ORIGINAL ARTICLE

Effect of selenium compound (selol) on the opioid activity in vincristine induced hyperalgesia

Magdalena Bujalska, Stanisław Witold Gumułka

Department of Pharmacodynamics, Medical University of Warsaw, Poland

Correspondence to: Magdalena Bujalska
Department of Pharmacodynamics, Medical University of Warsaw,
Krakowskie Przedmieście 26/28, 00-927 Warsaw 64, P.O. Box 3, Poland
tel./fax +48 (22) 826 13 66 or +48 (22) 826 10 88
e-mail: mbujalska@gmail.com

Submitted: 2008-03-16 Accepted: 2008-04-25 Published online: 2008-08-30

Key words: **vincristine; hyperalgesia; selol; opioids; rats**

Neuroendocrinol Lett 2008; **29**(4):552–557 **PMID:** 18766156 NEL290408A03

Abstract

PURPOSE: Effect of organoselenium compound (selol), on antinociceptive action of opioid agonists in vincristine neuropathic pain model was investigated.
METHODS: The changes in pain thresholds were determined using mechanical stimuli – the modification of the classic paw withdrawal test described by Randall-Selitto.
RESULTS: Daily administration of VIN (70 μg/kg, iv) resulted in progressive decrease of pain threshold. Neither morphine, fentanyl nor buprenorphine administered alone in 5 consecutives days modified the vincristine-induced hyperalgesia, whereas selol slightly increased the nociceptive threshold. Co-administration of selol with opioids markedly enhanced the analgesic activity of all three investigated compounds.
CONCLUSIONS: Therefore, concomitant administration of selenium and opioids may be beneficial in terminal neoplastic states.

Abbreviations:

BPR – buprenorphine,
CNS – central nervous system,
FEN – fentanyl,
MRF – morphine,
NO – nitric oxide,
ROS – reactive oxygen species,
SE – selol,
VIN – vincristine

INTRODUCTION

Severe oxidative stress – especially resulting in lipoperoxidation – leads to modifications of the genome, protein or structural carbohydrates (Khalil & Khodr, 2001). The peripheral and central nervous system is a rich source of lipids and therefore may be the predominant target of free radical-mediated lipid peroxidation (Khalil *et al*. 1999). Because of high lipid to protein ratio, myelin it can be easily damaged by reactive oxygen species (ROS). It was shown that immune-derived ROS and nitric oxide (NO) damage peripheral nerves and intensify pain (Gazda *et al*. 2001). Literature data indicate that free radical scavenger reduced hyperalgesia in models of neuropathic pain (Gazda *et al*. 2001; Khalil *et al*. 1999; Tal, 1996)

The role of selenium as an antioxidant is known. Selenium (IV) competes with sulfur and it is incorporated into the sulfur-containing amino acids, cystine and methionine and into the selenium-dependent enzymes, for example glutathione peroxidase, which protects cellular membranes and lipid-containing organelles from peroxidative damage (Koller & Exon 1986; Prabhu *et al*. 2002). Scarce available data suggest that selenium can produce antinociceptive activity in animal model of pain (Nogueira *et al*. 2003; Savegnago *et al*. 2006).

Neuropathy in the course of anticancer chemotherapy is accompanied by difficult to treat neuropathic pain. Classical analgetics, e.g. opioid receptor agonists – morphine, fentanyl as well as ago-antagonist with potent analgesic activity – buprenorphine, possess low activity in this kind of pain as it was observed in a previous study. However, opioids activity can be enhanced e.g. by magnesium ions^{+2} (Bujalska *et al.* 2008a). Since free radical scavengers seemed also to increase opioid analgesia it was of interest to clarify the influence of selol on the antinociceptive action of opioids in vincristine-induced hyperalgesia.

MATERIALS AND METHODS

Laboratory animals

Study was conducted according to the guidelines of the Ethical Committee for Experiments on Small Animals, Medical University of Warsaw. The aforementioned Committee approved the experimental protocols. Male Wistar rats (260–320 g) were housed in a room maintained at a temperature of 20 ± 2°C, under 12–12h light-dark cycle. Experimental groups consist of at least six rats. The animals had a free access to food and water.

