THE EFFECT OF COBALT CHLORIDE PRECONDITIONING ON PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY

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THE EFFECT OF COBALT CHLORIDE PRECONDITIONING ON PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY (Abstract) Introduction: Recent studies indicate that Cobalt Chloride (CoCl2) modulates mitochondrial activity. There is emerging data suggesting that Paclitaxel-induced peripheral neuropathy (PIPN) is a consequence of the drug's mitochondrial toxicity. AIM: to assess the effect of CoCl2 preconditioning on PIPN in an animal model. Material and method: PIPN was induced by 7 daily consecutive i.p. Paclitaxel (PXT) administrations. Male BALB/c mice were divided into three groups as follows: group A - CoCl2 (12.5 mg/kg b.w.) for three weeks (preconditioning) followed by 1 week of PXT, group B - saline for three weeks, followed by 1 week of PXT and group C - saline for four weeks. Thermal and mechanical allodynia were assessed by means of paw withdrawal latency (PWL). Results: In group A, CoCl2 preconditioning lead to a decrease in both thermal and mechanical PWLs. 7 days after the last dose of PXT, however, values returned to normal in group A and allodynia for both thermal and mechanical stimuli was noted in group B (p<0.05). Conclusions: CoCl2 preconditioning seems to protect against PIPN. Although CoCl2 administration decreased thermal and mechanical PWLs, subsequent P administration did not lead to the persistent mechanical and thermal allodynia that was noted in the P-alone group. Further studies are required for determining the exact relationship between CoCl2 and PIPN. Keywords: PACLITAXEL, PAINFUL NEUROPATHY, COBALT CHLORIDE

Painful peripheral neuropathy (PPN) is one of the most frequent side effects of Paclitaxel (PXT) – a chemotherapy drug used in the treatment of breast, lung, ovarian and germinal cancers. Its mechanism involves blocking the cell cycle, through centrosomal impairment, induction of abnormal spindles and suppression of spindle microtubule dynamics (1). This antitumor mechanism does not explain, however, the pathogenesis of PXT-induced peripheral neuropathy (PIPN). Recent research suggest that there is a connection between PIPN and its mitochondrial toxicity (2,3).

Cobalt chloride (CoCl2) is a chemical compound widely used in research due to its ability to induce chronic HIF-1 (hypoxia inducible factor-1) activation and, thus, to mimic cellular hypoxia by activating the genes responsible for adapting to low oxygen concentrations. Moreover, CoCl2 stabilizes this factor, which is usually inactivat-
ed once normal oxygen concentrations are again detected. Studies suggest that hypoxic preconditioning has cardio- (4), reno- (5) and neuroprotective (6) effects.

A 2012 study (7) indicates that CoCl2 preconditioning (due to hypoxia adaptation) leads to increased physical performance and enhances mitochondrial metabolism in mice. Other studies, such as the one of Jones et al. (8) demonstrate the protective effect of CoCl2 against perinatal hypoxic-ischemic lesions.

In a recent study, we showed that daily CoCl2 administration for 3 weeks has anti-inflammatory effects in a murine pain model and leads to a decrease in pain behavior, especially during the second phase of the formalin test (9).

The present study aims to investigate the effects of CoCl2 preconditioning on Paclitaxel-induced peripheral neuropathy.

MATERIAL AND METHODS

Animals: 24 male BALB/c mice, weighing 27 to 30 grams were used. Throughout the experiment, the animals had ad libitum access to water and food; the mice were habituated with the testing environment 2 hours prior to testing. The study design and experimental procedures described in this article are in accordance with the European Council Directive 2010/63/EU and follow the guidelines for investigations of experimental pain in conscious animals issued by the University of Medicine and Pharmacy “Gr. T. Popa”.

Drugs used in the experiment: CoCl2 (Sigma-Aldrich Chemie BmbH, Germany) and Paclitaxel (Sindan Pharma, Romania); the substances were administered intraperitoneally; doses are in mg per kg body weight (mg/ kg b.w.).

Thermal and mechanical allodynia were assessed by:

Hargreaves, or plantar, test (Hg) was used in order to assess thermal allodynia. Mice were placed in transparent Plexiglas cases. Underneath the heat-conducting floor, a mobile radiant heat source (Plantar Test-37370 UgoBasile) was placed directly under the animal’s paw; we measured the time between applying the heat stimulus and the moment when the animal withdraws its paw – response latency to thermal stimuli (paw withdrawal latency – PWL) (10).

The von Frey test, performed by means of the automatic von Frey method, (vF), was used to assess mechanical allodynia. Mice were placed in transparent cases with a wire-mesh floor. Underneath, a mechanical device with a thin filament (Dynamic Plantar Aesthesiometer 37450, UgoBasile) was used to apply increasing pressure onto the plantar side of the animal’s paw. We measured the time between applying pressure and the moment when the animal withdraws its paw – response latency to mechanical stimuli (paw withdrawal latency – PWL) (11).

