

THE EFFECT OF DEXTROMETHORPHAN, GABAPENTIN, AMITRIPTYLINE AND TRAMADOL ON A MOUSE MODEL OF VINCRISTINE - INDUCED PERIPHERAL NEUROPATHY

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Abstract

Painful peripheral neuropathy is a common side effect of cancer chemotherapy. In some cases, the pain diminishes within days or weeks after treatment, while in other cases is long-lasting. Since several different mechanisms are supposed to be involved in neuropathic pain, we evaluated the effect of dextromethorphan, gabapentin, amitriptyline and tramadol on vincristine-induced peripheral neuropathy. In the experiment we used adult male NMRI mice (25 – 35 g; $N = 72$) divided in six groups: control group (C), vincristine group (VCR), dextromethorphan and vincristine group (DMV), gabapentine and vincristine group (GBV), amitriptyline and vincristine group (AMV), tramadol and vincristine group (TRV). Vincristine (100 $\mu\text{g/kg-bw}$) was administered intraperitoneally daily for 11 days. Dextromethorphan (20 mg/kg-bw), gabapentin (150 mg/kg-bw), amitriptyline (25 mg/kg-bw) and tramadol (5 mg/kg-bw) were orally administered 11 days concomitantly with vincristine. The thermal sensitivity was evaluated in the 7th and 11th post-treatment days. We recorded the nociceptive reaction latencies in response to a given thermal stimulus 38°C (thermal *allodynia*) and 52°C (thermal hyperalgesia) using the hot plate test (Ugo Basile Hot Plate). No thermal allodynia was observed. On day 7 a statistically significant reduction of the nociceptive reaction latencies was observed on VCR group 5.74 ± 1.72 s. ($p < 0.001$), GBV group 6.32 ± 1.79 s. ($p < 0.001$), and AMV group 6.13 ± 2.25 s. ($p < 0.005$). Tramadol produced a statistically significant increase of nociceptive reaction latencies with 22.52 % ($p < 0.05$) compared to VCR group. These results show that tramadol, that has a double mechanism of action: it binds with a low affinity to μ -opioid receptors, and it activates central monoaminergic pathways inhibiting the neuronal uptake of serotonin and noradrenalin, is highly effective to reverse neuropathic hyperalgesia produced by vincristine to mice in the hot plate test.

Rezumat

Durerea determinată de neuropatia periferică este o reacție adversă, frecvent întâlnită în chimioterapie. În unele cazuri, durerea dispare în câteva zile sau săptămâni după tratament, dar în alte cazuri durerea este de lungă durată. Există numeroase mecanisme presupuse a fi implicate în patologia durerii neuropate. Prin urmare, ne-am propus să cercetăm efectul analgezic profilactic a patru medicamente cu potențial analgezic (dextrometorfân, gabapentin, amitriptilină și tramadol) în durerea neuropată indusă experimental de vincristină la șoarece. S-au utilizat șoareci adulți, șușa NMRI, sex

masculin, care au fost împărțiți în șase loturi: lot martor (C), lot vincristină (VCR), vincristină și dextrometorfan (DMV), lot vincristină și gabapentin (GBV), lot vincristină și amitriptilină (AMV), lot vincristină și tramadol (TRV). Vincristina (100 $\mu\text{g/kg corp}$) a fost administrată zilnic intraperitoneal, timp de 11 zile. Dextrometorfan (20 mg/kg corp), gabapentin (150 mg/kg corp), amitriptilină (25 mg/kg corp) și tramadol (5 mg/kg corp) s-au administrat zilnic *per os*, timp de 11 zile, concomitent cu vincristina. Sensibilitatea termică a fost evaluată post-tratament în zilele 7 și 11. Am înregistrat latențele reacției nociceptive ca răspuns la stimuli termici 38°C (alodinie termică) și 52°C (hiperalgezie termică), pe placa încălziată (testul *hot plate*, Ugo Basile). Nu s-a observat alodinie termică. În ziua 7 s-a observat reducerea semnificativă statistic a latențelor reacției nociceptive în lotul VCR, de $5,74 \pm 1,72$ s. ($p < 0,001$), în lotul GBV de $6,32 \pm 1,79$ s. ($p < 0,001$), și în lotul AMV de $6,13 \pm 2,25$ s. ($p < 0,005$). Tramadolul a produs o creștere semnificativă statistic a latenței reacției nociceptive cu 22,52% ($p < 0,05$), față de lotul VCR. Aceste rezultate arată că tramadolul, având mecanism dublu de acțiune, agonist pe receptorii opioizi μ și mecanism monoaminergic spinal, prin inhibarea recaptării serotoninei și noradrenalinei, prezintă eficacitate crescută în reducerea hiperalgeziei dată de vincristină, hiperalgezie evaluată la șoareci prin testul plăcii fierbinți.