Chemicals

Vincristine sulphate was purchased from Sigma Chemical Co., USA; morphine sulfate [(7,8-didehydro-4,5-epoxy-17-methylmorphina-3,6-diol)sulfate], fentanyl [N-(1-phenethyl-4-piperidyl)-N-phenyl-propanamide], buprenorphine hydrochloride [21-Cyclopropyl-7α-((S)-1-hydroxy-1,2,2-trimethylpropyl)-6,14-endo-etheno-6,7,8,14-tetrahydrooripavine hydrochloride] from "Polfa" Warsaw S.A., Poland, selenitetriglicerid named selol [(9E,11E,13E)-octadeca-9,11,13-trienoic acid 2-hexadecanoyloxy-3-{7-[5-((1E,3E)-nona-1,3-dienyl)-2-oxy-2λ^4-[1,3,2]dioxaselenolan-4-yl]-heptanoyloxy}-propyl ester were synthesized by the Department of Drug Analysis at Medical University of Warsaw, Poland (Jastrzębski *et al.* 1995; Jastrzębski *et al.* 1997; Suchocki *et al.* 2003; Suchocki, 2007).

Equipment

Analgesymeter exerted progressively increased pressure stimulus (type 7200, manufactured by Ugo-Basile Biological Research Apparatus, 21025 Comerio – Varese, Italy).

Chemotherapy (vincristine) - induced painful neuropathy

Vincristine neuropathy was induced as described by Aley *et al.* (1996), as well as in our previous paper (Bujalska *et al.* 2008). Vincristine sulphate was dissolved in distilled water to a stock concentration of 1 mg/ml and then stored at 4°C. Immediately before administration, the stock was diluted in distilled water to a concentration of 70 μg/ml. This solution was administered into the tail vein at a dose of 70 μg/kg. The administration of vincristine was performed daily – Monday through Friday – for 10 days (this phase of experiment lasted 12 days, no doses of the drug were given on neither Saturdays nor Sundays). The dosage calculations were based on daily body weight. Weight-matched control rats received injections of distilled water (Aley *et al.* 1996).

No weight gain was observed in rats receiving iv vincristine in a dose of 70 μg/kg.

Drugs administration

Vincristine (VIN) was administered as described above. Morphine (MRF), fentanyl (FEN), buprenorphine (BPR) were dissolved in 0.9% saline immediately before injection and applied intraperitoneally (ip). MRF was applied in a dose of 2.5 mg/kg, FEN and BPR were administered in the doses that corresponded to morphine's dose (FEN – 0.03125 mg/kg, BPR – 0.0375 mg/kg). Selol (SE) was diluted in vegetable oil and applied perorally (po) via gastric tube in a dose of 3 mg selenium/kg. Control animals were injected ip with 0.9% saline (control to MRF, FEN, and BPR) and/or were administered po with vegetable oil (control to selol) according to the same time schedule.

Time schedule

In chemotherapy induced neuropathy model, opioids were applied on 5 consecutive days (from 8 to 12 day of experiment) 10 minutes before vincristine. Selol was administered 30 minutes before MRF, FEN or BPR.

Measurement of the nociceptive threshold

The changes in pain thresholds were determined using mechanical stimuli – the modification of the classic paw withdrawal test described by Randall and Selitto (1957). In order to perform mechanical stimulation, a progressively increased pressure was applied to the dorsal surface of the rat's paw using an analgesymeter. The instrument used increased force on the paw at a rate of 32 grams per second. The nociceptive threshold was defined as force in grams, at which the rat attempted to withdraw its hind paw, and values of pressure were recorded at this moment. Nociceptive threshold was measured in duplicates and mean was drawn for further calculations.

A mean of nociceptive thresholds to mechanical stimuli measured on a first day of 29–days lasting study immediately before administration of vincristine alone or vincristine with investigated drugs constituted the baseline pain threshold (A). Consecutive measurements of nociceptive thresholds to mechanical stimuli (B) were conducted daily for 5 days (from 9 to 13 day of experiment) before administration of investigated compounds and then after drugs discontinuation (from day 15 to 29 of experiment). In all experimental sessions until end of the study, values of thresholds obtained (B) were compared to baseline (A) defined above.

In all experimental sessions, obtained thresholds (B) were compared to the baseline (A).

Changes in pain threshold were calculated as percentage of baseline value according to the following formula:

$$\text{\% of baseline pain threshold} = (\frac{B}{A} \bullet 100\%) - 100\%$$

A – pressure (in g), baseline pain threshold
B – pressure (in g) in consecutive measurements.

Percentage of baseline pain threshold values calculated as above for individual animals were subsequently used to calculate average values in particular experimental groups and for statistical analysis.

