

GABAPENTIN, ALONE AND ASSOCIATED WITH TRAMADOL REDUCES PERIPHERAL PACLITAXEL-INDUCED NEUROPATHY IN RATS

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Abstract

Paclitaxel is an antineoplastic drug widely used in metastatic breast cancer, in advanced and metastatic ovarian, and in pulmonary carcinoma [5, 6, 8, 9, 11]. Peripheral neurotoxicity is a common, dose-related side effect of paclitaxel therapy. We set out to investigate the efficacy of gabapentin, of tramadol and of the association of the two drugs in prophylaxy of pain induced with paclitaxel. Male Wistar rats were induced neuropathy by intraperitoneal (i.p.) administration of 2 mg/kg-bw paclitaxel, once daily, for 4 days. One group of animals served as control and the other three groups were administered, concomitantly with paclitaxel, with gabapentin 150 mg/kg-bw *per os*, tramadol 5 mg/kg-bw *per os* and gabapentin 150 mg/kg-bw + tramadol 5 mg/kg-bw *per os*. Assessment of allodynia and of hyperalgesia was performed before and 4 days after administration of paclitaxel by measuring mechanical-induced sensitivity to pain with Dynamic Plantar Aesthesiometer (Ugo Basile, Italy).

Both pretreatment with gabapentin and with association (gabapentin + tramadol) exerted a small but consistent ($p < 0.05$) reduction of applied force and of time to response compared to control group treated only with paclitaxel. Our research is a step forward in finding the suitable drug combination aiming at the prophylaxy of paclitaxel-induced neuropathy.

Rezumat

Paclitaxel este un medicament antitumoral utilizat pe scară largă în terapia cancerului de sân metastatic, a cancerului ovarian avansat și metastatic și în carcinomul pulmonar [5, 6, 8, 9, 11]. Terapia cu paclitaxel are ca efect advers, doză-dependent, neurotoxicitatea periferică. Ne-am propus să cercetăm eficacitatea tratamentelor cu gabapentin, tramadol și asocierea celor două medicamente, în prevenirea durerii neuropate. Neuropatia a fost indusă la șobolani masculi Wistar prin administrarea de paclitaxel 2mg/kg corp, i.p., timp de 4 zile consecutiv. Un lot a constituit lotul martor, alte trei loturi au primit tratament asociat cu paclitaxel: gabapentin 150 mg/kg corp p.o., tramadol 5 mg/kg p.o. și gabapentin 150 mg/kgcorp + tramadol 5 mg/kg corp p.o. Testările sensibilității tactile, a alodiniei și hiperalgeziei au fost efectuate înainte și după cele 4 zile de administrări, cu ajutorul aparatului *Dynamic Plantar Aesthesiometer* (Ugo Basile, Italy).

Tratamentele cu gabapentin și asocierea gabapentin + tramadol au determinat o reducere semnificativă statistic ($p < 0,05$) a timpului până la răspuns și a forței aplicate comparativ cu lotul martor, care a primit numai paclitaxel. Cercetările noastre sunt un pas înainte în găsirea unui tratament farmacologic potrivit pentru prevenirea neuropatiei induse de paclitaxel.

Keywords: paclitaxel, tactile allodynia, mechanical hyperalgesia, tramadol, gabapentin

Introduction

Chemotherapy-induced peripheral neuropathy is a major dose-limiting side effect of many commonly used chemotherapeutic agents, including platinum drugs, taxanes and vinca alkaloids. Paclitaxel is an antineoplastic agent isolated from the bark of the Pacific yew *Taxus brevifolia* in 1971 [7]. It is indicated for the treatment of ovarian, endometrial, breast and lung carcinomas and Kaposi's sarcoma [5, 8, 9].

