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## EFFICACY OF TRAMADOL IN COMBINATION WITH DOXEPIN OR VENLAFAXINE IN INHIBITION OF NOCICEPITIVE PROCESS IN THE RAT MODEL OF NEUROPATHIC PAIN: AN ISOBOLOGRAPHIC ANALYSIS

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Neuropathic pain constitutes a serious therapeutic problem. In most cases polytherapy is necessary. Tramadol and antidepressants have common mechanisms of action and are frequently used together in clinical practice, thus interaction between them is very important. In the present study isobolographic analysis for equivalent doses of drugs was applied to examine the nature of interaction between tramadol and doxepin or venlafaxine in a neuropathic pain model in rats. Allodynia and hyperalgesia were assessed after intraperitoneal administration of each drug alone or in combination. Dose response curves were obtained and ED<sub>50</sub> doses were calculated. All drugs were effective in reducing thermal hyperalgesia and mechanical allodynia, however doxepin was more effective than venlafaxine. Combined administration of tramadol and doxepin demonstrated synergistic action in reducing thermal hyperalgesia and additive action in reducing mechanical allodynia. Combined administration of tramadol and venlafaxine showed additive action in reducing hyperalgesia and allodynia. Moreover, combined administration of tramadol and doxepin was more effective than combined administration of tramadol and venlafaxine. The experiments demonstrated that the nature of interaction between tramadol and doxepin is synergistic, which is not the case for tramadol and venlafaxine, what provides a valuable information referring to clinical practice, rationalizing administration of such drug combination.

**Key words:** *neuropathic pain, drug interaction, tramadol, doxepin, venlafaxine, isobolographic analysis*

### INTRODUCTION

Neuropathic pain is a pain condition caused by damage of central or, more frequently, peripheral nervous system. The most characteristic clinical symptom of this kind of pain are spontaneous pain (1), hyperalgesia and allodynia easily demonstrated in various animal models (2-4). Pathomechanism of neuropathic pain differs significantly from that of inflammatory pain. It was shown that changes in spinal gene expression in these two models of chronic pain are different (5), therefore the response to antinociceptive drugs, especially opioids, is not the same (6). Neuropathic pain leads to a lowered morphine efficacy and a more rapid development of morphine tolerance (7-10). For that reason it is a common clinical practice to use analgesic drug combinations, like opioid and NSAIDs or antidepressants. Numerous studies demonstrated a synergistic antinociception of spinal administered combinations of morphine with NSAIDs agents (11) but there is no medical data on the nature of interaction between tramadol and doxepin or venlafaxine, antidepressant drugs recently used in clinic, however synergistic interaction of other antidepressant drugs and opioids has been proved by number of authors. Lopez-Munoz *et al.* performed a profound study on the interaction between dypyrone and morphine (12) between ketorolac and tramadol

(13) using surface of synergistic interaction analysis and isobolographic analysis. Pinardi *et al.* (14) proved synergistic interaction of tramadol and clomipramine and Wordliczek *et al.* (15) described synergistic action of morphine and intrathecally administered doxepin. Synergistic action of morphine with amitriptyline (16-18) or desimipramine (19, 20) was proved by number of authors. Taking into consideration complex mechanism of action of tramadol, which apart from being an activator of opioid antinociceptive system, also activates serotonergic and noradrenergic antinociceptive system (which is the basic mechanism of action of antidepressants) (21, 22), it seems reasonable to assume that the interaction with antidepressants may be more complex than in case of pure opioid receptor agonists. Moreover, it should be noticed that venlafaxine is a representative of newer generation of antidepressant drugs with increasing importance in the treatment of pain, however it lacks a great number of additional mechanisms of action typical for tricyclic antidepressants (23, 24) such as blocking of NMDA receptors, activation of adenosine antinociceptive system (25-27), blocking of α<sub>1</sub>-adrenergic, histaminic, muscarinic and nicotinic receptors and blocking of ion conduction in calcium, sodium and potassium channels (28, 29). Lack of these mechanisms in case of venlafaxine may be crucial when it is used together with tramadol, what has not been investigated

previously. Moreover, tramadol and venlafaxine share many similarities (30), what may account for different way of interaction than in case of tramadol and other antidepressants, which does not have so many common features. Considering the above mentioned aspects, which can influence the interaction between tramadol and doxepin and venlafaxine it seemed reasonable to perform the presented investigations.

## MATERIALS AND METHODS

### *Animals*

The experiments were performed on male Wistar rats weighing 200-300 g at the beginning of the study. They were housed in cages (6 animals in a cage) on a sawdust bedding under standard conditions (12 h light/dark cycle, lights on from 8:00 am) with food and water available *ad libitum*. All the experiments were conducted between 9:00 AM and 3:00 PM. Research was conducted at the Department of Pain Pharmacology of the Institute of Pharmacology of the Polish Academy of Sciences in Cracow. All experiments had the approval of the Local Bioethics Committee of the Institute of Pharmacology (Cracow, Poland) and were in accordance to "Ethical guidelines for investigations of experimental pain in conscious animals" (31).