Statistical analysis

The results are expressed as mean values ± standard error of the mean (S.E.M.). The statistical significance of differences between groups was evaluated by the t-Students test and the Newman-Keuls multiple-range test. $P \leq 0.05$ was accepted as statistically significant. All statistical calculations were performed using the computer software described by Tallarida and Murray (1986).

Percentage of hyperalgesia was calculated as above for individual animals and it was subsequently used to calculate average values in particular experimental groups and for statistical analyses.

RESULTS

Effect of chronic administration of vincristine alone on nociceptive thresholds to mechanical stimuli

Starting from the day 2, a statistically significant decrease of the nociceptive threshold was observed in vincristine treated animals. The decrease reached its nadir on day 9 of experiment and remained approximately stable until day 12. After discontinuation of vincristine administration from day 13, nociceptive thresholds gradually increased, and on day 23 returned to baseline values (Fig. 1).

Influence of selol on antinociceptive activity of morphine, fentanyl, and buprenorphine in vincristine-induced hyperalgesia model

Morphine (MRF), fentanyl (FEN), buprenorphine (BPR) and selol (SE) administered alone for 5 consecutives days did not significantly alter the VIN induced hyperalgesia. Pre-medication with SE increased the analgesic action of three investigated opioids. Cessation of SE and opioids administration resulted in gradual return of hyperalgesia (Fig. 2–4).

DISCUSSION

The development of vincristine model of chemotherapeutic-induced painful toxic neuropathy provides an opportunity to investigate mechanisms involved in this form of neuropathic pain (Chabner *et al.* 1996; Micromedex, 2002). Tanner *et al.* (1998) suggests that in rats vincristine causes disorganization of the axonal microtubule cytoskeleton, as well as an increase in the caliber of unmyelinated sensory axons. Topp *et al.* (2000) drew similar conclusions. These authors observed that VIN-induced hyperalgesia is accompanied by a decrease in microtubule density (probably due to swelling of axons), as well as an increase in tangentially oriented microtubules per axon compared to controls.

In this study, daily administration of VIN (70 µg/kg) resulted in progressive decrease of pain threshold.

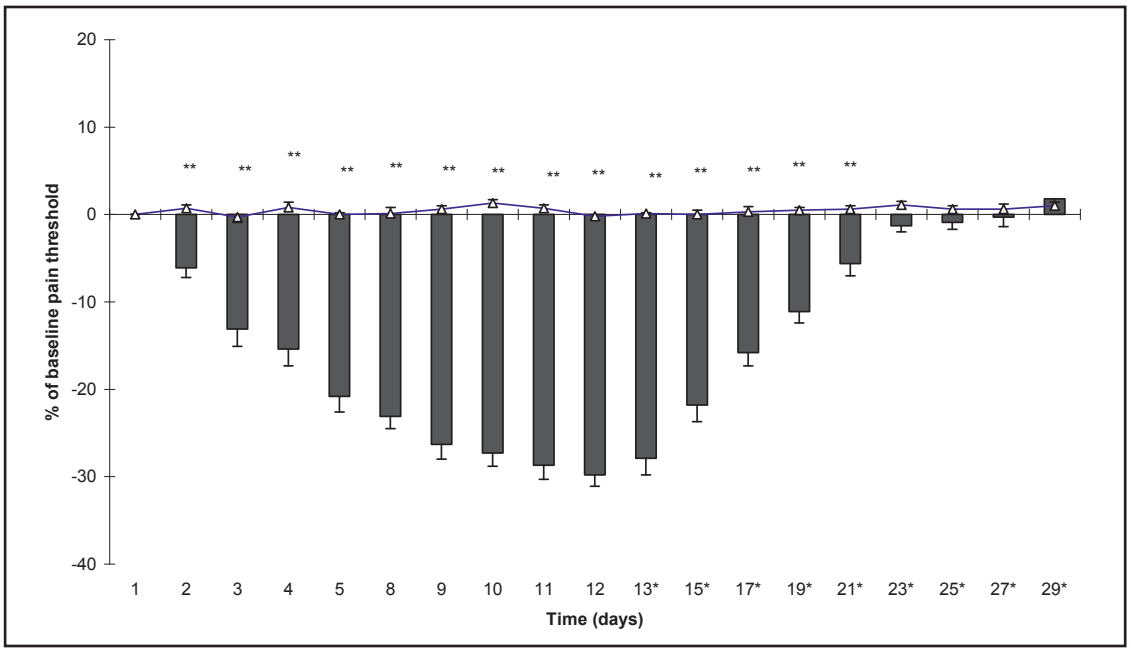

Fig. 1. Influence of vincristine (VIN) at a dose of 70 µg/kg iv on threshold to mechanical stimuli (days 1–29 of experiment). Values are means ± S.E.M. VIN vs. control $^{**}p \leq 0.01$.
Legend: ■ VIN; -△- control

