EXPERIMENTAL RESEARCH ON POSSIBLE OXALIPLATIN ANALGESIC EFFECT IN MICE

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Abstract. Pain is one of the main parameters used for evaluating the clinical efficacy of anticancer drugs. Previous research suggests that some oncologic drugs have an analgesic effect in acute administration, independent of the analgesic effect resulted from the tumor shrinkage. In this paper we analyzed the analgesic effect of oxaliplatin. We conducted two experimental tests: writhing test and hot plate test, using male swiss albino mice. The tested substance, oxaliplatin, was intraperitoneally administered, 1 hour before tests, in doses of 2 mg/kg body weight – bw (lethal dose 50/10) and 4 mg/kg bw (lethal dose 50/5), respectively. In writhing test, the number of abdominal contractions, when compared with the control group, increased for the high dose in a statistically significant manner. For the low dose of oxaliplatin, the number of writhes slightly decreased. In the hot plate test, none of the doses of oxaliplatin have produced statistically significant effects in the paw licking and jumping latency parameters. Further research on analgesic effects of platinum derivatives will clarify issues regarding the statistical significance.

Key words: analgesia tests, oxaliplatin, transient receptor potential vanilloid 1 (TRPV1).

INTRODUCTION

Pain is one of the main parameters used for evaluating the clinical efficacy of some anticancer drugs. Therefore, it may be possible that these oncologic drugs have an analgesic effect in acute administration, independent of the analgesic effect resulted from the tumor shrinkage. Our previous research seems to support this hypothesis.

For example, results from several studies demonstrate that chemotherapeutic agent gemcitabine has analgesic effect [5, 8, 17]. Additionally, previous experiments realized in our department (Department of Pharmacology and Pharmacotherapy from the "Carol Davila" University of Medicine and Pharmacy)

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have shown that fluorouracil (5-FU), another antineoplastic agent, also possesses a central antinociceptive effect [18]. The analgesic effect of both gemcitabine and 5-FU is probably due to an interference of the chemotherapeutic agent with the cyclic nucleotides (cGMP and cAMP, respectively). Because both 5-FU and gemcitabine are from the same pharmacologic class and influence the results from the hot plate test (which detects central nociception), we hypothesize that nucleoside analogues antineoplastic drugs may have a central analgesic effect.

Furthermore, we decided to study other classes of chemotherapeutic drugs for analgesic properties. Our department has previously conducted experimental pain tests using cisplatin, a DNA-damaging anticancer drug, known to cause neuropathic pain [1]. Our results indicated that, in acute administration, cisplatin has analgesic effects in the writhing test – a common method of analgesic assessment in which pain is induced through a peritoneal irritant (unpublished data).

In these conditions, we wanted to evaluate if oxaliplatin, a chemotherapeutic agent similar to cisplatin, has the same analgesic properties in acute administration and, if true, to establish a possible drug class effect.

Oxaliplatin is a third-generation platinum derivative antineoplastic agent which has a 1,2-diaminocyclohexane (DACH) carrier ligand. In combination with 5-fluorouracil/leucovorin (FOLFOX or FUFOX), oxaliplatin represents the standard treatment of advanced-stage colorectal cancer [2, 10]. This substance is a good drug choice for first-line therapy because of its decreased chance of resistance development [6].

The mechanism of action of oxaliplatin relies on the retention of DACH ring by activated oxaliplatin, which blocks DNA replication [16]. Amongst common adverse effects is painful neuropathy, which limits the dose adjustment of the cancer treatment [19]. The majority of the cases with neurotoxicity are reversible within 13 weeks from the end of the treatment [6]. Opioids can be used in managing FUFOX-induced neuropathy and therefore extend the duration of the treatment in patients [13].

In this study the analgesic properties of these drugs have been evaluated by using two commonly used behavioral methods which study nociception: the writhing test, which explores peripheral pain and the hot plate test, which explores central pain transmission and perception.

The writhing test is a chemical method which consists by intraperitoneal administration of agents (usually acetic acid or phenylquinone) which irritate serous membranes [9]. The common experimental protocol for writhing test in our department uses acetic acid of 10 mL/kg body weight (bw) and 0.75% solution as irritative agent [14]. The administration of the acetic acid causes contractions, twisting of abdominal muscles, and a reduction in motor activity and coordination [3]. The measurements are made by counting the occurrence of abdominal contractions per unit of time.

In this study, we decided to use the writhing test because of its sensitivity and capability of discerning analgesic effects of very low intensity. However, this method is generally less specific and gives positive response to spasmolytic drugs [4].