Paclitaxel binds to beta-tubulin of microtubules, which form the mitotic spindle [4], thereby stabilizing them and enhancing polymerization [7]. This mechanism plays a great role in paclitaxel's chemotherapeutic properties [10, 12]. The suppression of spindle microtubules halts mitosis at the metaphase-anaphase transition, inducing apoptosis [3]. Peripheral neurotoxicity is a serious dose-limiting side effect of paclitaxel therapy. It evokes an array of symptoms: numbness and tingling, mechanical allodynia, cold allodynia and on-going burning pain [2]. Unfortunately, paclitaxel-induced pain and sensory abnormalities can become chronic, persisting for months or years after finishing the paclitaxel therapy [12].

Models of chemotherapeutic drug-induced neuropathy have been used in order to study the mechanisms of neurotoxicity and to evaluate the efficacy of neuroprotective agents in the prevention of this toxicity.

We aimed to investigate the efficacy of gabapentin, tramadol and the association of the two in the prophylaxy of pain induced with paclitaxel on rats.

Materials and methods

Animals

Adult male Wistar rats (200-250 g, n = 32) were supplied by the rodent farm of the University of Medicine and Pharmacy "Carol Davila" and housed in groups of eight on sawdust bedding in plexiglass cages, having free access to water. Experiments were carried out between 8:00 a.m. and 2:00 p.m. All animals were habituated to the testing environment. The temperature and relative humidity were continuously monitored using an electronic hygro-thermometer. The temperature was between 20°C and 22°C and the relative humidity was generally maintained at 35-45%.

All procedures were carried out in accordance with the Directive 86/609/EEC of 24th November 1986, regarding the protection of animals used for experimental and other scientific purposes.

Rat model of paclitaxel-induced peripheral neuropathy

Paclitaxel, 2 mg/kg bw: Sindaxel[®] (Actavis) – 6 mg/mL paclitaxel in dehydrated citric acid, Cremophor EI and dehydrated ethanol), was diluted with saline solution. Paclitaxel was injected intraperitoneally (i.p.) once daily, for 4 days, following the method described by Flatters 2006 and modified [2]. Paclitaxel was administered in a dose of 0.1 mL/100g-bw.

Mechanical sensitivity

The mechanical sensitivity was assessed using a Dynamic Plantar Aesthesiometer (Ugo Basile, Italy), an automated version of the von Frey pain assessment apparatus. Animals were placed on an elevated wire mesh bottomed cage (22 x 16.5 x 14cm) and responded to puncture mechanical stimulation. The unit raised a metal needle (0.5 mm diameter) until it touched the plantar surface of the hindpaw and began to exert an upwards force until the paw was withdrawn or the preset cut-off was reached. Tactile allodynia was measured by assessing hindpaw withdrawal thresholds in response to a mechanical stimulus exerting a linearly increasing force (2 g/s; cut-off force: 20 g). For the mechanical hyperalgesia, we measured the response to mechanical stimulation (force increasing rate: 4 g/s, cut-off force: 40 g). Two readings were taken on each paw at each time point. Each left and right hindpaw was tested two times with a 3-5 min. interval between trials. The individual data are presented as the mean of four readings. The force and the time required to elicit a withdrawal response is measured, respectively, in grams and seconds.

Protocol

Behavioural testing was performed before (predrug), after the four doses of paclitaxel (day 4) and on days 6, 8 and 11 following paclitaxel administration.

The four animal groups received daily, for four days, the following:

- Group 1: paclitaxel group: distilled water, 1 mL/100 g-bw, p.o. and paclitaxel 2 mg/kg-bw, i.p.;
- Group 2: gabapentin -paclitaxel group: gabapentin 100 mg/kg-bw, p.o. and paclitaxel 2 mg/kg-bw, i.p.;
- Group 3: tramadol-paclitaxel group: tramadol 5 mg/kg-bw, p.o. and paclitaxel 2 mg/kg-bw, i.p.;
- Group 4: tramadol-gabapentin-paclitaxel group: gabapentin 100 mg/kg-bw, p.o., tramadol 5 mg/kg-bw, p.o. and paclitaxel 2 mg/kg-bw, i.p.