### *Drugs*

Intraperitoneal injections were performed on non-anesthetised rats. The following substances were used: tramadol hydrochloride (Tramal®, 50 mg/ml, solution for injections; Grunenthal Polska, Poland), venlafaxine hydrochloride (pure, water soluble powder, Wyeth, Poland), doxepin hydrochloride (pure, water soluble powder, Sigma Chemical, St Louis, USA), water for injection. Drugs and their combinations were dissolved in water for injection and were administered intraperitoneally in a volume of 4 ml/kg of rat's weight. Tramadol was injected in doses of 1, 5 and 20 mg/kg, doxepin in doses of 1, 5, 20 and 50 mg/kg and venlafaxine in doses of 1, 5, 20 and 50 mg/kg. To assess interaction between drugs, tramadol with doxepin and tramadol with venlafaxine were administered in fixed 1:1 weight ratio combination (1+1 mg/kg; 5+5 mg/kg and 20+20 mg/kg). In the control group water for injection was administered intraperitoneally.

### *Surgery*

A neuropathic pain model of chronic constriction injury (CCI) described by Bennett and Xie (32) was applied. Under pentobarbital anesthesia (60 mg/kg intraperitoneally) the biceps femoris and the gluteus superficialis were separated exposing right sciatic nerve of a rat. Four loose ligations were placed on the nerve using 4/0 silk surgical suture with 1 mm spacing. They were tied until they elicited a brief twitch in the respective hindlimb.

### *Behavioral testing*

To assess thermal hyperalgesia paw withdrawal latency test (PWD, Hargreaves test) was applied, in which rats were tested for paw withdrawal latency to a noxious thermal stimuli using Paw Withdrawal Apparatus (mod 22, IITC INC., Landing, NJ). Rats were placed 5 min before the experiment into individual plastic cages with a glass floor, where thermal stimulus in the form of radiant heat emitted from a focused projection bulb was applied. Noxious thermal stimulus was focused onto the plantar

surface of a hind paw until the animal lifted a paw away. A cut-off latency of 20 seconds was used to avoid tissues damage (23, 33). Mechanical allodynia was measured by the use of a set of calibrated nylon von Frey filaments (Stoelting, Chicago, IL, USA). Animals were placed in plastic cages with wire net floor 5 min before the experiment. Increasing strengths of filaments were applied sequentially to the midplantar surface of the hind paw. The intensity of mechanical stimulation was increased from 0.16 to 26 g in a graded manner using successively filaments with greater pressure until the hind paw was withdrawn (34, 35).

### *Experimental designs*

The animals were randomly assigned to groups of six. The experiments were performed according to the scheme presented on Fig. 1. Intensity of nociceptive reactions was assessed before administration of drugs (baseline) and 30, 60, 90 and 120 minutes after.

### *Statistical analysis*

For each drug or combination of drugs at all time points the results were presented as percent of maximal possible effect with standard error (% MPE $\pm$ SEM). Percent of maximal possible effect was calculated according the equation: % MPE = [(TR-BR)/(CUTOFF-BR)] x 100%, where TR is the respective test result, BR is the baseline result and CUTOFF is a set value established previously and it equals 26 grams of intensity for von Frey test (35) and 20 seconds latency for paw withdrawal latency test (36). To detect the difference between groups one way analysis of variance (ANOVA) was applied with the Bonferroni post hoc test. For each drug or combination of drugs the dose that produced a 50% antinociceptive response was calculated ( $ED_{50}$ ) using linear regression analysis according to the method described by Tallarida *et al.* (37, 38). To define the nature of interaction between drugs indices of interaction were calculated and isobolographic analysis was applied as described by Tallarida *et al.* (37-39). The differences were considered to be statistically significant with the p value lower or equal 0.05. Calculations were performed using statistical tools: STATISTICA and PharmToolsPro.

Interaction index ( $\gamma$ ) was calculated according to the equation:  $\gamma = a/A + b/B$  where A and B stand for theoretical doses of particular drugs, which would produce a 50% antinociceptive response ( $ED_{50}T$ ) in combination, provided that the interaction between them would have been additive, and a and b stand for experimental doses of drugs, which produce a 50% antinociceptive response when applied in combination ( $ED_{50}E$ ). When the value of  $\gamma$  is below 1 the interaction is synergistic; when the value is above 1 interaction is antagonistic; the value equal to one indicates additive interaction. (39).