The other method we choose to use, the hot plate test, has – on the contrary – an increased specificity and decreased sensitivity [3]. The hot plate test explores central mechanisms of analgesic activity and it consists of inserting mice, for specified amounts of time, into an open-ended transparent glass cylinder with a ground made of a metallic plate which is heated by a thermode or a boiling liquid [21]. The plate is heated (and maintained constant) at a temperature of 55 ± 0.5 °C. Two behavioral processes are observed during this time: paw licking and jumping from the plate. Licking is a fast reaction to nociceptive thermal stimuli and is an indicator of pain threshold. Jumping represents a more elaborated response, with a latency, and shows pain tolerance.

MATERIALS AND METHODS

ANIMALS

In the writhing test, 11 mice have been used for each tested group. In the hot plate test, 12 mice have been used for each tested group. The tested groups were control, low dose oxaliplatin, high dose oxaliplatin.

The animals used in the experiments were male Swiss albino mice, weighing 20–25 g, two months old, and were kept under normal conditions of temperature, and day/night alternation, with food and water supplied *ad libitum*. The animals have been brought in the laboratory one week before the beginning of each experiment.

All experiments took place between 9 am and 5 pm. The experiments were conducted according to the agreement of the Institutional Ethic Committee, previously obtained, respecting directive 86/609/EU. The animals were obtained from the hatchery of the "Carol Davila" University of Medicine and Pharmacy, Bucharest. After 24 hours from the end of the experiments, the animals were euthanized. The mice were not used in two consecutive experiments.

The active substance used was oxaliplatin – eloxatin, 50 mg concentrated for intravenous infusion, Aventis. When the drug is administered intraperitoneally, lethal dose (LD50) for oxaliplatin in mice is 19.8 mg/kg. The tested doses of oxaliplatin were oxaliplatin 2 mg/kg (LD50/10), respectively oxaliplatin 4 mg/kg (LD50/5).

PROTOCOLS

Three groups of mice were used in the writhing test. Group 1 received saline, group 2 received oxaliplatin 2 mg/kg, and group 3 received oxaliplatin 4 mg/kg. The concentration of oxaliplatin solutions was calculated in such a manner that each mouse received 0.1 mL solution per 10 g bw.

All substances, including the saline (control group), were administered intraperitoneally (i.p.). Time between two consecutive substance administrations (i.p.) was 5 minutes. One hour after the oxaliplatin or saline administration, each animal received an i.p. – injection 0.1 mL/10 g bw with acid acetic solution of 0.75% v/v. Five minutes after the acetic acid administration, each animal was put in an exploration cage and the number of writhes over a 5 minute period was determined. A writhe was considered and counted when the animal touched the floor of the cage with its abdomen and spread the rear legs relative to front legs.

Three groups of animals were used in the hot plate test. Group 1 received saline. Group 2 received oxaliplatin 2 mg/kg, and group 3 received oxaliplatin 4 mg/kg. The concentration of oxaliplatin solutions was calculated in such a manner that each mouse received 0.1 mL solution per 10 g bw. All substances were administered as i.p. injections. One hour after the i.p. injection each animal was placed upon a plate that was heated to a temperature of 55±0.5 °C. For each animal, the latency (in seconds and hundredths of a second) of fore paw licking (first reaction) and the delay of jumping from the hot plate (second reaction) were recorded. The time of latency is defined as the time period between the zero point, when the animal is placed on the hot plate surface, and the time when the animal licks its paw or jumps off to avoid thermal pain.

STATISTICAL ANALYSIS

Data were presented as the average value obtained for each group, for each variable and experiment. The standard deviation and the standard errors were calculated for each group. The statistical significance of the results was studied with ANOVA and appropriate post hoc test (Tukey test) (if Levene test was significant, for homogeneous groups, *i.e.* $p > 0.05$). Only the values of $p < 0.05$ (obtained with ANOVA and appropriate post-hoc tests) were considered statistically significant.

RESULTS

The results of the writhing test are presented in Figure 1 and the results of the hot plate test are shown in Figure 2 for the first parameter (fore paw licking latency) and in Figure 3 for the second parameter (jumping latency).