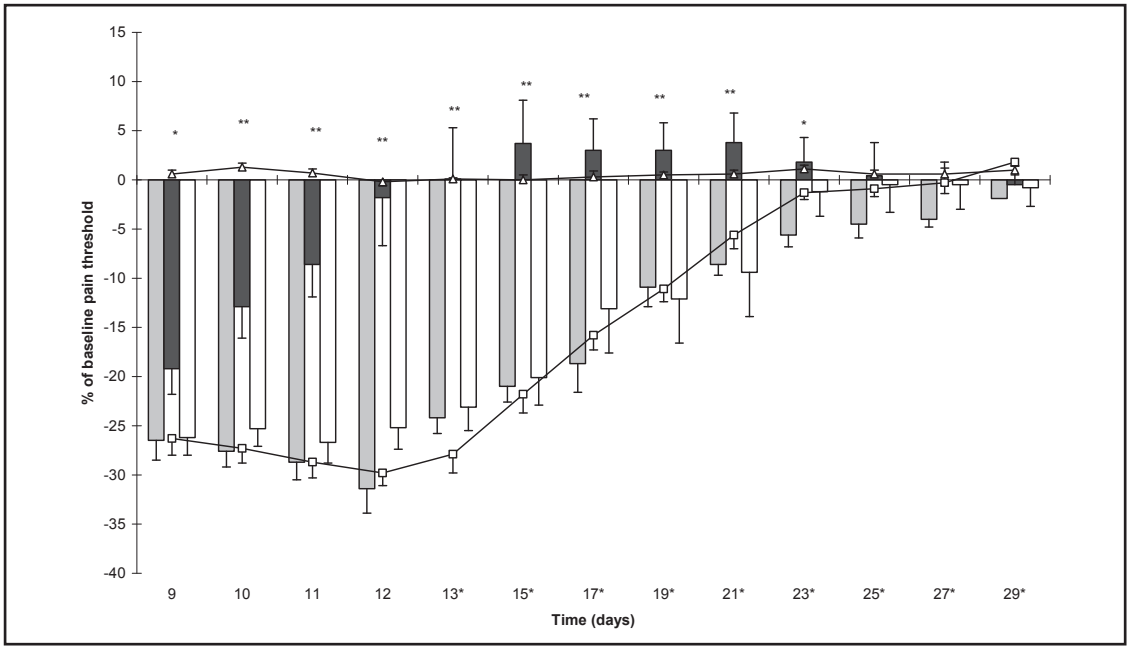

Fig.2. Effect of selol (SE) at a dose of 3 mg selenium/kg po on analgesic activity of morphine (MRF) at a dose of 2.5 mg/kg ip in VIN treated rats. MRF was applied on 5 consecutive days (from 8 to 12 day of experiment) 30 minutes after SE and 10 minutes before VIN. Days 9–13* – measurements of prolonged activity of investigated drugs; days 15*–29* - after discontinuation of administration. Values are means ± S.E.M. MRF vs. SE+MRF $^{**}p \leq 0.01$; $^{*}p \leq 0.05$.
Legend: MRF; SE+MRF; SE; VIN; control