Isobolographic analysis allows graphical interpretation of the nature of interaction. It was performed for drug combinations in 1:1 weight ratio at  $ED_{50}$  effect level. Isobograms were constructed by plotting on the axes of ordinates and abscissa respectively doses of drugs, which administered alone gave a desired (in this case 50%) response in decreasing pain intensity ( $ED_{50}$ ). The diagonal line connecting the plotted points constitutes a theoretical additive line (line of additive effect). Coordinates of all the points on this line correspond with doses of drugs, which administered together produce the desired antinociceptive effect with the assumption of additive interaction between them. Coordinates of points below the additive line correspond with doses of drugs in combination when synergistic interaction is observed. Coordinates of points above the additive line correspond with doses of drugs in combination when antagonistic interaction is observed. All points were plotted with

95% confidence intervals (vertical and horizontal bars on isobolograms). Statistical significance was determined by comparison between experimental and theoretical ED<sub>50</sub> points using Student's *t*-test. If the difference was not statistically significant the nature of interaction was considered to be additive.

## RESULTS

### *Antinociceptive efficacy of the drugs*

The obtained results demonstrated effectiveness of all drugs administered alone in reducing thermal hyperalgesia and mechanical allodynia in animal neuropathic pain model. Dose-dependency was detected for each of the drugs. The results were significantly different from basal nociceptive threshold for experimental groups as well as from the control group. The maximal antinociceptive effect of tramadol was observed 30 and 60 minutes after drug administration for thermal hyperalgesia and mechanical allodynia respectively. The maximal antinociceptive effect of doxepin and venlafaxine was observed 30 minutes after drug administration in both tests. The antinociceptive effect lasted 120 minutes after drug administration for tramadol, doxepin and venlafaxine, however it was gradually decreasing 60 minutes after drug administration (data not shown). Doses of the drugs that produced a 50% decrease in pain intensity (ED<sub>50</sub>) were calculated for each drug

or combination of drugs from the dose-response curves 30 minutes after drug administration. Quantities of drugs were kept at the same 1:1 fixed weight ratio. The ED<sub>50</sub> doses of drugs for reducing thermal hyperalgesia and mechanical allodynia are presented in *Table 1*. The ED<sub>50</sub> were lowest for tramadol, and significantly lower for doxepin than venlafaxine what stands for greater antinociceptive effect of doxepin in comparison to venlafaxine (Student *t*-test). The ED<sub>50</sub> were significantly lower for mechanical allodynia than for thermal hyperalgesia, what accounts for greater activity of all the drugs in reducing intensity of allodynia (Student's *t*-test).

### *Analysis of interaction*

Isobolograms were constructed and indices of interaction were calculated for combination of tramadol with doxepin and tramadol with venlafaxine in 1:1 weight ratio at ED<sub>50</sub> effect level 30 minutes after drug administration, when the antinociceptive effect of most drugs was most significant. Theoretical and experimental ED<sub>50</sub> doses for drug combinations are presented in *Table 1*. Isobolographic analysis demonstrated that combined administration of tramadol and doxepin has synergistic action in reducing thermal hyperalgesia (*Fig. 2*) and additive action in reducing mechanical allodynia (*Fig. 3*). This was also confirmed by analysis of dose response curves (*Figs. 2A, 3A*) and calculation of interaction indices which equaled 0.39 for thermal hyperalgesia and 0.93 for mechanical allodynia (*Table 2*).

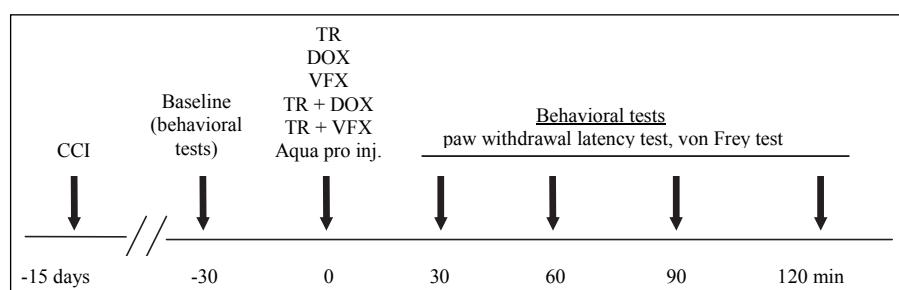
*Table 1.* Doses of tramadol, doxepin, venlafaxine and their theoretical and experimental combinations that produced 50% antinociceptive response (ED<sub>50</sub>± SEM) in reducing thermal hyperalgesia and mechanical allodynia 30 minutes after drug administration.