Keywords: vincristine, hot plate test, tramadol, gabapentin, dextromethorphan, amitriptyline

Introduction

Pain remains difficult to control for a significant proportion of patients with cancer. Neuropathic pain, defined as “pain initiated or caused by primary lesion or dysfunction in the nervous system”, occurs in nearly 40% of patients with cancer pain [3,6]. Several mechanisms have been postulated to cause neuropathic pain. In the context of cancer, neuropathic pain may be a late effect of treatment with vinca alkaloids, taxanes, platinum-derived compounds, radiotherapy, or surgery. The dose limiting toxicity of many of these chemotherapeutic agents is peripheral neuropathy [8,11,12,15]. The mechanisms underlying chemotherapy-induced peripheral neuropathy are diverse and depend on the drug. Microtubule-targeting agents (e.g., paclitaxel, vincristine) display axonal toxicity, with the longest axons being the first affected [12]. Peripheral nerve biopsies from patients with vincristine-evoked painful neuropathy revealed axonal and mitochondrial swelling, whereas microtubule alterations were not evidenced [14]. The current consensus is that the pain management for chemotherapy-induced peripheral neuropathy should be guided by the same principles as other types of neuropathic pain and include opioid analgesics, antidepressants and anticonvulsants.

Tricyclic antidepressants (TCAs), though often the first choice treatment in most patients, have significant side effects including sedation and various cardiovascular issues, and they often require several days of

treatment prior to producing positive effects [7,16,18]. Anticonvulsants, like the TCAs, are only partially effective in the majority of patients suffering from chemotherapy induced pain [2,18]. Opioids, though often prescribed for moderate to severe pain, are sometimes avoided because of their potential for dependence and tolerance, scheduling issues and side effects [5,10].

Tramadol is a therapeutic option for the control of neuropathic pain in patients with cancer [1]. In our previous research prophylactic administration of tramadol (5 mg/kg-bw) reduced statistically significant also the allodynia induced by paclitaxel. Tramadol (5 – 80 mg/kg-bw) alone also reduced painful sensitivity of thermal stimulus in mice [4].

Materials and Methods

Animals

Adult male NMRI mice (25 – 35 g; N = 72) were supplied by the rodent farm of the University of Medicine and Pharmacy “Carol Davila” and housed in groups of twelve on sawdust bedding in plexiglass cages, having free access to water. Experiments were carried out between 8:00 a.m. and 12:00 a.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.

Mice model of vincristine-induced peripheral neuropathy

Vincristine, 0.1 mg/kg-bw: Sindovin® (Actavis) – 1 mg/mL vincristine sulfate was diluted with saline solution. Vincristine was injected intraperitoneally (i.p.) once daily, for 7 days, following the method described by Kiguchi in 2008 [9] and after that we continued for 11 days. Vincristine was administered in a dose of 0.1 mL/10g-bw.

Thermal sensitivity

The thermal sensitivity was assessed using Hot Plate (Ugo Basile, Italy). Response latency was determined (licking paws, shaking paws, or jumping) to thermal stimulation. Increased thermal pain sensitivity was observed by decreasing the time of occurrence of the first sign of pain. We applied the stimulus of 38°C to evaluate allodynia and the stimulus of 52°C,

to assess thermal hyperalgesia. Each mouse was subjected to a single determination [17].