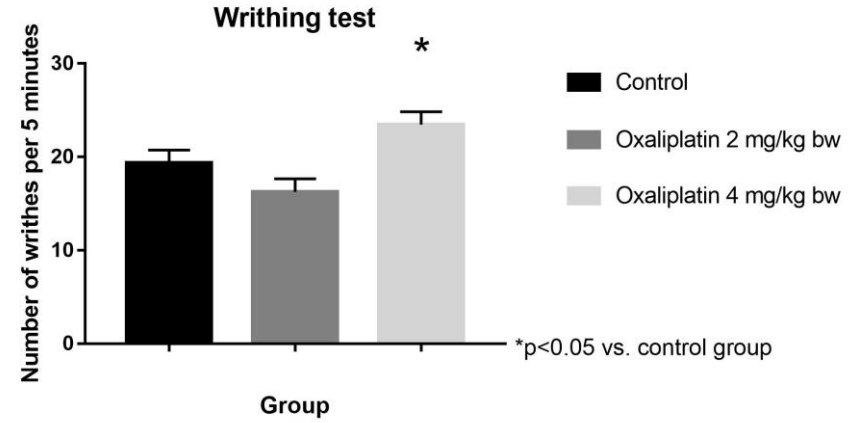

Fig. 1. The relationship dose-effect (number of writhes) when two doses of oxaliplatin were used (2 mg/kg body weight, bw and 4 mg/kg bw, respectively).

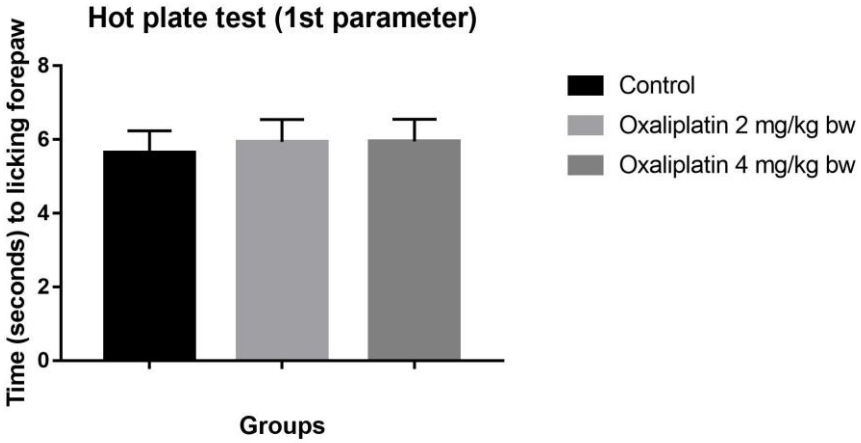

Fig. 2. The effect (fore paw licking latency) when two doses of oxaliplatin were used (2 mg/kg bw and 4 mg/kg bw).

In the writhing test, when oxaliplatin in dose of 2 mg/kg bw was administered, the number of writhes was lower than the number of writhes from the control group (16.27 ± 1.45 writhes / 5 minutes *vs* 19.36 ± 1.45 writhes / 5 minutes). However, the difference was not statistically significant ($p = 0.32$).

When oxaliplatin in dose of 4 mg/kg bw was administered, the number of writhes was greater when compared with those from the control group

(23.45 ± 1.45 writhes / 5 minutes *vs* 19.36 ± 1.45 writhes / 5 minutes). In this case, the results were statistically significant ($p = 0.022$).

In the hot plate test, for the fore paw licking latency (first parameter) there are no significant differences *vs*. control group (ANOVA). Levene test indicates $p > 0.05$. Consequently, we could make the assumption that all the standard errors of mean are equal and the statistical program indicates for Tukey test these standard errors (5.64 ± 0.63 seconds control group *vs* 5.94 ± 0.63 seconds oxaliplatin 2 mg/kg bw *vs* 5.95 ± 0.63 seconds oxaliplatin 4 mg/kg bw).

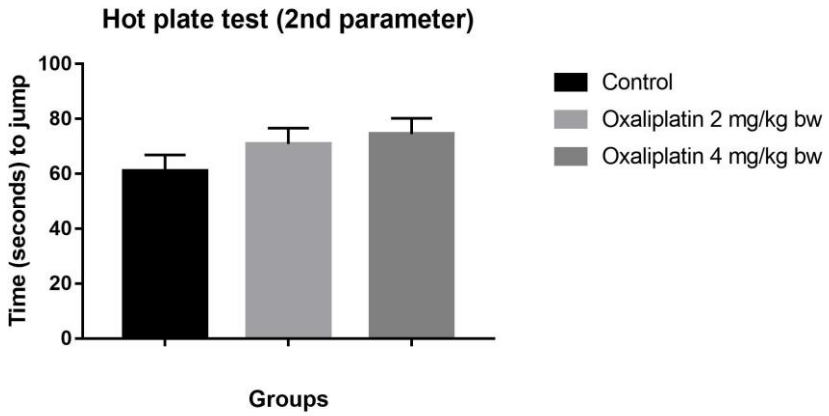

Fig. 3. The effect (jumping latency) when two doses of oxaliplatin were used (2 mg/kg bw and 4 mg/kg bw).