	Thermal hyperalgesia (paw withdrawal latency test)	Mechanical allodynia (von Frey test)
<b>Tramadol</b>	14,68± 7,35	4,67± 1,75
<b>Doxepin</b>	26,44 ± 9,81	10,22 ± 2,97
<b>Venlafaxine</b>	51,28 ± 16,91	18,31 ± 14,24
<b>Experimental TR + DOX</b>	6,93 ± 3,44*	5,94 ± 2,67
<b>Theoretical TR + DOX</b>	18,88 ± 6,89*	6,41 ± 1,80
<b>Experimental TR + VFX</b>	14,48 ± 8,61	11,31 ± 6,82
<b>Theoretical TR + VFX</b>	22,82 ± 10,87	7,44 ± 2,58

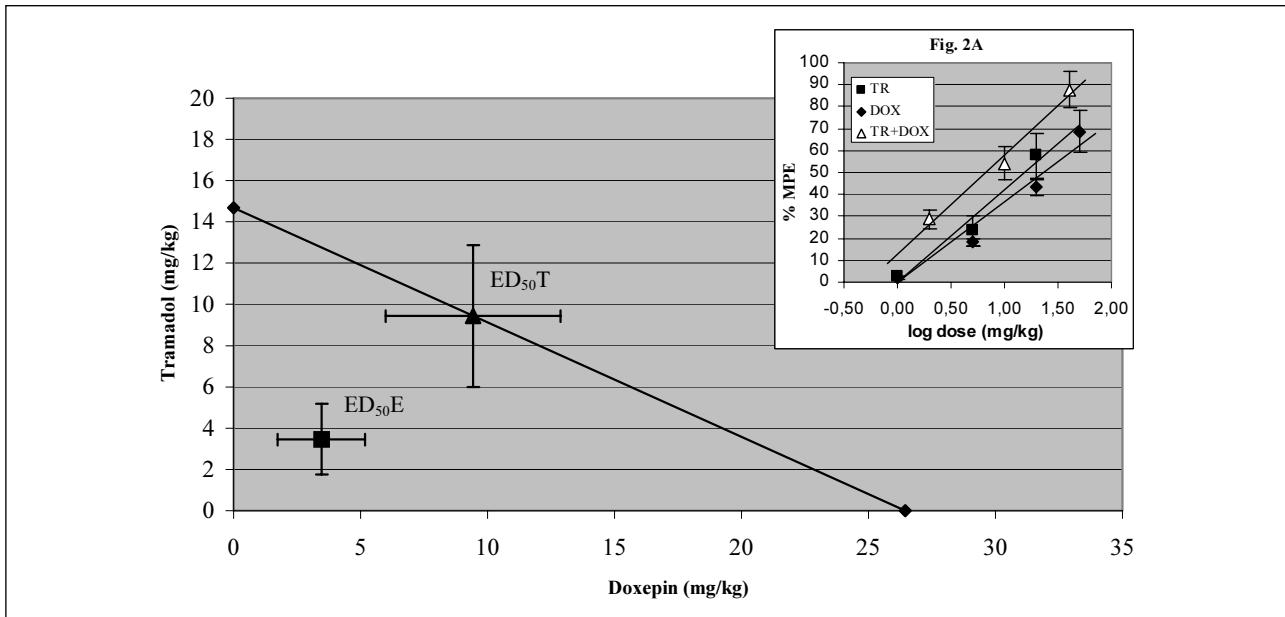
\* p<0.05 between experimental and theoretical ED<sub>50</sub> values (Student's *t*-test)

*Table 2.* Indices of interaction for combination of tramadol with doxepin and combination of tramadol with venlafaxine in reducing thermal hyperalgesia and mechanical allodynia at ED<sub>50</sub> effect level.

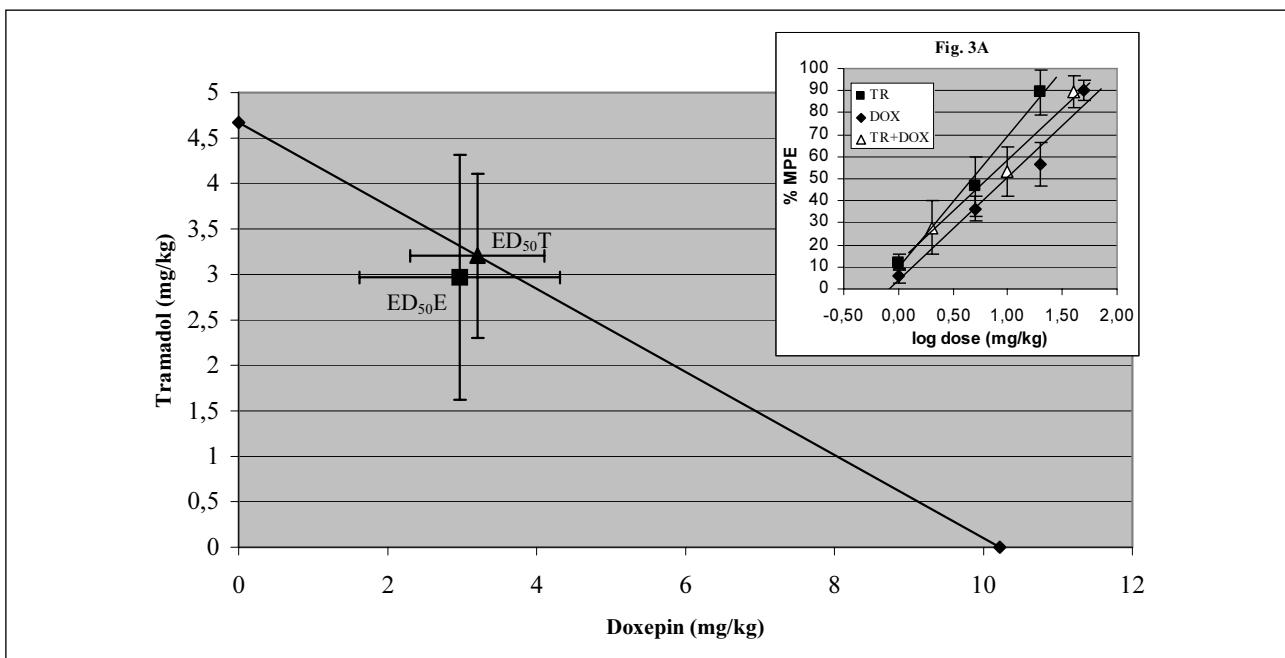
Drug combinations	Thermal hyperalgesia (paw withdrawal latency test)	Mechanical allodynia (von Frey test)
Tramadol and Doxepin	0.39	0.93
Tramadol and Venlafaxine	0.64	1.52