Experimental protocol

The animals were divided into six groups of 12 mice each, and were administered daily, for eleven days, the following:

- group 1: control group (C) - distilled water 0.1 mL/10 g-bw, p.o. and saline solution 0.1 mL/10 g-bw, i.p.;
- group 2: vincristine group (VCR) - distilled water 0.1 mL/10 g-bw, p.o., and vincristine 0.1 mg/kg-bw, i.p.;
- group 3: dextromethorphan-vincristine group (DMV) - dextromethorphan 20 mg/kg-bw, p.o. and vincristine 0.1 mg/kg-bw, i.p.;
- group 4: gabapentine-vincristine group (GBV) - gabapentine 150 mg/kg-bw, p.o. and vincristine 0.1 mg/kg-bw, i.p.;
- group 5: amitriptyline-vincristine group (AMV) - amitriptyline 25 mg/kg-bw, p.o. and vincristine 0.1 mg/kg-bw, i.p.;
- group 6: tramadol-vincristine group (TRV) - tramadol 5 mg/kg-bw, p.o. and vincristine 0.1 mg/kg-bw, i.p.

Behavioural measurements were performed at two different temperatures of the hot-plate: 38°C and 52°C. The temperature of the plate was monitored at all times. Thermal sensitivity was assessed initially and post-treatment after 7 days and 11 days by the hot plate test (Ugo Basile Hot Plate).

Statistical analysis

Chi-squared test was used for statistical evaluation of thermal allodynia. Chi-squared test calculates the significant difference between the two activities expressed as a percentage [13].

Data were presented as mean $\pm$ SD (standard deviation) of 12 animals per group. Results were statistically processed using Microsoft Excel 2007 and GraphPad Prism 5 software. Multiple group comparisons were performed using Student *t* test or Mann Whitney test. All results were considered statistically significant when $p < 0.05$.

Results and Discussion

Hot plate thermal allodynia

Taking into account that allodynia induced by antineoplastic drugs reduces the time to response to the thermal stimulus, experimental results presented in table I underline the increase of thermal pain sensitivity following vincristine administration.

Table I

Percentage modifications of animals allodynia (38°C) following vincristine and each of the co-administered: dextromethorphan, gabapentine, amitriptyline and tramadol. Group comparisons were performed using Chi-squared test.

<i>Behavioural thermal allodynia in response to 38°C thermal stimulus</i>				
Group		Basal	After 7 days	After 11 days
C	Percentage of animals responding at 38°C thermal stimulus, under 60 seconds	25% (3/12)	0% (0/12)	8.33% (1/12)
	Change vs basal Chi-squared test		1.52	0.3
VCR	Percentage of animals responding at 38°C thermal stimulus, under 60 seconds	16.66% (2/12)	33.33% (4/12)	18.18% (2/11)
	Effect % vs basal Chi-squared test		2	0.42
	Effect % vs control Chi-squared test	0	7.5(*p<0.01)	1.74
DMV	Percentage of animals responding at 38°C thermal stimulus, under 60 seconds	0% (0/12)	8.33% (1/12)	16.66% (2/12)
	Effect % vs basal Chi-squared test		4.17(*p<0.05)	4.90(*p<0.05)
	Effect % vs control Chi-squared test	1.52	4.17(*p<0.05)	1.52
	Effect % vs VCR Chi-squared test	0.54	1.01	0.2
GBV	Percentage of animals responding at 38°C thermal stimulus, under 60 seconds	16.66% (2/12)	8.33% (1/12)	25% (3/12)
	Effect % vs basal Chi-squared test		0	1.01
	Effect % vs control Chi-squared test	0	4.17(*p<0.05)	2.7
	Effect % vs VCR Chi-squared test	0.3	1.01	0.81
AMV	Percentage of animals responding at 38°C thermal stimulus, under 60 seconds	33.33% (4/12)	0% (0/12)	16.66% (2/12)
	Effect % vs basal Chi-squared test		2.7	0.22
	Effect % vs control Chi-squared test	0.8	-	1.52
	Effect % vs VCR Chi-squared test	2	2.7	0.21
TRV	Percentage of animals responding at 38°C thermal stimulus, under 60 seconds	16.66% (2/12)	0% (0/12)	16.66% (2/12)
	Effect % vs basal Chi-squared test		0.54	0.3
	Effect % vs control Chi-squared test	0	-	1.52
	Effect % vs VCR Chi-squared test	0.3	2.7	0.21

After 7 days of vincristine administration in a dose of 0.1 mg/kg-bw/day, the percentage of animals in response to 38°C thermal stimulus, increased statistically significant compared to control group. Vincristine induced the thermal allodynia (Figure 1). This thermal sensitivity is not registered in the control group. Amitriptyline (25 mg/kg-bw) and tramadol (5 mg/kg-bw) treatment have proven analgesic effect in thermal allodynia induced by vincristine (0.7 mg/kg-bw cumulative dose).