Statistical analysis

Data were presented as mean $\pm$ SEM (standard error of the mean) of 8 animals per group. Results were statistically processed using Microsoft

Excel 2007 and GraphPad Prism 5 software. Multiple group comparisons were performed using One-Way analysis of variance ANOVA followed by Dunnett's and Bonferroni's test. All results were considered statistically significant when $p < 0.05$.

Results and discussion

Taking into account that allodynia induced by antineoplastic drugs (taxanes) reduces both the time to response and also the force applied for paw retraction [2], experimental results presented in Tables I and II underline a progressive increase of tactile pain sensitivity following paclitaxel administration, together with a statistically significant reduction in the same sensitivity as gabapentin-tramadol association is co-administered.

Table I

Modifications in time to response during allodynia (2 g/s) following paclitaxel and each of the co-administered prophylactics: gabapentin, tramadol and association.

Behavioural tactile allodynia in response to stimulus 2 g/s – time (s)						
		Basal	4 days	6 days	8 days	11 days
Paclitaxel 2 mg/kg-bw i.p.	Mean $\pm$ SEM	29.95 $\pm$ 6.87	13.71 $\pm$ 2.21	13.8 $\pm$ 3.58	18.64 $\pm$ 3.39	16.07 $\pm$ 2.58
	Effect % vs baseline		-54.22	-53.92	-37.76	-46.34
	ANOVA followed by Dunnett's vs baseline(p)					
			$p < 0.05^*$	$p < 0.05^*$	ns	ns
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg-bw p.o.	Mean $\pm$ SEM	25.94 $\pm$ 4.62	37.74 $\pm$ 8.73	26.59 $\pm$ 4.62	29.77 $\pm$ 4.49	13.92 $\pm$ 1.1
	Effect % vs baseline		45.49	2.51	14.76	-46.34
	ANOVA followed by Dunnett's vs baseline(p)					
			ns	ns	ns	ns
	Effect % vs paclitaxel		99.71	56.43	52.53	0.01
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
Paclitaxel 2 mg/kg-bw i.p. + Tramadol 5 mg/kg-bw p.o.	Mean $\pm$ SEM	27.69 $\pm$ 3.71	40.69 $\pm$ 7.64	30.78 $\pm$ 7.68	27.42 $\pm$ 9.6	18.77 $\pm$ 4.64
	Effect % vs baseline		46.95	11.16	-0.98	-32.21
	ANOVA followed by Dunnett's vs baseline(p)					
			ns	ns	ns	ns
	Effect % vs paclitaxel		101.17	65.08	36.79	14.13
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg bw. p.o. + Tramadol 5 mg/kg-bw p.o.	Mean $\pm$ SEM	27 $\pm$ 5.06	34.39 $\pm$ 4.32	44.33 $\pm$ 9.26	31.39 $\pm$ 5.23	23.99 $\pm$ 5.74
	Effect % vs baseline		27.37	64.19	16.26	-11.15
	ANOVA followed by Dunnett's vs baseline(p)					
			ns	ns	ns	ns
	Effect % vs paclitaxel		81.59	118.11	24.02	35.20
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
			$p < 0.01^{**}$	$p < 0.01^{**}$	ns	ns

*ns – not significant

Table II

Modifications in applied force during allodynia (2 g/s) following paclitaxel and each of the co-administered prophylactics: gabapentin, tramadol and association.