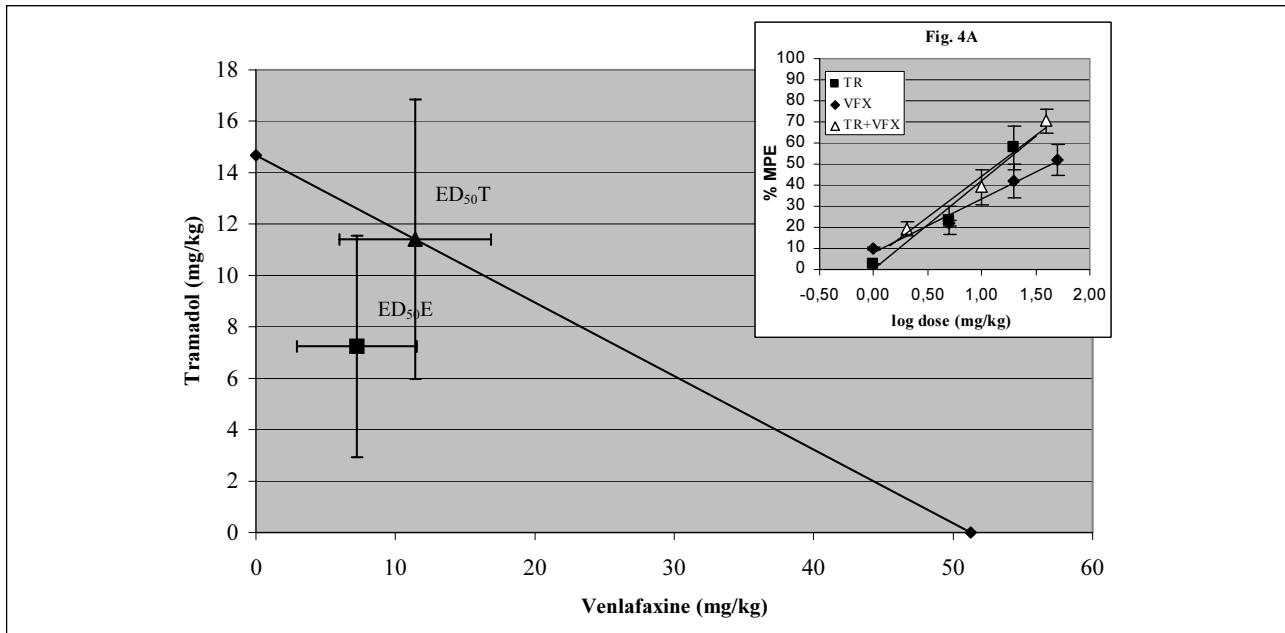
*Fig. 1.* Experimental scheme. CCI: surgical procedure (chronic constriction injury); TR: tramadol; DOX: doxepin; VFX: venlafaxine, Aqua pro inj.: bidestilled water for injection. All drugs and vehicle were injected intraperitoneally.