In the hot plate test, oxaliplatin produced no statistically significant effects for jumping latency (second parameter), for both administered doses (61.07 ± ± 6.07 seconds control group *vs* oxaliplatin 2 mg/kg bw 70.85 ± 6.07 *vs* oxaliplatin 4 mg/kg bw 74.43 ± 6.07).

DISCUSSION

In the writhing test, the number of abdominal contractions, when compared with the control group, increased for the high dose in a statistically significant manner. For the low dose of oxaliplatin, the number of writhes slightly decreased (compatible with an analgesic effect), but the results were not statistically significant. These data, in which oxaliplatin was algogenic in mice, correlate with clinical findings which state that oxaliplatin produces neuropathic pain, especially at high doses [20].

Although there are no published data, our previous research has shown an analgesic effect in the writhing test for cisplatin. Taking into consideration our results we could hypothesize that the analgesic effect of cisplatin is not a class effect of platinum derivatives.

Animal studies have been employed in order to explain the mechanisms by which oxaliplatin causes hyperalgesia and neuropathic pain. Results suggest that TRPV1 (transient receptor potential vanilloid 1) contributes to mechanical hyperalgesia evoked by oxaliplatin [7].

TRPV1 is a Ca^{2+} permeable non-selective cation channel which is localized in neurons both in the central nervous system and periphery. This ionic channel is activated by physical and chemical stimuli and plays an important role in detection of nociceptive thermal inflammatory pain [15]. TRPV1 antagonists have shown efficacy in reducing nociception from inflammatory and neuropathic pain models in rats [11]. However, some agonists of TRPV1, like capsaicin, also produce analgesic effect [12]. This suggests that TRPV1 may be involved both in hyperalgesia and analgesia.

Therefore, we can speculate that the slight decrease of the number of writhes at the low dose of oxaliplatin is due to the same TRPV1 channel that was algogenic at high dose of oxaliplatin. More research in this field is needed.

In the hot plate test none of the parameters was affected in a statistically relevant manner.

CONCLUSIONS

1. In the writhing test, the high dose of oxaliplatin (4 mg/kg bw) has statistically significantly increased the number of writhes.

2. For the low dose of oxaliplatin (2 mg/kg bw), the number of writhes slightly decreased, but the results were not statistically significant.

3. In the hot plate test, for none of the doses administered, oxaliplatin has not modified – in a statistically significant manner – neither the jumping from the hot plate, nor the fore paw licking latencies.

4. Further research on analgesic effects of platinum derivatives will complete the pharmacological profile of these substances.

REFERENCES

1. AMPTOULACH S., N. TSAVARIS. Neurotoxicity caused by the treatment with platinum analogues, *Chemother. Res. Pract*., 2011, **2011**, 843019.

