Intravenous Immunoglobulin Suppresses Chemotherapy-induced Peripheral Neurotoxicity via Macrophage Modulation in Rats and Mice

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Research Article

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Abstract

Chemotherapy-induced peripheral neurotoxicity (CIPN) is a serious adverse effect that leads to treatment discontinuation by patients receiving anticancer therapy. Treatment discontinuation is a serious and life-threatening problem for patients with cancer; hence, there is a need for drugs that suppress the induction of CIPN by anticancer drugs. Intravenous immunoglobulin (IVIg) reportedly suppresses bortezomib-induced CIPN in a rat model. Here, using rodent models, we showed that IVIg can also suppress peripheral neurotoxicity induced by anticancer drugs other than bortezomib. Furthermore, the suppressive effect of IVIg disappeared when macrophages were depleted. These findings indicate two novel independent possibilities of alleviating CIPN using IVIg. First, CIPN can be controlled by macrophage modulation. Second, IVIg directly combined with anti-cancer drugs can avoid restrictions on the use of anti-cancer drugs due to CIPN inductions. However, further research is necessary for the bench-to-bedside translation of these novel applications of IVIg. Our findings lay a strong foundation for research on IVIg therapeutics.

Introduction

Patients with anticancer chemotherapy-induced peripheral neurotoxicity (CIPN) experience compromised daily life activities and lowered quality of life post treatment ¹⁻³. Consequently, treatment must be interrupted even if a patient is responding well to a drug. This causes undue psychological stress in patients who must deal with CIPN-related symptoms and effective drug discontinuation. Although some drugs have been approved to treat CIPN (such as duloxetine, pregabalin, and tricyclic antidepressants), there is no drug that shows a remarkable effect. Moreover, the pathogenesis of CIPN remains unclear ⁴.

Intravenous immunoglobulin (IVIg) was developed as a drug for patients with antibody deficiency ⁵; however, currently, IVIg is also used to treat many autoimmune disorders, such as Kawasaki disease and idiopathic immune thrombocytopenic purpura ^{6,7}. The proposed mechanisms underlying IVIg action in autoimmune diseases include the modulation of Fc receptors, interference with the cytokine network and complement proteins, provision of anti-idiotypic antibodies, suppression of lymphocyte effector function ^{6,8,9}, and presentation of regulatory T cell-inducing epitope termed Tregitope ¹⁰. Additionally, sialylated immunoglobulin G (IgG) contained in IVIg indirectly upregulates the inhibitory IgG Fc receptor FcγRIIB on effector macrophages ⁷. Although IVIg has several functions and is effective against many diseases, its precise action mechanism remains unclear.

Recently, Meregalli et al. reported that IVIg exerts suppressive effects on bortezomib-induced CIPN in rat models ¹¹. Additionally, they observed that IVIg reduced the infiltration of M1 macrophages into the peripheral nerves. To the best of our knowledge, their study is the first to report the effectiveness of IVIg in alleviating CIPN. However, IVIg is generally not effective for CIPN, because CIPN can also be caused by anticancer drugs other than bortezomib. Recently, our group focused on the multi-functional effects of IVIg and studied the mechanisms underlying the effects under various conditions such as infectious diseases ¹²⁻¹⁵, recurrent pregnancy loss ¹⁶, and neurological disease ^{17,18}. In the present study, we

focused on whether the suppressive effects of IVIg can be extended to peripheral neurotoxicity induced by other chemotherapy drugs and whether the reduced infiltration of macrophages observed in response to IVIg administration is involved in alleviating CIPN. Therefore, we aimed to clarify the effects of IVIg on peripheral neurotoxicity induced by chemotherapeutics and studied the role of macrophages in IVIg-mediated suppression of CIPN.

Results

IVIg suppresses peripheral neurotoxicity induced by chemotherapeutics other than bortezomib

We examined the effects of IVIg on peripheral neurotoxicity induced by drugs other than bortezomib using rodents. We studied the effect of IVIg on paclitaxel-induced peripheral neurotoxicity (PIPN) and doxorubicin-induced peripheral neurotoxicity (DIPN) in rats. Our results showed that IVIg alleviated mechanical allodynia in PIPN (Fig. 1a) and DIPN (Fig. 1b) rats. Additionally, in the mouse PIPN model, IVIg alleviated mechanical allodynia (Fig. 2a) and heat hyperalgesia (Fig. 2b).

Role of macrophages in IVIg action to alleviate CIPN

To clarify the role of macrophages in the action of IVIg, we investigated the influence of macrophage depletion on the suppressive effects of IVIg in the rat PIPN model. Interestingly, IVIg failed to suppress PIPN in the rat model when macrophages were depleted (Fig. 3).