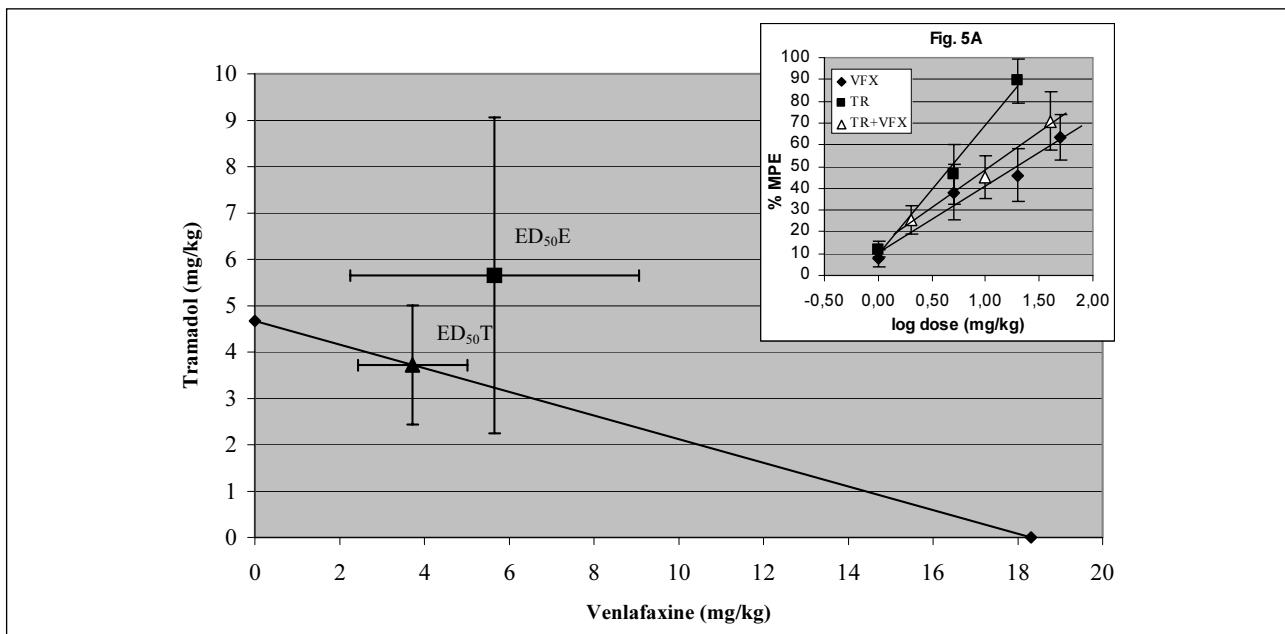
*Fig. 2.* Isobolographic representation of the interaction between tramadol and doxepin at ED<sub>50</sub> effect level in reducing thermal hyperalgesia (paw withdrawal latency test) 30 minutes after drug administration and dose-response curves from which the isobolograms derive (*Fig. 2A*). The diagonal line connecting equi-effective doses of the drugs is a theoretical additive line. ED<sub>50</sub>E (experimental ED<sub>50</sub>) indicates experimental doses of tramadol and doxepin, which produce a 50% antinociceptive response in combination. ED<sub>50</sub>T (theoretical ED<sub>50</sub>) indicates theoretical doses of tramadol and doxepin, which would produce a 50% antinociceptive response in combination, provided that the interaction between them would have been additive. The ED<sub>50</sub>E point is located below the theoretical additive line what indicates synergistic interaction between tramadol and doxepin and the result is statistically significant ( $p \leq 0.05$ ; Student's *t*-test). The vertical and horizontal bars indicate 95% confidence intervals. *Fig. 2A*. The graph represents log dose-response curves for intraperitoneal administration of tramadol, doxepin and combination of tramadol and doxepin in 1:1 weight ratio. Each dose point on the graph corresponds to the mean %MPE  $\pm$  SEM.



*Fig. 3.* Isobolographic representation of the interaction between tramadol and doxepin at ED<sub>50</sub> effect level in reducing mechanical allodynia (von Frey test) 30 minutes after drug administration and dose-response curves from which the isobolograms derive (*Fig. 3A*). The diagonal line connecting equi-effective doses of the drugs is a theoretical additive line. ED<sub>50</sub>E (experimental ED<sub>50</sub>) indicates experimental doses of tramadol and doxepin, which produce a 50% antinociceptive response in combination. ED<sub>50</sub>T (theoretical ED<sub>50</sub>) indicates theoretical doses of tramadol and doxepin, which would produce a 50% antinociceptive response in combination, provided that the interaction between them would have been additive. The ED<sub>50</sub>E point is located near the theoretical additive line what indicates additive interaction between tramadol and doxepin. (the difference between ED<sub>50</sub>E and ED<sub>50</sub>T is not statistically significant;  $p > 0.05$ ; Student's *t*-test). The vertical and horizontal bars indicate 95% confidence intervals. *Fig. 3A*. The graph represents log dose-response curves for intraperitoneal administration of tramadol, doxepin and combination of tramadol and doxepin in 1:1 weight ratio. Each dose point on the graph corresponds to the mean %MPE  $\pm$  SEM.