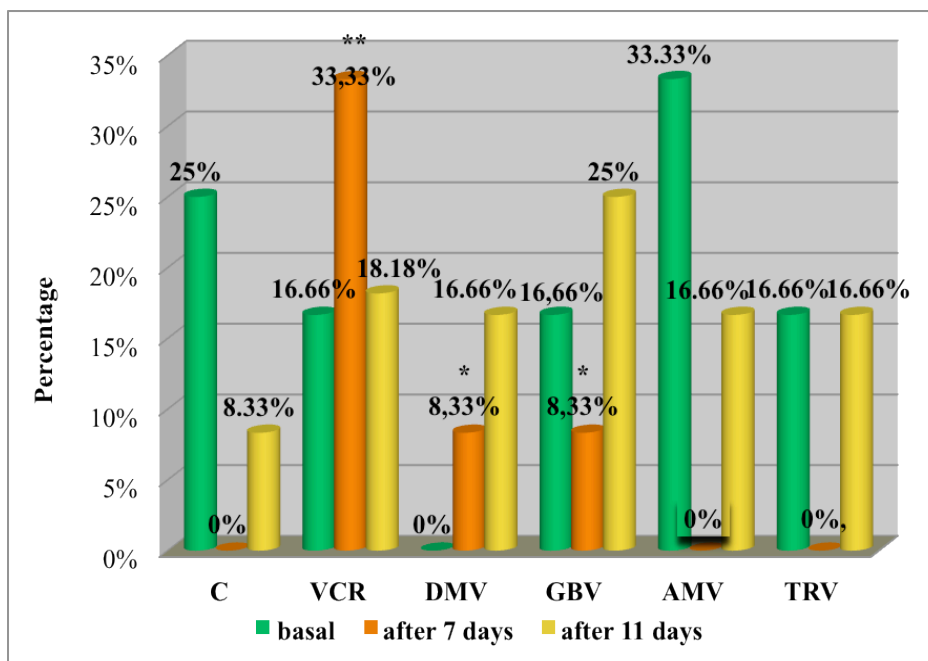


Figure 1

Basal (pre-drug), after 7 days and after 11 days values (first, second and third column, respectively) percentage of animals responding at 38°C thermal stimulus of saline (control) and vincristine-treated mice. Group comparisons were performed using Chi-squared test, compared to control group, * $p < 0.05$, ** $p < 0.01$.

Hot plate thermal hyperalgesia

Taking into account that hyperalgesia induced by antineoplastic drugs reduces the time to response to thermal stimulus (52°C), the experimental results presented in table II underline the increase of thermal pain sensitivity following vincristine administration.

Table II
Modifications of the response to 52⁰C thermal stimulus during hyperalgesia following vincristine and each of the co-administered: dextromethorphan, gabapentine, amitriptyline and tramadol.