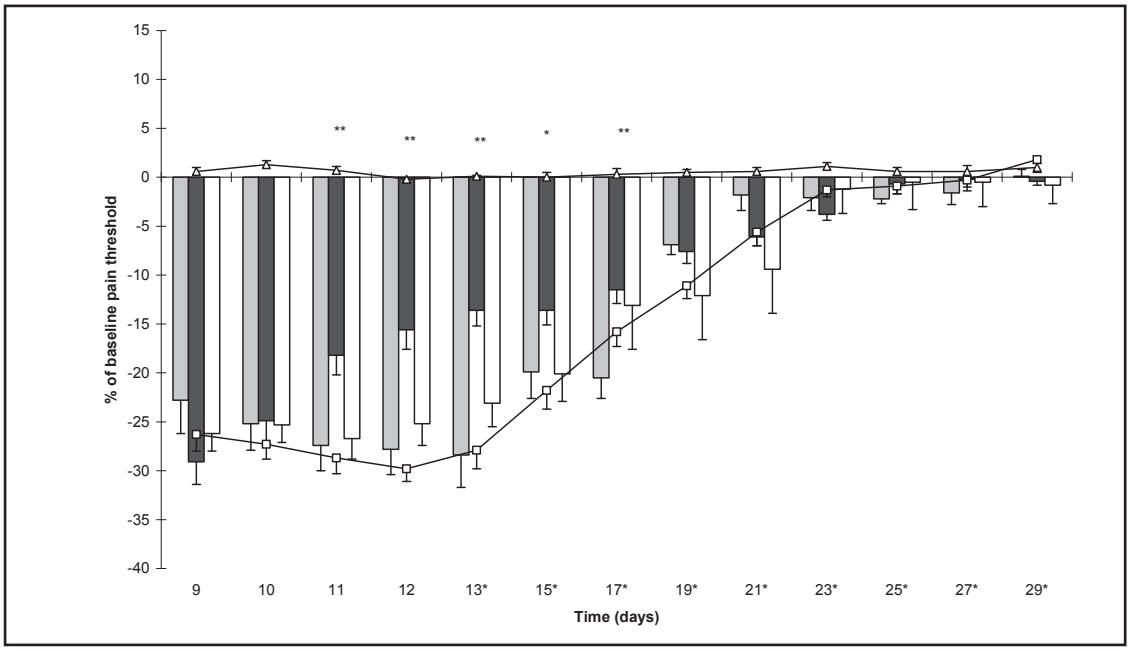

Fig. 3. Enhancement of fentanyl (FEN) produced analgesia (0.031 mg/kg ip) after selol (SE) administration in VIN treated rats. FEN was applied on 5 consecutive days (from 8 to 12 day of experiment) 30 minutes after SE and 10 minutes before VIN. Days 9–13* – measurements of prolonged activity of investigated drugs; days 15*–29* - after discontinuation of administration. alues are means ± S.E.M. FEN vs. SE+FEN $^{**}p \leq 0.01$; $^{*}p \leq 0.05$.
Legend: FEN; SE+FEN; SE; VIN; Control

Diminishing of the pain threshold was significant on day 2. Thereafter nociceptive threshold progressively decreased until day 9 of the experiment and then remained approximately stable until withdrawal of the drug. It is interesting to note that the hyperalgesia was reversible and after cessation of drug administration (after 12 days) nociceptive threshold gradually returned to initial values.

The results presented here are similar to those reported by Aley and co-workers (1996) who demonstrated the appearance of hyperalgesia in response to mechanical stimulus after administration of vincris-