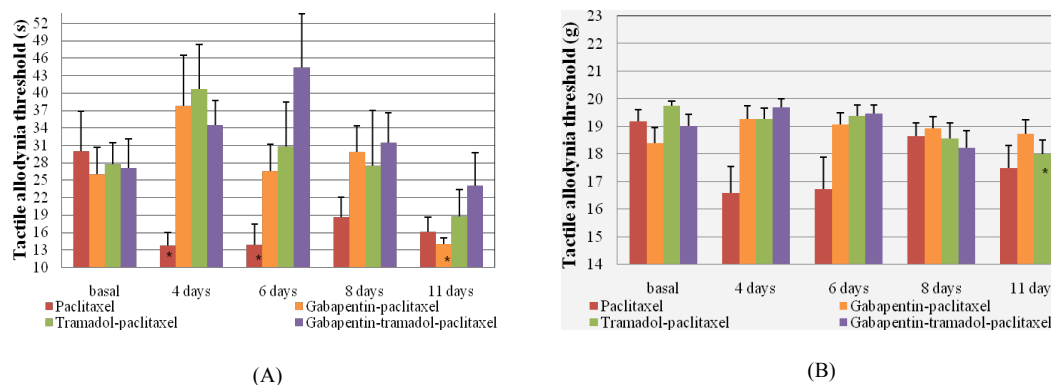
Behavioural tactile allodynia in response to stimulus 2g/s – force (g)						
		Basal	4 days	6 days	8 days	11 days
Paclitaxel 2 mg/kg-bw i.p.	Mean±SEM	19.19±0.42	16.58±0.97	16.74±1.16	18.64±0.49	17.49±0.82
	Effect % vs baseline		-13.60	-12.77	-2.87	-8.86
	ANOVA followed by Dunnett's vs baseline(p)					
			ns*	ns	ns	ns
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg-bw p.o.	Mean ±SEM	18.39±0.56	19.26±0.49	19.06±0.43	18.93±0.43	18.73±0.51
	Effect % vs baseline		4.73	3.64	2.94	1.85
	ANOVA followed by Dunnett's vs baseline(p)					
			ns	ns	ns	ns
	Effect % vs paclitaxel		18.33	16.41	5.8	10.71
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
Paclitaxel 2 mg/kg-bw i.p. + Tramadol 5 mg/kg-bw p.o.	Mean ±SEM	19.7 ±0.16	19.27±0.41	19.38 ±0.4	18.57±0.55	17.99±0.51
	Effect % vs baseline		-2.43	-1.87	-5.97	-8.91
	ANOVA followed by Dunnett's vs baseline(p)					
			ns	ns	ns	p<0.05*
	Effect % vs paclitaxel		11.17	10.89	-3.11	-0.05
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg-bw p.o. + Tramadol 5 mg/kg-bw p.o.	Mean ±SEM	19.01±0.44	19.6 ±0.31	19.46±0.32	18.22±0.63	18.46±0.55
	Effect % vs baseline		3.58	2.37	-4.16	-2.89
	ANOVA followed by Dunnett's vs baseline(p)					
			ns	ns	ns	ns
	Effect % vs paclitaxel		17.18	15.13	-1.29	5.97
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
			p<0.01**	p<0.05*	ns	ns

*ns – not significant

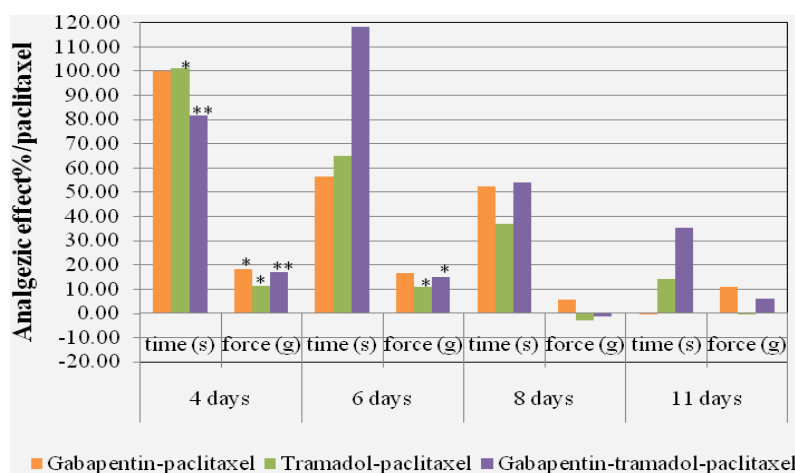
Effect of the treatment on tactile allodynia is represented in figure 1 A-B which shows the mechanical response on allodynia 2g/s, in the four animal groups, throughout the experiment. The mechanical response to 2g/s was assessed by two parameters: time to response (s) and applied force (g).