*Fig. 4.* Isobolographic representation of the interaction between tramadol and venlafaxine at ED<sub>50</sub> effect level in reducing thermal hyperalgesia (paw withdrawal latency test) 30 minutes after drug administration and dose-response curves from which the isobolograms derive (*Fig. 4A*). The diagonal line connecting equi-effective doses of the drugs is a theoretical additive line. ED<sub>50</sub>E (experimental ED<sub>50</sub>) indicates experimental doses of tramadol and venlafaxine, which produce a 50% antinociceptive response in combination. ED<sub>50</sub>T (theoretical ED<sub>50</sub>) indicates theoretical doses of tramadol and venlafaxine, which would produce a 50% antinociceptive response in combination, provided that the interaction between them would have been additive. The ED<sub>50</sub>E point is located below the theoretical additive line what indicates synergistic interaction between tramadol and venlafaxine, however the result is not statistically significant ( $p>0.05$ ; Student's *t*-test). The vertical and horizontal bars indicate 95% confidence intervals. *Fig. 4A*. The graph represents log dose-response curves for intraperitoneal administration of tramadol, venlafaxine and combination of tramadol and venlafaxine in 1:1 weight ratio. Each dose point on the graph corresponds to the mean %MPE $\pm$ SEM.



*Fig. 5.* Isobolographic representation of the interaction between tramadol and venlafaxine at ED<sub>50</sub> effect level in reducing mechanical allodynia (von Frey test) 30 minutes after drug administration and dose-response curves from which the isobolograms derive (*Fig. 5A*). The diagonal line connecting equi-effective doses of the drugs is a theoretical additive line. ED<sub>50</sub>E (experimental ED<sub>50</sub>) indicates experimental doses of tramadol and venlafaxine, which produce a 50% antinociceptive response in combination. ED<sub>50</sub>T (theoretical ED<sub>50</sub>) indicates theoretical doses of tramadol and venlafaxine, which would produce a 50% antinociceptive response in combination, provided that the interaction between them would have been additive. The ED<sub>50</sub>E point is located above the theoretical additive line what indicates antagonistic interaction between tramadol and venlafaxine, however the result is not statistically significant ( $p>0.05$ ; Student's *t*-test). The vertical and horizontal bars indicate 95% confidence intervals. *Fig. 5A*. The graph represents log dose-response curves for intraperitoneal administration of tramadol, venlafaxine and combination of tramadol and venlafaxine in 1:1 weight ratio. Each dose point on the graph corresponds to the mean %MPE $\pm$ SEM.

Combined administration of tramadol and venlafaxine showed additive action in reducing mechanical allodynia and thermal hyperalgesia (*Figs. 4, 4A, 5, 5A*). Indices of interaction for combination of these drugs are shown in *Table 2*.

Combined administration of tramadol and doxepin was more effective in reducing thermal hyperalgesia than combined administration of tramadol and venlafaxine at equivalent doses (data not shown).

## DISCUSSION

The objective of this study was to determine the nature of interaction between tramadol and antidepressant drugs as they are commonly used in clinical practice and there is limited evidence on the subject in medical literature. The best currently available tools such as isobolographic analysis and calculation of interaction indices were used (13, 37, 39). The experiments showed that the nature of interaction between tramadol and doxepin is synergistic in reducing thermal hyperalgesia and additive in reducing mechanical allodynia at the 50% level of effect. Another interesting finding, which can be derived from our experiments is stronger reduction by all drugs (used alone and in combinations) of mechanical allodynia in comparison to thermal hyperalgesia. Accordingly, Pedersen *et al.* (40) proved in an animal model of neuropathic pain (CCI) that fluoxetine (SSRI) and bupropion (dopamine and noradreanline reuptake inhibitor) reduced mechanical allodynia, but they influenced thermal hyperalgesia only in minimal degree, however venlafaxine and reboxetine (noradrenaline reuptake inhibitor) reduced thermal hyperalgesia, but did not influence mechanical allodynia in this experiment. Similarly, in other studies tricyclic antidepressants influenced thermal hyperalgesia in a stronger degree, while their action on mechanical allodynia was insignificant or did not appear (27, 41). Taking these results into consideration it should be stated that there is a great discrepancy between antidepressant drugs as regards different behavioral test. In our experiments stronger reduction of mechanical allodynia was observed. Our findings can be supported by similar results obtained by Abdi *et al.* (42), which demonstrated reduction of mechanical allodynia by amitryptyline. Complexity of analyzed processes can be emphasized by results of other authors, in which tricyclic antidepressants were most effective in tests, where pain reactions were elicited by chemical factors, and significantly less effective in thermal tests (43). The above results highlight the difficulty in correlating antinociceptive effects of drugs from different antidepressant classes across a range of animal pain models and suggest that antidepressants that variously affect both noradrenaline and serotonin levels have more potent and efficacious antinociceptive effects than SSRIs (as exemplified by citalopram), against a range of pain-like behaviours in an animal (41).