2. ANDRÉ, T., C. BONI, L. MOUNEDJI-BOUDIAF, MATILDE NAVARRO, J. TABERNERO, T. HICKISH, CLARE TOPHAM, MARTA ZANINELLI, P. CLINGAN, J.BRIDGEWATER, ISABELLE TABAH-FISCH, AIMERY DE GRAMONT, Multicenter international study of oxaliplatin/5-fluorouracil/leucovorin in the adjuvant treatment of colon cancer (MOSAIC) Investigators. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer, *N. Engl. J. Med*., 2004, **350**, 2343–2351.
3. BARS, D., MANUELA GOZARIU, S.W. CADDEN, Animal models of nociception, *Pharm. Rev*., 2001, **53**, 597–652.
4. BERGE, O.G., Predictive validity of behavioural animal models for chronic pain, *Br. J. Pharmacol*., 2011, **164,** 1195–1206.
5. BURRIS, H.A. 3rd, M.J. MOORE, J. ANDERSEN, M.R. GREEN, M.L. ROTHENBERG, M.R. MODIANO, M.C. CRIPPS, R.K. PORTENOY, A.M. STORNIOLO, P. TARASSOFF, R. NELSON, F.A. DORR, C.D. STEPHENS, D.D. VON HOFF, Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial, *J. Clin. Oncol*., 1997, **15,** 2403–2413.
6. CASSIDY, J., J.L. MISSET. Oxaliplatin-related side effects: characteristics and management, *Semin. Oncol*., 2002, **29,** 11–20.
7. CHEN, K., Z.F. ZHANG, M.F. LIAO, W.L. YAO, J. WANG, X.R. WANG, Blocking PAR2 attenuates oxaliplatin-induced neuropathic pain via TRPV1 and releases of substance P and CGRP in superficial dorsal horn of spinal cord, *J. Neurol. Sci*., 2015, **15,** 62–67.
8. FULGA, CARMEN, A. ZUGRAVU, I. FULGA, The analgesic effect of gemcitabine in mice, *Rom. J. Intern. Med.*, 2006, **44**, 335–350.
9. GAWADE, S.P., Acetic acid induced painful endogenous infliction in writhing test on mice, *J. Pharmacol. Pharmacother*., 2012, **3,** 348.
10. GROTHEY, A., Clinical management of oxaliplatin-associated neurotoxicity, *Clin. Colorectal Cancer*., 2005, **Suppl 1,** S38–S46.
11. JHAVERI, M.D., S.J. ELMES, D.A. KENDALL, V. CHAPMAN, Inhibition of peripheral vanilloid TRPV1 receptors reduces noxious heat-evoked responses of dorsal horn neurons in naïve, carrageenan-inflamed and neuropathic rats, *Eur. J. Neurosci.*, 2005, **22,** 361–370.
12. KNOTKOVA, HELENA, M. PAPPAGALLO, A. SZALLASI. Capsaicin (TRPV1 Agonist) therapy for pain relief: farewell or revival?, *Clin. J. Pain*., 2008, **24**, 142–154.
13. NAGASHIMA, M., M. OOSHIRO, A. MORIYAMA, Y. SUGISHITA, K. KADOYA, A.SATO, T. KITAHARA, R. TAKAGI, T. URITA, Y. YOSHIDA, H. TANAKA, T. OSHIRO, S. OKAZUMI, R. KATOH, Efficacy and tolerability of controlled-release oxycodone for oxaliplatin-induced peripheral neuropathy and the extension of FOLFOX therapy in advanced colorectal cancer patients, *Support. Care Cancer*, 2014, **22**, 1579–1584.
14. PĂUNESCU, H., OANA ANDREIA COMAN, L. COMAN, F. DRĂGHIA, CRISTINA MUŞA, ISABEL GHIȚĂ, I. FULGA, The influence of cannabinoid receptor antagonist AM281 on paracetamol effect in the writhing test in mice, *Therapeutics, Pharmacology and Clinical Toxicology*, 2011, **15,** 126–131.
15. PREMKUMAR, L.S., P. SIKAND, TRPV1: A target for next generation analgesics, *Curr Neuropharmacol.*, 2008, **6,** 151–163.
16. RAYMOND, E., S. FAIVRE, J.M. WOYNAROWSKI, S.G. CHANEY, Oxaliplatin: mechanism of action and antineoplastic activity, *Semin. Oncol*., 1998, **Suppl 5,** 4–12.
17. ROMANUS, D., H.L. KINDLER, L. ARCHER, E. BASCH, D. NIEDZWIECKI, J. WEEKS, D. SCHRAG, Cancer and leukemia group B. Does health-related quality of life improve for advanced pancreatic cancer patients who respond to gemcitabine? Analysis of a randomized phase III trial of the cancer and leukemia group B (CALGB 80303), *J. Pain. Symptom Manage*., 2012, **43**, 205–217.
18. STOLERU, SMARANDA, H. PĂUNESCU, A. ZUGRAVU, SORINA VASILE, OANA ANDREIA COMAN, I. FULGA, The analgesic effect of 5-fluorouracil in mice, *Modern Medicine*, in press.

19. VENOOK, A.P., A.H., KO, M.A. TEMPERO, J. UY, T. WEBER, M. KORN, E.K. BERGSLAND, Phase II trial of FOLFOX plus bevacizumab in advanced, progressive neuroendocrine tumors, *J. Clin. Oncol.* 2008, **26**, 15545.
20. WEICKHARDT, A., K. WELLS, W. MESSERSMITH, Oxaliplatin-induced neuropathy in colorectal cancer, *J. Oncol*., 2011, **2011,** 1–7.
21. WOOLFE G., A.D. McDONALD, The evaluation of the analgesic action of pethidine hydrochloride (Demerol), *J. Pharmacol. Exp. Ther*., 1944, **80**, 300–307.