	Group					
	C	VCR	DMV	GBV	AMV	TRV
Basal Mean ± SD	8.89 ±1.967	9.26 ±1.295	9.21 ±2.596	9.05 ±0.9223	8.70 ±2.126	9.37 ±2.559
<i>ANOVA with Tukey's Multiple Comparison Test</i>	ns (p= 0.9719)					
After 7 days Mean ± SD	7.29 ±2.372	5.74 ±1.721	7.77 ±3.27	6.32 ±1.793	6.13 ±2.258	7.92 ±2.611
<i>Effect % vs baseline</i>	-18.01	-38.00	-19.73	-32.65	-29.57	-15.48
<i>Student T test</i>	ns 0.1262	*** < 0.0001	ns 0.2628	*** 0.0002	* 0.0123	ns 0.1628
<i>Effect % vs control group</i>		-19.99	-1.72	-14.64	-11.56	2.53
<i>Student T test</i>		ns 0.1190	ns 0.7154	ns 0.3107	ns 0.2778	ns 0.5777
<i>Effect % vs VCR group</i>			18.27	5.35	8.43	22.52
<i>Student T test</i>			ns 0.0955	ns 0.4611	ns 0.6660	* 0.0355
After 11 days Mean ± SD	7.24 ±1.128	6.86 ±2.38	7.68 ±2.964	7.82 ±1.795	6.87 ±3.043	7.96 ±2.436
<i>Effect % vs baseline</i>	-18.56	-25.88	-16.65	-13.58	-21.07	-15.04
<i>Student T test</i>	ns 0.1162	** 0.0091		ns 0.0547	ns 0.1119	ns 0.2026
<i>Mann Whitney test</i>			ns 0.9211			
<i>Effect % vs control group</i>		-7.32	1.91	4.98	-2.51	3.52
<i>Student T test</i>		ns 0.6620		ns 0.3895	ns 0.7180	ns 0.4019
<i>Mann Whitney test</i>			ns 0.6668			
<i>Effect % vs VCR group</i>			9.23	12.29	4.80	10.84
<i>Student T test</i>				ns 0.3196	ns 0.9974	ns 0.3332
<i>Mann Whitney test</i>			ns 0.6713			

ns – not significant

The response to 52⁰C thermal stimulus increases after 0.7 mg /kg-bw cumulative dose of vincristine, in the following order VCR < AMV < GBV < C < DMV < TRV (Figure 2).

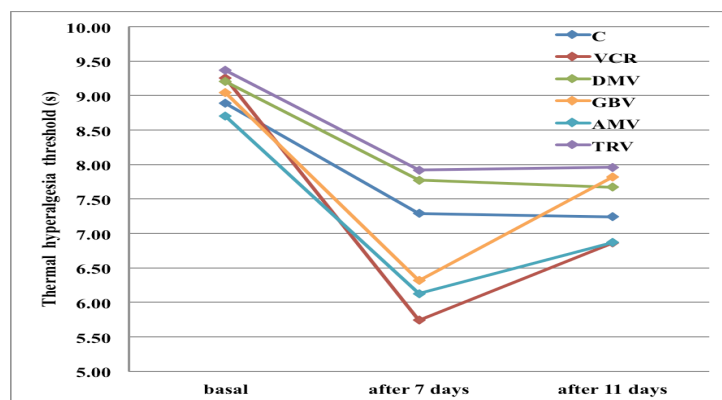


Figure 2.

Basal (pre-drug), after 7 days and after 11 days behavioural thermal hyperalgesia in response to 52°C thermal stimulus throughout the experiment.

After 7 and 11 days of vincristine 0.1 mg/kg-bw, the thermal response to 52°C decreased statistically significant compared to basal response. In the VCR group the decrease is highly significant after 7 days (-38.00%, $p < 0.001$) and statistically significant after 11 days (-25.88%, $p < 0.01$). It highlights the vincristine-induced thermal hyperalgesia (Figure 3).

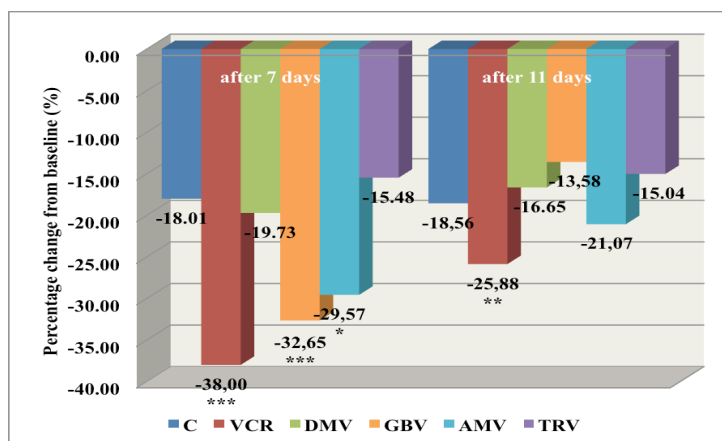


Figure 3

The percentual change from baseline in response to 52°C thermal stimulus (thermal hyperalgesia) after 7 days and after 11 days. Group comparisons were performed using t Student test or Mann Whitney test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Gabapentin (150 mg/kg-bw) and amitriptyline (25 mg/kg-bw) don't reduce vincristine-induced thermal hyperalgesia. Tramadol (5 mg/kg-bw) and dextromethorphan (20 mg/kg-bw) reduce the thermal hyperalgesia

induced by vincristine cumulative dose of 0.7 mg/kg-bw. All groups that received the analgesic protection have positive values after 7 and 11 days of treatment, compared with the VCR group (Figure 4).