Time to response and the applied force that elicited the response decrease significantly compared to basal, following administration of paclitaxel i.p., 2 mg/kg-bw, even after discontinuation of the substance (tables I and II).

The association of gabapentin-tramadol significantly increases the time to response at applying 2 g/s constant force (p<0.01) following 4 consecutive days of paclitaxel. In addition, the effect of reduction of pain sensitivity persists significantly (p<0.01) for another 2 days after discontinuing the prophylactic association of drugs (table I).

**Figure 1**

Behavioural tactile allodynia in response to stimulus 2g/s throughout the experiment. The figure shows the mean $\pm$ SEM of the time to response (A) and applied force (B), to mechanical stimulation 2g/s. Group comparisons were performed using One-Way analysis of variance ANOVA followed by Dunnett's test compare to baseline, * $p < 0.05$.

**Figure 2**

Analgesic effect (%) of each treatment (gabapentin 100mg/kg-bw; tramadol 5mg/kg-bw and the association gabapentin 100mg/kg-bw + tramadol 5mg/kg-bw) in the prophylaxy of pain (**tactile allodynia**) induced with paclitaxel. Group comparisons were performed using one-way analysis of variance ANOVA followed by Bonferroni's test in comparison to paclitaxel, * $p < 0.05$; ** $p < 0.01$.

It is worth noting that 7 days after discontinuation of prophylactic treatment for the allodynia induced with paclitaxel, pain sensitivity increases nearing values observed exclusively with the antineoplastic drug.

Effect of treatment on mechanical hyperalgesia

Following four consecutive doses of paclitaxel (2 mg/kg-bw, i.p.) tactile sensitivity of the animals increased (tables III and IV), as time to paw retraction at constant 4 g/s force decreases (table III).

The most intense effect of reduction of hyperalgesia was noted for the group treated with tramadol 5 mg/kg-bw (figure 3), probably through its complex analgesic mechanism, both as opioid and as monoaminergic (inhibition of noradrenaline reuptake on monoaminergic descending pathways of pain) agonist.

Table III

Modifications of time to response during hyperalgesia (4 g/s) following paclitaxel and each of the co-administered prophylactics: gabapentin, tramadol and association

Behavioural tactile hyperalgesia in response to stimulus 4 g/s - time (s)						
		Basal	4 days	6 days	8 days	11 days
Paclitaxel 2 mg/kg-bw i.p.	Mean $\pm$ SEM	11.31 $\pm$ 1.67	7.5 $\pm$ 0.81	7.44 $\pm$ 0.93	6.1 $\pm$ 0.59	6.44 $\pm$ 0.83
	Effect % vs baseline		-33.69	-34.22	-46.07	-43.06
	ANOVA followed by Dunnett's vs baseline(p)					
			p<0.05*	p<0.05*	p<0.01**	p<0.01**
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg-bw p.o.	Mean $\pm$ SEM	11.22 $\pm$ 0.97	8.48 $\pm$ 0.42	10.03 $\pm$ 0.97	7.82 $\pm$ 0.5	6.93 $\pm$ 0.54
	Effect % vs baseline		-24.42	-10.61	-30.30	-38.24
	ANOVA followed by Dunnett's vs baseline(p)					
			p<0.05*	ns	p<0.05*	p<0.001***
Paclitaxel 2 mg/kg-bw i.p. + Tramadol 5 mg/kg-bw p.o.	Effect % vs paclitaxel		9.27	23.61	15.76	4.82
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
			ns	ns	ns	ns
			ns	ns	ns	ns
Paclitaxel 2 mg/kg-bw i.p. + Tramadol 5 mg/kg-bw p.o.	Mean $\pm$ SEM	11.65 $\pm$ 2.91	8.08 $\pm$ 0.66	10.45 $\pm$ 1.67	7.11 $\pm$ 0.27	7.95 $\pm$ 0.71
	Effect % vs baseline		-30.64	-10.30	-38.97	-31.76
	ANOVA followed by Dunnett's vs baseline(p)					
			p<0.05*	ns	p<0.05*	p<0.05*
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg-bw p.o. + Tramadol 5 mg/kg-bw p.o.	Effect % vs paclitaxel		3.04	23.92	7.10	11.30
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
			ns	ns	ns	ns
			ns	ns	ns	ns
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg-bw p.o. + Tramadol 5 mg/kg-bw p.o.	Mean $\pm$ SEM	11.75 $\pm$ 1.07	9.14 $\pm$ 0.74	9.26 $\pm$ 0.63	7.61 $\pm$ 0.41	7.8 $\pm$ 0.8
	Effect % vs baseline		-22.21	-21.19	-35.23	-33.62
	ANOVA followed by Dunnett's vs baseline(p)					
			ns	ns	p<0.01**	p<0.01**
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg-bw p.o. + Tramadol 5 mg/kg-bw p.o.	Effect % vs paclitaxel		11.47	13.03	10.83	9.44
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
			ns	ns	ns	ns
			ns	ns	ns	ns

