second examination followed six months from the start of opioid use for severe pain. During the examination, blood for biochemical analysis was collected, patients completed a Pain Detect questionnaires, DN4, and Lanss Pain Scale, and clinical parameters were measured.

Bicinchoninic acid was used for blood plasma protein determination. Glutathione peroxidase, glutathione reductase and glutathione-S-transferase activities were determined according to the procedures given by the kit manufacturer (Sigma-Aldrich, Germany). Superoxide dismutase activities were set according to the SOD-Assay KIT-WST (Fluka, Japan). The concentration of reduced glutathione (GSH) was determined by the method originally described by Floreani et al. (1997).

Descriptive statistics were used to characterise groups of patients. A T-test was used to compare values within groups against the corresponding control. Intragroup differences at two sampling times were determined by one-way analysis of variance followed by Tukey-post hoc test. Intergroup differences within parameters were detected by a Mann-Whitney test. Differences were considered significant at $p < 0.05$, $p < 0.01$, $p < 0.001$.

3. RESULTS AND DISCUSSION

After screening the patients who met the conditions and completed both samplings (also after 6 months), 37 patients were included in the study. The measurements of antioxidant parameters in plasma were compared with a group of 42 healthy individuals. Results (Figure 1 and 2) show that SOD activities were decreased in each treatment group when compared to control before the start of therapy and after 6 months regardless of the form of drug administered and the type of opioid. Activities of GPx and GR were increased in comparison to control at both time points. The activities of both GPx and GR enzymes have an even greater tendency to increase 6 months after opioid treatment in all groups. In group 3, there was a statistically significant increase in GPx ($p=0.0044$) over 6 months (Table 1). Morphine treatment affects antioxidant enzyme activities, as morphine-dependent rhesus macaques have been observed with GPx, SOD increased after 140 days of morphine treatment (Pérez-Casanova et al., 2008). Other studies have shown that these enzyme activities were reduced by morphine (Payabvash et al., 2006; Sumathi et al., 2011; Zhou et al., 2011; Roziski et al., 2013). It becomes obvious that this can be affected by many different factors including dosage, exposure time, and species exposed.

The catalytic activity of SOD is the dismutation of the superoxide radical ($O_2^{\cdot-}$) to hydrogen peroxide. Hydrogen peroxide as well as other hydro peroxides is converted to water or the corresponding alcohols by GPx. Glutathione is co-oxidized in the reaction, and reduced back by GR, thus providing an effective antioxidant response. The increased activities of these two glutathione-related enzymes are confirmation of oxidative stress conditions in patients suffering from pain, and very likely suppression or inactivation of SOD. Moreover, $O_2^{\cdot-}$ as well as peroxynitrite anion (ONO_2^-), are known as pro-nociceptive species (Salvemini and Newmann, 2009; 2010). Under conditions of oxidative stress, inflammation contributes even more significantly to raising the concentration of $O_2^{\cdot-}$ through several sources. In the respiratory chain within mitochondria, these include lipoxygenase, cyclooxygenase, NOX enzyme induction, and concomitantly the formation of the ONO_2^- as a result of thiol oxidation in xanthine dehydrogenase, and uncoupling of NOS activity (Vašková et al., 2016).

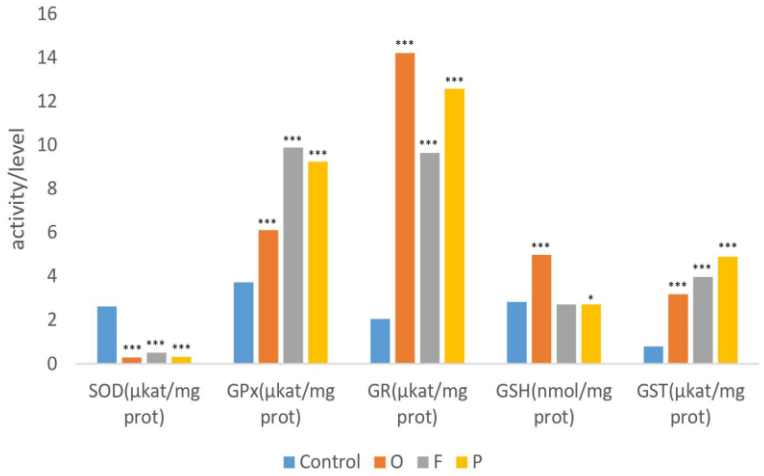

Figure 1 Antioxidant enzyme activities and reduced glutathione concentrations in patients treated with opioids for severe pain compared to healthy subjects at the beginning of treatment period. Statistical significance at *$p < 0.05$ a ***$p < 0.001$