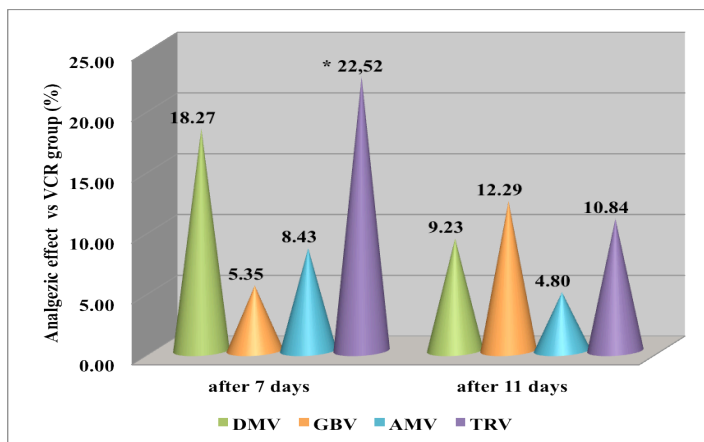


Figure 4

Analgesic effect (%) of each co-treatment (dextromerthorphan 20 mg/kg-bw/day; gabapentin 150 mg/kg-bw/day; amitriptyline 25 mg/kg-bw/day; tramadol 5 mg/kg-bw/day) in the treatment of pain (thermal hyperalgesia) induced by vincristine. Group comparisons were performed using t Student test or Mann Whitney test, in comparison to the vincristine group (* $p < 0.05$).

Tramadol (5 mg/kg-bw) increased statistically significant (22.52%, $p < 0.05$) the response to 52°C thermal stimulus. This indicates that tramadol reduced the thermal hyperalgesia and provided protection for the given cumulative dose of vincristine at 0.7 mg/kg bw (Figure 4).

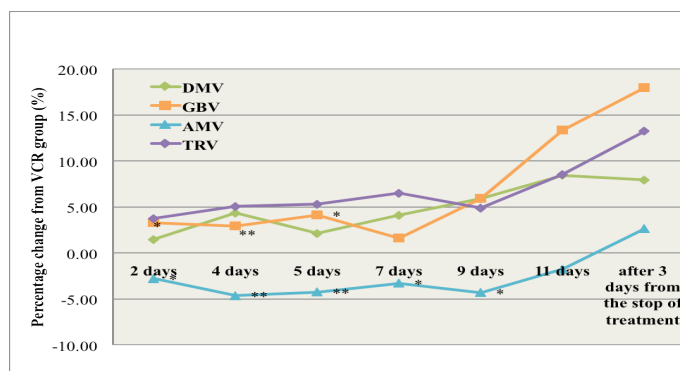


Figure 5

The percentual change of the animals weight compared with the VCR group in co-treatment groups during the experiment. Group comparisons were performed using t Student test or Mann Whitney test, * $p < 0.05$; ** $p < 0.01$.

The weight of the VCR group animals decreased statistically (-10.75% $p < 0.05$) compared with control group at the time the cumulative dose of vincristine reached 1.1 mg/kg-bw. Tramadol (5 mg/kg-bw), dextromethorphan (20 mg/kg-bw) and gabapentin (150 mg/kg-bw) have the advantage of reducing or preventing chemotherapy-induced weight reduction. But amitriptyline (25 mg/kg-bw) augmented the weight reduction induced by vincristine (Figure 5).

Conclusions

These results support that drugs with a complex mechanism of action: tramadol (an opioid agonist and activator of the spinal monoaminergic inhibition of pain by inhibiting the norepinephrine and serotonin re-uptake) and dextromethorphan (an opioid agonist and antagonist of the NMDA glutamatergic receptor) reduce the vincristine-induced thermal hyperalgesia.

Further research will target the combination of analgesic effect of drugs with complex or different mechanism of action, for combating neuropathic pain induced by vincristine.

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