Recombinant human erythropoietin counteracts cisplatin-induced visceral hyperalgesia

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Abstract: Objective Cisplatin exerts its cytotoxic effect through distinct DNA lesions, leading to peripheral neuropathy. The risk of sensory neuropathy is a common problem during cancer treatment with cisplatin, leading to somatic hyperalgesia. Yet, data focussing on cisplatin-induced impairment of the autonomic nervous system are limited. The present study was aimed to investigate the effect of recombinant human erythropoietin (rhEPO) on cisplatin-induced visceral hyperalgesia.

Methods C57BL/6 mice were treated either with cisplatin (2 mg/kg, once per week) or with cisplatin (2 mg/kg, once per week) plus rhEPO (40 µg/kg, 3 times per week) for 8 weeks. Controls were treated with saline. To quantify the visceromotor response (VMR) at week 9, standardized electrodes were implanted into the external oblique musculature for electromyographic recordings. After that, animals were decapitated and dorsal root ganglia (DRG) was removed for transmission electron microscopy studies.

Results Cisplatin-treated mice showed a significant increase of VMR compared to the controls [(7080 ± 969) vs (2864 ± 279); \( P < 0.001 \)], while rhEPO dramatically counteracted this effect [(2962 ± 336) vs (7080 ± 969); \( P < 0.001 \)]. Transmission electron microscopy revealed cisplatin-induced structural lesions of nuclear membrane in DRG cells, which could be ameliorated by rhEPO. Conclusion Erythropoietin can significantly ameliorate the cisplatin-induced visceral hyperplasia and DRG nuclear membrane structure damage in mice, indicating a neuroprotective role of erythropoietin.

Keywords: cisplatin; dorsal root ganglia; erythropoietin; visceral neuropathy

1 Introduction

Cisplatin is widely used in the treatment of solid tumors. However, its high efficacy in cancer treatment is usually accompanied with severe neurotoxic side effects, affecting the peripheral nervous system. The sensory neuropathy is a common problem that often leads to therapy withdrawal. The paradoxical combination of sensory loss and reduction in nociceptive threshold results in a painful somatic hypersensitivity. Although an animal model of nociceptive peripheral neuropathy has been introduced\(^1\), data concerning cisplatin-dependent impairment of the autonomic nervous system and the strategies to circumvent the visceral neuropathy are scarce and mostly focussed on inhibiting sero-
tonergic pathways [2]. There is growing evidence that cation channels are involved in the process of neuropathic pain due to cisplatin [3,4].

The precise mechanisms of cisplatin-induced neurotoxicity remain still unclear. Cisplatin exerts its cytotoxic effect through the interaction with cellular DNA and the formation of distinct platinum (Pt)-DNA adducts [5]. Accumulation and persistence of DNA adducts in the cell nucleus can lead to cell apoptosis [6,7]. There is growing evidence that nuclei of DRG neurons are the primary targets of cisplatin-induced neurotoxicity [8,9]. Recently, we have found that repeated exposure to cisplatin can lead to sensory neuropathy in wild type (DNA repair proficient) mice and more severely in knock-out [xeroderma pigmentosum complementation group A deficient (XPA−), xeroderma pigmentosum complementation group C deficient (XPC−), DNA repair deficient] mice [10]. However, neurons in the spinal cord are protected by the intact blood-brain barrier, resulting in an approximately 40% decrease in Pt-DNA adducts [10].

Moreover, accumulating evidence has indicated that recombinant human erythropoietin (rhEPO) possesses neuroprotective properties [11,12]. In the peripheral nervous system, erythropoietin and its receptor are expressed in DRG neurons, axons and Schwann cells [13]. More recently, rhEPO has been demonstrated to produce neuroprotective effect against cisplatin-induced neurotoxicity, without affecting Pt-DNA adduct accumulation in DRG neurons [14].

Cisplatin-induced significant impairment of sensory nerve function is associated with structural damage in somatic nerves and mitochondria. Administration of rhEPO can rescue the nerves from damage, consequently leading to significant improvement in electrophysiological parameters and a tendency toward improvement of the withdrawal threshold. Besides, rhEPO does not have any effect on the nerve conduction velocity when administered alone [14].

Based on our previous study on large myelinated somatic fibers, the present study was aimed to extend the understanding of the neuroprotective effects of erythropoietin against cisplatin-induced visceral hyperalgesia. The mouse model of cisplatin-induced visceral hyperalgesia was employed to assess the visceromotor response (VMR) and neuronal cell structural changes in DRG neurons in vivo. Since cisplatin causes a systemic side effect, here thoracolumbar DRGs, instead of visceral nerve fibers, were examined.

2 Materials and methods
2.1 Animals and study design
2.1.1 Animals Male C57BL/6J mice from the Institute of Cell Biology were used throughout the experiment. Mice were housed under specific pathogen free (SPF) conditions with access to 1314 fortified standard diet (Altromin, Germany) and water ad libitum, under a 12:12 h dark/light cycle. Mice were aged 20 weeks at the time of behavioral testing. All experiments were approved by the state animal welfare board (G-033/05Z).

2.1.2 Study design Mice were randomly divided into 3 groups (groups A-C), and the number of animals was 17 in group A, 32 in group B and 21 in group C, respectively. Differences in group size were related to an overall animal dropout rate of approximately 15%, due to the drug intolerance, anaesthesiological and surgical complications, or electromyographic (EMG) electrode dislocation. All mice were treated for 8 weeks and were 20 weeks old at the time of examination. Mice in group A were treated with cisplatin (2 mg/kg body weight, i.p., Platinex®, Bristol, Munich, Germany) once per week. To prevent renal damage, the mice were injected with 500 µL saline i.p. after each cisplatin injection. Mice in group B were treated with cisplatin (2 mg/kg body weight, i.p.) once per week and additionally with rhEPO (40 µg/kg body weight, s.c., Neorecormon®, Roche, Basel, Switzerland) 3 times per week. These mice were injected with 500 µL saline i.p. after each cisplatin injection. Mice in group B were treated with cisplatin (2 mg/kg body weight, i.p.) once per week and additionally with rhEPO (40 µg/kg body weight, s.c., Neorecormon®, Roche, Basel, Switzerland) 3 times per week. These mice were injected with 500 µL saline i.p. as well to prevent renal damage. In group C, mice were only treated with saline (500 µL, i.p.) once per week as a control. In week 9, colorectal distension was conducted in all the groups. After that, mice were sacrificed and DRGs were removed from thoracic and lumbar regions for histological analysis. Since erythropoietin administration does not affect the peripheral nervous system [15,16], a fourth group was abdicated to minimize the number of experimental animals.

2.2 Assessment of VMR to colorectal distension
2.2.1 Surgical preparation Under ketamine hydrochloride (1 mL/kg body weight, i.p., Ketamin 10%; Sanofi-Ceva,