Reactive oxygen species (ROS) formation has been observed even at low doses of morphine in vascular endothelial cells (mouse and human) (Hsiao et al., 2009). Macrophages have also been found to be morphine-induced generating $O_2^{\cdot-}$ (Bhat et al., 2004). Other morphine-induced sources are activated nitric oxide synthase (NOS) and NADPH oxidase. Activation of NOS leads to increase NO production and subsequent SOD nitration. Inactivation of SOD leads to the formation of ONO_2^- in a reaction between $O_2^{\cdot-}$ and NO. Although $O_2^{\cdot-}$ is an initiator of the formation of other ROS, ONO_2^- together with NO depletion have deleterious effects on tissues and their functions (Vašková et al., 2016). All these ROS are involved in the pain sensitisation, opiate-induced hyperalgesia and antinociceptive tolerance (Salvemini et al., 2009).

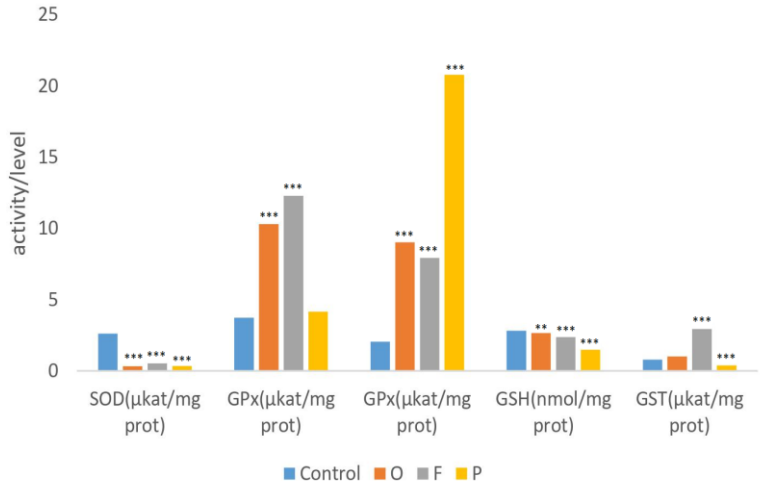

Figure 2 Antioxidant enzyme activities and reduced glutathione concentrations in patients treated with opioids for severe pain compared to healthy subjects 6 months after starting therapy. Statistical significance at **p < 0.01 and ***p < 0.001.

SOD is inactivated *in vivo* through nitration with NO-derived oxidants (ONO_2^-) and hydroxylation (hydroxyl radical) (Janssen-Heininger et al., 2005). Salvemini et al., (2011) reported MnSOD inactivation as an essential element for increased production of $O_2^{\cdot-}$ and ONO_2^- in nociceptive signalling from the results of several studies of their research group. Inactivation of NOS or inhibition of nitration and inactivation of SOD made it possible to prevent the formation of morphine-induced antinociceptive tolerance (Muscoli et al., 2007).

It was found that hydrogen peroxide is capable of SOD inactivation, yet too slow to be the cause of inactivation under physiological conditions (Escobar et al., 1996). However, relatively higher concentrations of hydrogen peroxides (and hydroperoxides) are capable of substrate peroxidase inactivation (Olorunniji et al., 2009), which was not confirmed by the results of our study. Increased activities of GPx, synergistically acting GR together with lowered concentrations of GSH (especially 6 months after starting therapy) only support the response to oxidative stress conditions (Figure 1 and 2). Levels of GSH were significantly higher when compared with control (p < 0,001) in group treated with oxycodone, hydromorphone at the beginning, and unchanged in group with fentanyl, they markedly decreased 6 months after starting therapy (p < 0,001).