The average of three different measurements for each paw (right and left) was used for statistical evaluation, both for thermal and for mechanical allodynia. Hyperalgesia was quantified as the percentage decrease in withdrawal latency (DWL) when compared to baseline:

\[ \% \text{DWL} = \frac{(\text{baseline value} - \text{measured value}) \times 100}{\text{baseline value}} \]

Study design: PIPN was induced by intraperitoneal administration of 2 mg/kg b.w. PXT for 7 consecutive days. The mice were divided into 3 groups (n = 8 mice/group) as follows: group A – CoCl2 (12.5 mg/kg b.w.) daily, for 3 weeks (preconditioning), then PXT for a week; group
B–3 weeks of daily saline administration, then PXT for a week; group C–4 weeks of daily saline administration. The CoCl2 dose was selected in accordance with published data (9). Thermal and mechanical allodynia were evaluated at the beginning of the experiment (baseline), then weekly during CoCl2/saline treatment and twice a week after PXT administration and until the end of the experiment. All tests were performed before the daily administration of CoCl2/PXT/saline. The animals were monitored for 7 weeks.

Statistical analysis: The results are expressed as DWL or as average±standard error. Statistical evaluation was performed by means of the SPSS v.20 software – ANOVA analysis with post-hoc Tukey test. Significance level was set at .05.

RESULTS

**Effects on mechanical alldynia**

Chronic CoCl2 administrations lead to a progressive decrease of the response latency for mechanical stimuli (vF test). Thus, after two weeks of treatment, PWL average for group A was 6.91±0.12s, while in groups B and C, the average values were 7.84±0.15s and 7.96±0.41s, respectively (p<0.02); after three weeks, the average PWL for group A was 5.77±0.26s, while for groups B and C, PWL averages were 7.94±0.08s and 7.31±0.47s, respectively (p<0.01). At that time, the CoCl2 treatment group had a response latency 24.8% lower compared to the beginning of the experiment (fig. 1). ANOVA analysis indicated a significant substance effect for days 14 and 21 of the experiment with F(2, 5.3)=4.73, p=0.02 at day 14 and F(2, 19.9)=12.56, p=<0.0001 at day 21.

After the last PXT dose (for groups A and B), in the 28th day of the experiment, the response latency of CoCl2 preconditioned mice increased close to baseline values. Thus, in the last day of chemotherapy (CHT), the average was 7.63±0.51s for group A, 7.33±0.43s for group B and 7.41±0.4s for group C (p=0.05). Two weeks after CHT, in the Paclitaxel-alone group (group B), a significant decrease of response latency for mechanical stimuli was noted, with average values of 5.69±0.53s, 27.5% lower than baseline values (p=0.02); at that time, the average value for group A was 8.01±0.69s and the average value for group C (control group) was 7.52±0.42s. Subsequently, sensitivity to mechanical stimuli increased only for PXT treated mice, with a maximum 41% decrease in response latency compared to baseline (p=0.002); for the CoCl2 preconditioned group, maximum decrease in averages after PXT treatment was 2.9% when compared with baseline (7.44±0.5s). At the end of the experiment, the average values of group B remained 13% lower than baseline, while in the other two groups, average values varied by 1-3% when compared with baseline (fig. 1).

There were no statistically significant differences between baseline values in the three groups.

**Effects on thermal alldynia**

Chronic CoCl2 administration determined a transient decrease of response latency to thermal stimuli (Hg test). After the first week of CoCl2 treatment, PWL average in group A was 6.34±0.23s, while in groups B and C is was 8.06±0.43s and 7.92±0.23s respectively (p=0.03). ANOVA analysis indicated a significant substance effect for day 7, with (F(2, 14.6)=9.08, p=0.001). Subsequently, response latency values of CoCl2 treated mice increased and, after the third week, reached a higher
than baseline average (fig. 2.).

After the last PXT dose (for groups A and B), in the 28th day of the experiment, the response latency of CoCl2-treated mice remained constant, while for the mice treated with PXT alone, a decrease of 18.65% was noted. Starting with the 6th day after CHT, the difference between group B and the other two groups became statistically significant. Mice treated only with PXT had lower response latencies, with up to 45% decrease when compared to baseline (p<0.05 for all measurements) (fig. 2.). At that time point, in group A, average values were similar to baseline. In the last day of the experiment, the average value in group B was 3.92±0.43s, while in groups A and C, it was 10.81±0.92s and 9.01±0.81s, respectively.

There were no statistically significant differences between baseline values in the three groups.

![Graph](image1.png)

**Fig. 1.** The effect of CoCl2 on response latency after mechanical stimuli. For each group, treatment and motorization time-frames are mentioned. * - p<0.05 - Tukey post-hoc

![Graph](image2.png)

**Fig. 2.** The effect of CoCl2 on response latency after thermal stimuli. For each group, treatment and motorization time-frames are mentioned. * - p<0.05 - Tukey post-hoc
DISCUSSION

CoCl2 induced thermal and mechanical allodynia after one week of administration; thermal allodynia was transient, while mechanical allodynia persisted until the end of CoCl2 treatment (3rd week of the experiment). These results are in accordance with published data (9) and indicate a neurotoxic effect of CoCl2, possibly due to the substance’s affinity for the nervous tissue (12). However, after PXT administration, response latency after both thermal and mechanical stimuli returned to baseline values in CoCl2 group and significantly decreased in the PXT-alone group. In group B, a significant and persistent thermal and mechanical allodynia appeared after CHT, with response latencies 41% lower than at baseline. The protective effect of CoCl2 preconditioning can be explained by CoCl2’s stimulating effects on mitochondria.