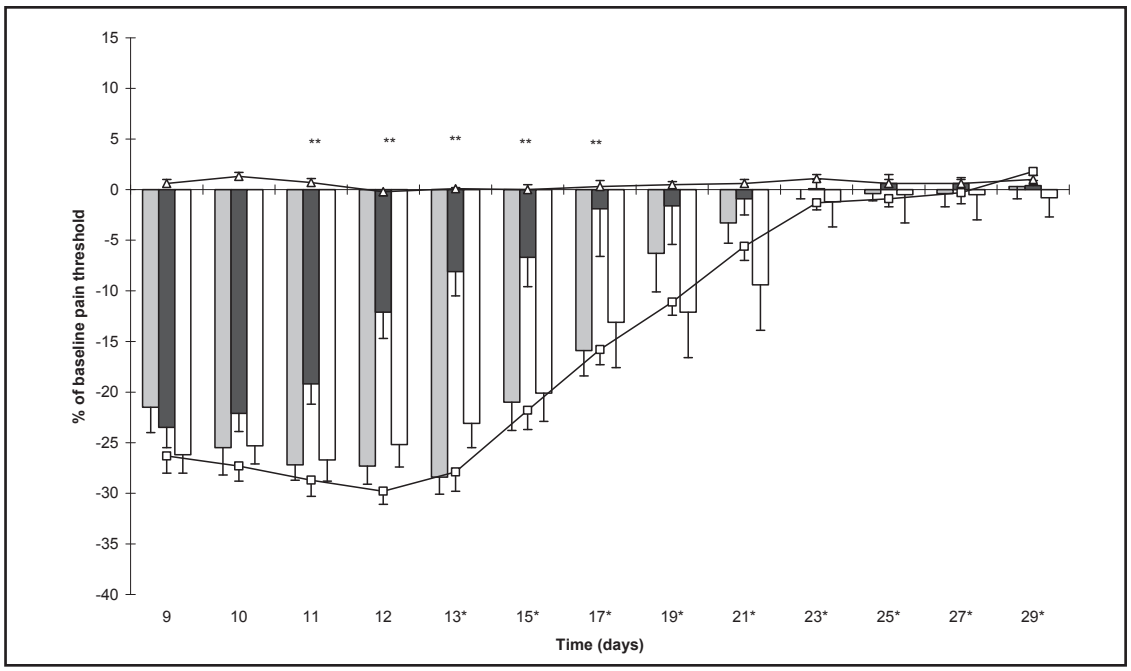

Fig. 4. Enhancement of buprenorphine (BPR) produced analgesia (0.038 mg/kg ip) after selol (SE) administration in VIN treated rats. BPR was applied on 5 consecutive days (from 8 to 12 day of experiment) 30 minutes after SE and 10 minutes before VIN. Days 9–13* – measurements of prolonged activity of investigated drugs; days 15*–29* - after discontinuation of administration. Values are means ± S.E.M. BPR vs. SE+BPR **p≤ 0.01;
Legend: BPR; SE+BPR; SE; STZ; control

tine at doses of 100 μg/kg and 200 μg/kg. No significant differences in the intensity of hyperalgetic effects between doses were observed in this study. However, unlike animals receiving VIN at 100 μg/kg dose, rats given 200 μg/kg loss on average 12.5% of body weight during the experiment but regained weight when the drug application was stopped. In the present study, intravenous administration of vincristine at a dose of 70 μg/kg prevented the physiological weight gain in rats. The animals gradually increased weight after withdrawal.

Considering its complex mechanism, alleviation of neuropathic pain creates an important problem for medicine. Available in literature data (Zurek *et al.* 2001) and results of our previous study (Bujalska *et al.* 2008a) show a limited efficacy of opioids in relieving of neuropathic pain. In the present study, morphine, fentanyl, buprenorphine also did not modify VIN induced hyperalgesia. Insensitivity of neuropathic pain to opioid analgesic is difficult to explain. Ulugol *et al.* 2002) suggested that the loss of opioid receptors expressed on C-fibre afferents, excessive activation of NMDA receptors, increase in the levels of cholecystokinin and accumulation of morphine-3-glucuronide may lead to reduced sensitivity to morphine in neuropathic pain. Some authors explain limited efficacy of opioids in neuropathic pain by an increase of NO synthesis (Grover *et al.* 2000; Xiangqi & Clark, 2001).

It was suggested that ROS (superoxide, hydrogen peroxide and hydroxyls) and peroxynitrite play an important role in development of neuropathic pain, although the mechanism or mechanisms involved in this process remain to be elucidated (Gazda *et al.* 2001; Khalil *et al.* 1999; Raghavendra *et al.* 2003; Rokyta *et al.* 2003; Wagner *et al.* 1998).

It was suggested that antioxidants, when properly used, may not only protect CNS against free radicals, but they are also able to decrease the pain sensation (Chung, 2004; Wagner *et al.* 1998). Selenium possess antioxidant activity, however its effect on analgesic action of opioid was not systematically investigated. Rokyta *et al.* (2003) showed that among other antioxidants (vitamins C, E, A) administration of selenium also led to the decrease of the antinociceptive dose of morphine. Antioxidant properties of selol were demonstrated in several previous studies (Jastrzębski *et al.* 1995; Jastrzębski *et al.* 1997; Suchocki *et al.* 2003; Suchocki, 2007).

In the current study, prolonged pre-medication with selol increased the analgesic action of morphine, fentanyl and buprenorphine in vincristine – induced hyperalgesia,

One of the possible explanations of the observed potentiation of antinociceptive opioids activity in vincristine hyperalgesia by selol are the antioxidant properties of this compound. However, other mechanism(s) e.g. inhibition of iNOS (Kim *et al.* 2004; Prabhu *et al.* 2002; Yun *et al.* 2007) and/or COX-2 expression (Tetsuka *et al.* 1996; Zamamiri-Davis *et al.* 2002) must also be taken into consideration.

Nevertheless, results of this study indicate that selol significantly increases analgesic activity of opioids in vincristine model of chemotherapeutic-induced painful toxic neuropathy. This observation can be clinically rel-

evant since selol possess anticancer activity (Suchocki *et al*. 2007). Therefore, concomitant administration of selenium and opioids may be beneficial in terminal neoplastic states.

Acknowledgments

The authors thank Dr. P. Suchocki from Department of Drug Analysis, Medical University of Warsaw for the generous gift of selol.

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