There is limited evidence in medical literature on the nature of interaction between tramadol and doxepin and venlafaxine, however synergistic action of opioids with other antidepressant drugs was proved by number of authors. Synergistic interaction of tramadol and clomipramine (14), morphine with clomipramine and amitryptyline (16-18), morphine with desimipramine (19, 20) and morphine with intrathecally injected doxepin (15) were demonstrated by other authors and these studies support results obtained in our work showing that the interaction between antidepressants and opioids may be synergistic. As regards the interaction between tramadol and venlafaxine no synergy was noticed and the interaction had additive nature both in reducing thermal hyperalgesia and mechanical allodynia. There is no scientific evidence available to support the results obtained in this work, however such interaction has not been studied previously in

valuable preclinical experiments. It should be noticed that both substances have much similar chemical structure. Both have methoxyphenyl, N,N-dimethylamino and hydroxycyclohexyl groups and present comparable topographical displays (30). Additionally, it has been shown that both tramadol and venlafaxine have similar prominent side effects such as nausea, headache and dizziness. Both drugs are metabolized by cytochrome P450 2D6 and both have active O-desmethylmetabolites (30). The above listed similarities between these two drugs may be responsible for observed in our experiments lack of synergy, since synergy more likely may be expressed after co-administration of two drugs with diverse mechanism of action. Other antidepressants does not share such similarity with tramadol, what may account for the differences in the nature of interaction of tramadol with venlafaxine and of tramadol with other antidepressants. The interaction between other opioids (not tramadol) and venlafaxine may however be different, as other opioids lack the specific mechanisms of action characteristic for tramadol (such as activation of serotonergic and noradrenergic antinociceptive systems), which has been shown previously by other authors. Schreiber *et al.* (44) investigated interaction between venlafaxine and opioid receptors agonists in hot plate test in mice. They demonstrated synergistic action of venlafaxine with  $\delta$ -opioid receptor agonist (DPDPE),  $\kappa_1$ - and  $\kappa_3$ -opioid receptor agonists. In case of  $\mu$ -opioid receptor agonist (morphine) a trend toward synergistic action was observed, however it was not statistically significant. Our findings seem to be of great importance as they may indicate that there is less benefit from the combined use of tramadol and venlafaxine than from tramadol and doxepin. It must be stressed however, that other ways of interaction may occur for different levels of effect (20% or 80% of maximal possible antinociceptive effect) or for different drug combination ratios.

Synergism between tramadol and doxepin and the lack of such synergism between tramadol and venlafaxine probably may also be explained by other mechanisms of action of tricyclic antidepressant drugs, like interaction with NMDA receptors or adenosine system. Activation of NMDA receptors is one of the major factors responsible for development of central sensitization and has a key role in the development of neuropathic pain (3, 45, 46). Activation of NMDA receptors can also be responsible for decrease of the efficacy of opioid drugs in neuropathic pain (3, 45, 47). Previous animal studies have shown that NMDA receptor blocker, amantadine, administered with antidepressants increases their antidepressive activity (48, 49). Moreover, administration of NMDA receptor antagonists potentiates the antinociceptive activity of morphine in neuropathic pain (50, 51). Mechanisms responsible for this interaction probably take place on spinal level, because intrathecal administration of morphine with NMDA receptor antagonist in various models of neuropathic pain decreased the intensity of thermal hyperalgesia and mechanical allodynia (50, 52, 53). Synergistic effect of morphine and NMDA receptor antagonist was reversed by naloxone what points to the involvement of opioid system (51, 54). Opioids decrease secretion of excitatory amino acids from primary afferents, and their action is additionally limited by the blockade of postsynaptic NMDA receptors (54). Inhibition of activation of NMDA receptors by tricyclic antidepressants was evidenced by many scientists (25, 26). The mechanism described above can explain synergism between doxepin (tricyclic antidepressant, which additionally blocks NMDA receptors) and tramadol (which acts through activation of opioid receptors). Venlafaxine, a representative of newer generation of antidepressants, does not interact with NMDA receptor, and it may be the reason that in our study venlafaxine did not show synergistic action with tramadol. Another mechanism involved in the interaction of doxepin and tramadol may be the adenosine system. Tricyclic antidepressants

enhance the activity of the adenosine system probably by inhibition of its reabsorption (27), and the mechanism can be responsible for the synergistic action of tricyclic antidepressants which enhance the activity of the adenosine system in combination with tramadol. Such synergy was observed in our studies only for doxepin, while venlafaxine, which is deprived of interaction with adenosine system, did not show synergism with tramadol.

In conclusion, our results demonstrate that combined use of tramadol and doxepin effects in synergistic action of the drugs and thus rationalizes use of such drug combination in clinical practice. Combined use of tramadol and venlafaxine shows no synergy, what suggests that there is less clinical benefit from such drug combination compared to other options (such as combination of tramadol and doxepin).

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