Several studies suggest that PXT induced neuropathy is a result of its mitochondrial toxicity. For example, a paper published in 2014 indicated that, by blocking the sigma-1 receptors (which are involved in mitochondrial calcium homeostasis) in a mouse PXT neuropathy model, PIPN is can be prevented (13). Another study revealed the presence of mitochondrial anomalies in A and C fibers by microscopy analysis of the saphenous nerve; this led the authors to suggest that mitochondrial modifications are the main mechanism responsible of PIPN (3). Janes et al. suggested that the presence of PIPN is strongly related to mitochondrial superoxide dismutase inactivation and to decreased ATP production in the axons of the primary sensitive neurons (14).

There are several in vitro studies highlighting the effect of PXT on mitochondria. For instance, Xiang et al. performed in-vitro administration of PXT to isolated mitochondria and measured the organelle’s membrane potential (ΔΨm) by means of flow citometry. The results showed a dose-dependent decrease in potential value; these changes were not observed when the experiment was carried out with Cisplatin or actinomycin D (15).

Another in-vitro study revealed the direct influence of PXT on mitochondria isolated from human cancer cells (neuroblastoma), in which PXT administration lead to the release of cytochrome C. Thus, the authors suggested that even the apoptotic effect of PXT may result from its direct interaction with the mitochondria (16).

Even though the exact mitochondrial component responsible for PIPN is unknown, all the above-mentioned studies point toward a common mechanism: mitochondrial toxicity in the axons of the primary sensitive nerves following a loss of mitochondrial bioenergetics (14). Furthermore, a recent study (17) assessed the effect of two mitochondrial modulators - ascorbic acid and curcumin – on pain; results showed that these substances decreased pain behavior in the second phase of the formalin-induced pain test, considered to be an equivalent of neuropathic pain (18).

Our results can also be explained by the beneficial effects of hypoxic preconditioning in itself. In humans, training in a hypoxic environment was associated with improvements in physical performance (7). Oxidative stress generated during CoCl2 administration is involved in nociceptive processing (19), and may also lead to adaptive processes that subsequently protect against PIPN. Furthermore, a recent study suggests that HIF activity in itself protects against thermal pain (21). Taking into account the fact that ischemia is known to play a part in other types of neuropathy, such as diabetic (22) or optic (23), adapting...
to hypoxia may be involved in PIPN pathogenesis.

**CONCLUSIONS**

CoCl2 preconditioning protects against PIPN. Although CoCl2 administration had an initial hyperalgesic affect, subsequent PXT treatment did not lead to persistent thermal and mechanical allodynia. By contrast, mice that were not preconditioned and were treated with PXT alone experienced intense and persistent allodynia for both thermal and mechanical stimuli. Further studies are required for establishing the exact relationship between CoCl2 and PIPN.

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### POSSIBLE IMPLICATIONS OF Ni ON ORAL IL-1B-INDUCED INFLAMMATORY PROCESSES

Nickel (Ni) is one of the main metal elements in orthodontic and prosthetic devices. Different effects of Ni are described ranging from an induction of local inflammation to allergy and cancerous/mutagenic properties. Inflammatory reactions are frequently observed in the oral cavity, but the interrelationship of Ni with those events is still unknown. The authors carried out an in vitro study to analyze the impact of Ni on inflammation processes. Human gingival fibroblasts (HGFs) \((n = 6)\) were exposed to a pro-inflammatory environment using interleukin-1 beta (IL-1β) and additionally stimulated with different Ni(II) concentrations \((4000 \text{ng/ml})\). At varying time points the expression of pro- and anti-inflammatory as well as matrix degeneration proteins, i.e. MMPs, were analyzed. Proliferation assays, wound healing tests and the detection of NF-κB activation were conducted. Unstimulated HGFs served as control. The experiments showed that low clinical average Ni(II) levels did not alter pro-inflammatory cytokines significantly compared to control \((p > 0.05)\). Instead, a 10-fold higher dose up-regulated these mediators significantly in a time-dependent manner \((p < 0.01)\). This was even more pronounced combining both Ni(II) concentrations with an inflammatory condition \((p < 0.001)\). The mRNA data were supported by proliferation and wound closure assays \((p < 0.001)\). However, the combination of both stimuli induced contradictory results. Analyzing NF-κB activation revealed that the results may be in part attributed to NF-κB. The authors of this study suggested that Ni(II) has various modifying effects on IL-1β-induced inflammatory processes depending on the concentration (Lina Gölz, Stefan Bayer, Ludger Keilig, Andreas Jäger, Helmut Stark, Christoph Bouraue, Werner Götz, Stilla Frede, Jochen Winter, Dominik Kraus. Possible implications of Ni(II) on oral IL-1β-induced inflammatory processes. *Dental Materials* 2014; 30(12):1325-1335).