Discussion

Here, to the best of our knowledge, we first established that IVIg exerts suppressive effects on both PIPN and DIPN. Briefly, we have shown that IVIg suppresses PIPN and DIPN via macrophage modulation using rats or mice. Meregalli et al. reported that IVIg exerts a similar effect on bortezomib-induced peripheral neurotoxicity¹¹. These results indicate that IVIg could have a suppressive effect on peripheral neurotoxicity caused by anticancer drugs (regardless of drug types, such as platinum compounds, antitubulins, thalidomide, and bortezomib)^{1,2}. However, the mechanism of CIPN induction varies with the anticancer drug. Thus, IVIg does not directly interfere with anticancer drugs and may act on a factor, which is common in the CIPN pathway.

If IVIg can be proven to suppress CIPN, it can be used in combination with anticancer drugs that are effective in patients with CIPN. As IVIg does not cause serious adverse effects and is a multifunctional drug, combination therapy is a rational strategy. Moreover, IVIg is a polyclonal preparation comprising antibodies pooled and concentrated from the plasma of thousands of healthy individuals. Thus, they are highly effective as an immune replacement therapy in immunocompromised patients with cancer receiving chemotherapy. Therefore, IVIg administration could prove beneficial owing its dual roles of suppressing CIPN and alleviating immune deficiency caused by chemotherapy.

Notably, our results showed that in the absence of macrophages, IVIg loses its CIPN-suppressive effect. Macrophages are the key coordinators of the immune system. Furthermore, IVIg can normalize immune system-related abnormalities. Thus, we speculate that the immunomodulatory effects of IVIg are mediated by macrophages. Although the development of PIPN after macrophage depletion remains unclear, it is possible that in our study, clodronate failed to completely deplete the macrophages in the peripheral neurons; therefore, further exploratory investigations are required. Moreover, the subset of macrophages essential for IVIg action may differ from those required for the onset of PIPN. Nevertheless, based on our results, we can conclude that macrophages are essential for IVIg action.

Our study outcomes emphasize a strong relationship between IVIg action and macrophages. Interestingly, we also confirmed the same phenomenon in a model of recurrent miscarriage, a condition completely different from CIPN. In a model of recurrent miscarriage¹⁶ depleting macrophages eliminated the effect of IVIg (Supplementary Fig. 1). Thus, the action of IVIg may not change with the model. Moreover, in our previous study in a mouse model of chronic inflammatory demyelinating polyneuropathy, we found that IVIg can modulate macrophage levels¹⁷. IVIg reportedly exhibits immunostimulatory effects in infectious diseases and an immunosuppressive effect in autoimmune diseases. These seemingly contradictory effects can be explained by understanding how IVIg modulates macrophages, which are the key coordinators of the immune system. Thus, IVIg may act by normalizing the immune system via macrophage modulation. Thus, our study provides new insights to elucidate the mechanisms underlying the dual roles of IVIg.

Here, we demonstrated two distinct and important possibilities of therapeutic application of IVIg suppression in CIPN. First, we found that IVIg acts on macrophages to normalize immunity. Thus, our study further validates that macrophages could be a new target for the development of novel multifunctional drugs. Second, our results showed that a combination therapy may be developed using IVIg to treat cancer and reduce adverse effects. To date, all studies in this field have focused on the direct effects of drugs on cancer. The range of cancer therapy will expand in the future if IVIg is found useful in combination therapy with drugs that are effective in combating cancer. We expect to examine this possibility in the future to expedite therapeutic strategies for cancer patients.

Finally, our data will help to elucidate the action mechanism of IVIg, and its future therapeutic applications. Moreover, to our knowledge, the current therapeutic guidelines for CIPN do not include IVIg therapy. In the present study, we conducted experiments only on rodents, but further studies in other animals are needed to verify the effectiveness of IVIg therapy in alleviating CIPN, and our study emphasizes this urgent need.

Materials And Methods

Laboratory animals and code of ethics

CrI:CD (SD) male rats (7 weeks) and CrI:CD1 (ICR) male mice (6 weeks) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). The rats were maintained under specific pathogen-free conditions and a 12-h light/dark cycle; they were used in experiments after 7 weeks of age. All animal procedures were approved by the Animal Care and Use Committee of Japan Blood Products organization. The study was performed in accordance with the guidelines of the Animal Care and Use Committee of Japan Blood Products Organization, which conforms to the policies of the Japan Health Sciences Foundation (The business of the "Japan Health Sciences Foundation" was taken over by the "Japan Pharmaceutical Information Center (<https://www.japic.or.jp/calac/english.html>)" on 2021 / mar / 31.). This study is reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org>).

Induction of CIPN model

Paclitaxel (Bristol-Myers Squibb, Princeton, NJ USA) was intraperitoneally injected into rats (1 g/kg on days 0, 1, 2, 3, 4, 7, 8, 9, 10, 11, 14, and 15) or mice (3 mg/kg on days 0, 1, 2, 3, and 4) to induce PPIN. Paclitaxel was dissolved in 8.3% Cremophor EL (Nacalai Tesque, Kyoto, Japan), 8.3% ethanol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), and 83.4% sterile saline (Otsuka Pharmaceutical Factory, Tokushima, Japan).

Doxorubicin (Nippon Kayaku, Tokyo, Japan), an anticancer drug, was intravenously injected into rats (6 mg/kg on day 0). Control group animals were administered the same amount of the solvent or saline used to dissolve the drugs.