Table 1 Further description of the results of the antioxidant parameters in patients treated with opioids for severe pain compared to healthy subjects before at the beginning (0) and 6 months of treatment (6). Significance at b <0.01, c<0.05

Group	t	SOD (μkat/mg prot) med (min-max)	GPx (μkat/mg prot) med (min-max)	GR (μkat/mg prot) med (min-max)	GSH (nmol/mg prot) med (min-max)	GST (μkat/mg prot) med (min-max)
O	0	0.24 (0.16-0.99)	6.61 (0.99-11.6)	14.06 (1.37-27.29)	9.76 (0.78-12.23)	3.35 (0.97-4.53)
	6	0.24 (0.22-0.61)	5.60 (0.94-29.11)	9.18 (4.65-13.12)	1.86 (1.70-5.21)	0.55 (0.20-2.74)
F	0	0.22 (0.19-1.21)	9.98 (1.09-26.94)[b]	9.23 (3.32-19.07)	2.51 (1.01-4.38)	3.08 (0.15--10.36)
	6	0.27 (0.19-1.39)	7.00 (1.12-29.11)[b]	9.75 (1.83-10.39)	2.51 (0.56-3.96)	1.55 (0.60-9.01)
T	0	0.23 (0.17-1.21)	6.72 (2.23-21.04)	12.52 (2.53-22.58)	2.39 (0.76-4.83)	4.19 (0.18-12.23)[c]
	6	0.24 (0.21-0.63)	5.45 (0.69-6.35)	22.32 (11.7-28.34)	1.33 (1.19-1.97)	0.44 (0.19-0.56)[c]

Unlike other opioids, morphine is more associated with the induction of oxidative stress either by the formation of ROS or reduction the activity of antioxidants (Skrabalová et al., 2013; Zahmatkesh et al. 2017). Several studies demonstrated that both acute and chronic morphine exposure can lead to significant reductions in GSH levels in rodent and human brains, serum and liver (Abdel-Zaher et al., 2010; Cemek et al., 2011; Guzmán et al., 2006; Mannelli et al., 2009; Ozmen et al., 2007; Payabvash et al., 2006; Skoulis et al., 1989; Sumathi et al., 2011; Todaka et al., 2005). Methadone and buprenorphine treatment showed similar results (Leventelis, et al., 2019).

In addition to the examined function of glutathione within this study, this molecule is much more widely involved in redox reactions, e.g. reacting directly with $O_2^{\cdot-}$, H_2O_2 and NO, participating in disulphide interchange, amino acid transport into the cells in the γ-glutamyl cycle and conjugation with electrophilic compounds catalysed by GST. A significant increase in GST activities was found in every group at the beginning of treatment (Figure 1). After 6 months, there was a decrease in GST activity in group 2 with no difference from the control (Figure 2). Yet, in the group treated with tapentadol, activities decreased in comparison with control, and there was the significant difference between times of sampling ($p=0.0019$) in this group (Table 1). Myers et al., (2010) found a six-fold increase in protein level and expression of liver GST isoenzyme after administration of oxycodone to rats for 8 days. However, in the case of this study, the first measurements were not made after the short-term effect of the opioids, but before their action. Observed increases in activities of GST may be due to the response to the oxidative stress conditions in patients. In 6 month of opioid administration, there was decrease in GST activity, in all three groups, however most in group 4. Comparable effect in long-term morphine use was showed on rat model study (Samarghandian et al., 2014).

4. CONCLUSION

Although some studies pointed to lower levels of adverse effects in comparison to equivalent doses of opioids such as morphine, the results of our study did not show significant changes between the monitored antioxidant parameters in the three various opioid treatment groups over a period of half a year. In all three groups, a very similar pattern of action was found on antioxidant enzymes, conjugation with glutathione and the effect of reduced glutathione levels.

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Author contributions

KL, MI and JV conceived and designed the study. Data collection and measurements were performed by DO, ŠJ and MI. The manuscript was written by DO, JV and SF, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethical approval

The study was approved by the Ethic Committee of Slovak Medical University in Banská Bystrica 28/11/2016, the Ethic Committee of East Slovak Institute of Cardiovascular Diseases in Košice no. 1/2019/VUSCH/EK and the Ethic Committee of Faculty of Medicine Pavol Jozef Šafárik University in Košice no. 1N/2017 approved the study.

Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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