ns – not significant

Table IV
Modifications of applied force eliciting response during hyperalgesia (4 g/s)
following paclitaxel and each one of the co-administered prophylactics:
gabapentin, tramadol and association

Behavioural tactile hyperalgesia in response to stimulus 4 g/s - force (g)						
		Basal	4 days	6 days	8 days	11 days
Paclitaxel 2 mg/kg-bw i.p.	Mean $\pm$ SEM	36.81 $\pm$ 1.12	31.44 $\pm$ 2.81	28.07 $\pm$ 2.89	26.4 $\pm$ 1.69	24.63 $\pm$ 2.67
	Effect % vs baseline		-14.59	-23.74	-28.28	-33.09
	ANOVA followed by Dunnett's vs baseline(p)					
			p<0.05*	ns	p<0.05*	p<0.01**
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg-bw p.o.	Mean $\pm$ SEM	36.89 $\pm$ 0.99	32.02 $\pm$ 1.13	33.32 $\pm$ 1.5	31.58 $\pm$ 1.63	30.21 $\pm$ 1.44
	Effect % vs baseline		-13.20	-9.68	-14.39	-18.11
	ANOVA followed by Dunnett's vs baseline(p)					
			p<0.05*	ns	p<0.05*	p<0.01**
	Effect % vs paclitaxel		1.39	14.07	13.89	14.98
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
Paclitaxel 2 mg/kg-bw i.p. + Tramadol 5 mg/kg-bw p.o.	Mean $\pm$ SEM	38.88 $\pm$ 0.53	32.03 $\pm$ 2	33.62 $\pm$ 2.01	29.14 $\pm$ 0.71	30.48 $\pm$ 2.11
	Effect % vs baseline		-17.62	-13.53	-25.05	-21.6
	ANOVA followed by Dunnett's vs baseline(p)					
			ns	ns	p<0.01**	p<0.01**
	Effect % vs paclitaxel		-3.03	10.21	3.23	11.48
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
Paclitaxel 2 mg/kg-bw i.p. + Gabapentin 100mg/kg-bw p.o. + Tramadol 5 mg/kg-bw p.o.	Mean $\pm$ SEM	38.79 $\pm$ 0.54	33.8 $\pm$ 1.73	33.65 $\pm$ 1.32	30.44 $\pm$ 1.66	29.81 $\pm$ 2.93
	Effect % vs baseline		-12.86	-13.25	-21.53	-23.15
	ANOVA followed by Dunnett's vs baseline(p)					
			ns	ns	p<0.05*	p<0.01**
	Effect % vs paclitaxel		1.72	14.49	6.75	9.94
	ANOVA followed by Bonferroni's vs paclitaxel(p)					
			ns	ns	ns	ns

ns – not significant

The mechanical response on hyperalgesia 4 g/s, on the four animal groups, throughout the experiment is presented in figure 3A-B.