Administration of IVIg

IVIg (Japan Blood Products Organization, Tokyo, Japan) was intravenously administered on days 0, 7, 14, and 21 at 1 g/kg to the rats treated with paclitaxel or doxorubicin and on days 0 and 5 at 1 g/kg to mice treated with paclitaxel. Control group animals were administered the same amount of saline.

Depletion of macrophage

To deplete macrophages, clodronate liposome (Hygieia Bioscience, Osaka, Japan) was intravenously injected on days 2, 5, 12, and 19 at 5.6 mg/body.

Mechanical allodynia and heat hyperalgesia

Neuropathic pain was evaluated using the dynamic test in accordance with a previously published method^{19,20}. Animals were acclimated to the corresponding behavioral test environments, and baseline responses were measured before anticancer drug administration. The occurrence of neuropathic signs was monitored after 3, 4, and 5 weeks (rat) or 1 and 2 weeks (mouse) of anticancer drug injection.

Mechanical allodynia was assessed using a 26-g Semmes-Weinstein Von Frey Anesthesiometer (Tactile Test; Muromachi Kikai, Tokyo, Japan). Briefly, the animals were placed in a compartment with a wire mesh bottom, and later, a Semmes-Weinstein monofilament was applied to the plantar surface of their hind paws with a progressive increase in puncture pressure. The sensory threshold was recorded, and the

mechanical sensitivity was determined by calculating the mean value of 10 repeated applications per footpad. Mechanical measurements were assessed via verification by two experimenters.

Heat hyperalgesia was measured as the latency to express pain-related behavior using a hot plate (54.5°C). The following behaviors were observed: 1) licking of foot, 2) fluttering of foot, 3) raising of foot, 4) jumping, and the latency until one of them was expressed (latency to paw withdrawal) was measured for individual animals.

Statistical Analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM). The results are representative of two or more independent experiments. Means were compared using Student's *t*-test. First, the control group and the anticancer drug-administered group were tested. After confirming that the model was established, the anticancer drug-administered group and the IVIg-administered group were tested. Statistical analyses were performed using SAS9.3 software (SAS Institute Inc, NC, USA), and results with $P < 0.05$ (two-tailed) indicated significance.

Declarations

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Author contributions

MK conceived and designed the study and collated and analyzed the data. JT interpreted the data, directed the research, and wrote the manuscript draft.

Competing interest statement:

The authors declare no competing interests.

Data availability:

Authors provide adequate assurances that they can comply with the publication's requirements for sharing materials.

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Figures

Figure 1a

b

Figure 1

IVIg suppresses peripheral neurotoxicity induced by antineoplastic drugs other than bortezomib in rat models Representative results of the mechanical allodynia monitoring experiments to evaluate neuropathic signs after 3, 4, and 5 weeks of anticancer drug injection are shown. (a) Rats were intraperitoneally injected with paclitaxel (1 g/kg) to develop the PIPN model. (b) Rats were intravenously injected with doxorubicin (6 mg/kg) to develop the DIPN model; IVIg (1 g/kg) was intravenously administered on days 0, 7, 14, and 21 [data are presented as mean $\pm$ SEM {n = 10 (control group) or 15 (DIPN and PIPN groups)}, ##P < 0.01 (vs. same time vehicle group by Student's t-test), *P < 0.05 and **P < 0.01 (vs. same time saline group by Student's t-test), n.s. = not significant]

Figure 2a

b

Figure 2

IVIg suppresses PIPN in mice Representative results of the experiments to monitor the occurrence of neuropathic signs—mechanical allodynia (a) and heat hyperalgesia (b)—after 7, 10, and 14 d of anticancer drug injection are shown; IVIg (1 g/kg) was intravenously administered on days 0 and 5. Mice were intraperitoneally administered paclitaxel (3 mg/kg) to induce PIPN [data are presented as mean $\pm$ SEM (n = 15), #P < 0.05 and ##P < 0.01 (vs. same time vehicle group by Student's t-test), *P < 0.05 and **P < 0.01 (vs. same time saline group by Student's t-test), n.s. = not significant. Results are representative of two or more independent experiments]

Figure 3

Figure 3

Suppressive effect of IVIg disappeared following macrophage depletion Representative results of the experiments to monitor the occurrence of neuropathic signs of mechanical allodynia after 3, 4, and 5 weeks of anticancer drug injection are shown; IVIg (1 g/kg) was intraperitoneally administered on days 0, 7, 14, and 21; clodronate liposomes (5.6 mg/body) were intraperitoneally administered 2 d before the administration of IVIg. Rats were intraperitoneally injected with paclitaxel (1 g/kg) to induce PIPN [data are presented as mean $\pm$ SEM {n = 5 (control group) or 8 (non-control groups)}, ##P < 0.01 (vs. same time vehicle group by Student's t-test), *P < 0.05 (vs. same time saline group by Student's t-test), \$P < 0.05 (vs. same time saline vehicle by Student's t-test), n.s. = not significant. Results are representative of two or more independent experiments]

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