It can be observed that the co-administration of gabapentin (100 mg/kg-bw p.o.) and tramadol (5 mg/kg-bw) also accentuates tactile pain sensitivity, statistically significant against basal (tables III and IV), whilst being below the one in the group exclusively treated with paclitaxel. The same effect is observed after discontinuation of the prophylactic treatments.

The results suggest that in treating hyperalgesia induced with antineoplastic agents like taxanes one should use analgesics or co-analgesics in larger doses compared to the doses used in treating allodynia.

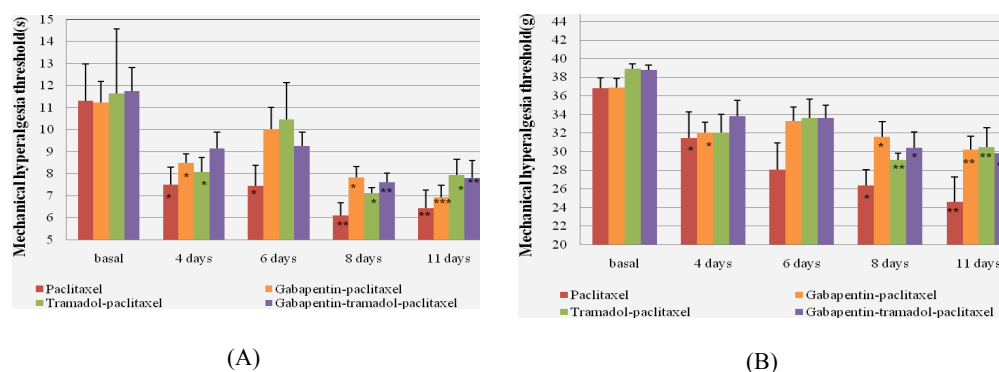


Figure 3

Behavioural mechanical hyperalgesia in response to stimulus 4 g/s throughout the experiment. The figure shows the mean $\pm$ SEM of the response, time (A) and force (B), to mechanical stimulation 4 g/s. Group comparisons were performed using One-way analysis of variance ANOVA followed by Dunnett's test compare to baseline, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

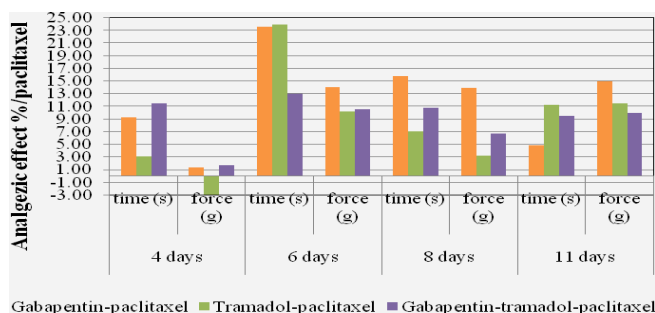


Figure 4

Analgesic effect (%) of each co-treatment (gabapentin 100mg/kg-bw; tramadol 5mg/kg-bw and the association gabapentin 100 mg/kg-bw + tramadol 5 mg/kg-bw) in the prophylaxy of pain (mechanical hyperalgesia) induced with paclitaxel. Group comparisons were performed using One-Way analysis of variance ANOVA followed by Bonferroni's test in comparison to paclitaxel.

Conclusions

Research demonstrated that after the administration of paclitaxel, the sensitivity to mechanical-induced pain increases statistically significant, both in allodynia parameter (applied force reduced by 15.54%; $p < 0.01$) and in hyperalgesia parameter (time to response reduced by 53.76% compared to basal).

Prophylactic administration of gabapentin, tramadol or the association gabapentin-tramadol reduced statistically significant the allodynia induced by paclitaxel. Further researches on the prophylactic

approach of hyperalgesia induced by antineoplastic agent should focus on increasing the doses of the active prophylactic drugs used in the present experimental study.

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