ORIGINAL ARTICLE

Arch. Immunol. Ther. Exp., 2006, **54**, 51–53
PL ISSN 0004-069X

DOI 10.1007/s00005-006-0005-3

Therapeutic effects of cisplatin on rat experimental autoimmune encephalomyelitis

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Received: 2005.02.23, **Accepted:** 2005.08.10, **Published online first:** 2006.02.13

Abstract

Introduction: Experimental autoimmune encephalomyelitis (EAE) is a prototypic Th1-mediated autoimmune inflammatory disease of the central nervous system (CNS), and serves as a model for the human demyelinating disease, multiple sclerosis. Cisplatin is a drug widely used in the treatment of a variety of human neoplasias, such as advanced bladder carcinoma, adrenal cortex carcinoma, breast cancer, head and neck or lung carcinoma. Cisplatin binds to DNA and interferes with cellular repair and other mechanism, which eventually result into cell death. It is known that cisplatin can induce immunosuppressive effects through inhibition of T cell activity. Therefore we analyzed the anti-inflammatory effects of cisplatin in a rat EAE model.

Materials and Methods: EAE was induced in male LEW rats by immunizing with a synthetic peptide of guinea pig myelin basic protein. The development of EAE and neurological signs were evaluated by a standard protocol. Immunohistochemistry was applied to show immune cell infiltration into the CNS.

Results: Early treatment of EAE rats with cisplatin effectively ameliorated the development of disease and provided a significant protective effect compared to control rats. Further, histological analysis demonstrated that the formation of the typical perivascular cuffs and brain infiltration of monocytes and lymphocytes were complete absent in cisplatin treated rats, while abundant T cell infiltration was seen in the CNS of EAE rats.

Conclusions: Our data show that cisplatin has protective effects in EAE, indicating that cisplatin could be a candidate in the treatment of human CNS autoimmunity.

Key words: experimental allergic encephalomyelitis, rat, cisplatin, suppression.

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INTRODUCTION

Cisplatin (cis-DDP, *cis*-diamminedichloroplatinum (II)) is one of the most effective and widely used antineoplastic drugs and has been in clinical use for more than 20 years. It has shown activity against a broad spectrum of tumors, especially in the treatment of testicular and ovarian carcinoma [1]. Cisplatin generates its antitumor properties by induction of programmed cell death, or apoptosis, predominantly by forming intra- and interstrand DNA and DNA-protein cross-links [2, 3, 8]. The aim of this study was to elucidate the immunosuppressive potential of cisplatin in autoimmunity using a rat model of acute experimental autoimmune encephalomyelitis (EAE).

MATERIALS AND METHODS

Male LEW rats (200–250 g) were immunized with a synthetic peptide of guinea pig myelin basic protein (gpMBP) as described [6]. The rats were scored daily for development of EAE and neurological signs were scored as follows: 0 – no clinical signs, 1 – loss of tail tone (flaccid tail), 2 – tail weakness plus hind-limb paresis (ataxia), 3 – moderate hind-limb paralysis, 4 – tetraparesis, and 5 – moribund. The rats were randomly assigned to treatment groups (24 rats/group) and received injections of 1 ml of saline (0.9 w/v of NaCl) or 1 ml of cisplatin solution (10 mg/kg/rat) on day 9 and day 11. Three rats from each group were sacrificed on days 13 and 16 after immunization for histological

analysis. The animals were deeply anesthetized with diethylether and perfused intracardially with 4% paraformaldehyde (PFA) in 100 mM phosphate-buffered saline (0.2 M NaCl, 0.0056 M NaH_2PO_4, 0.0144 M Na_2HPO_4, pH 7.4). The spinal cords and brains were removed and post-fixed in 4% PFA overnight at 4°C.

The spinal cords were divided into 8-mm segments and embedded in paraffin and serially sectioned (2–5 μm) and mounted sequentially on silan-covered slides. Representative sections were stained by hematoxylin and eosin. Spinal cord and brain tissue sections for immunohistochemistry were boiled (in a 600 W microwave oven) for 15 min in citrate buffer (2.1 g sodium citrate/l, pH6). Endogenous peroxidase was inhibited by 1% H_2O_2 in methanol for 15 min. Slices were incubated with 10% normal pig serum (Biochrom, Berlin, Germany) to block non-specific binding of immunoglobulins and then exposed to the mouse monoclonal antibodies ED1 (1:100; Serotec, Oxford, Great Britain) and endothelial-monocyte-activating polypeptide II (EMAP II) (1:100; BMA, Augst, Switzerland) to stage activation of monocytes/microglial cells [6], and W3/13 (1:100; Serotec, Oxford, Great Britain) against the pan-T cell marker CD43. Antibody binding to rat tissue was visualized with a biotinylated rabbit anti-mouse IgG $F(ab)_2$ antibody fragment (Dako, Hamburg, Germany). To visualize bound antibody complexes, sections were incubated with a Streptavidin-Avidin-Biotin complex (Dako, Hamburg, Germany), followed by development with diaminobenzidine substrate (Fluka, Neu-Ulm, Germany). Finally, sections were counterstained by hematoxylin. Negative controls consisted of sections incubated in the absence of the primary antibody.

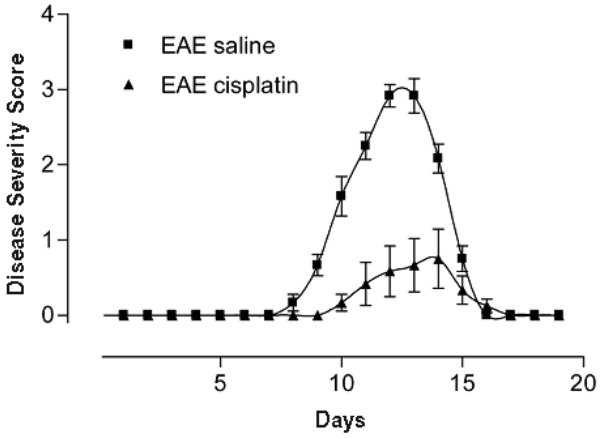

Fig. 1. Disease Severity Scores of EAE. Rats were immunized with a synthetic peptide of gpMBP and received injections of saline (■) or 10 mg/kg/rat of cisplatin (▲) at day 9 and day 11. Data points represent means ±SEM (n=12/group, randomly chosen rats for histology were not represented to avoid missing data).

RESULTS

In this study, cisplatin was used early after the onset of clinical signs of EAE. The first signs of neurological disease (weakness of the tail tip) could be observed in some rats already on day 9 after immunization, and cisplatin was injected the same day. A second injection on day 11 was necessary to drastically ameliorate the development of neurological disease (Fig. 1). All rats recovered from disease by day 16. The rats were observed for up to two months, but relapse of disease was not observed. As treatment with cisplatin provided a significant protection against the development of severe neu-

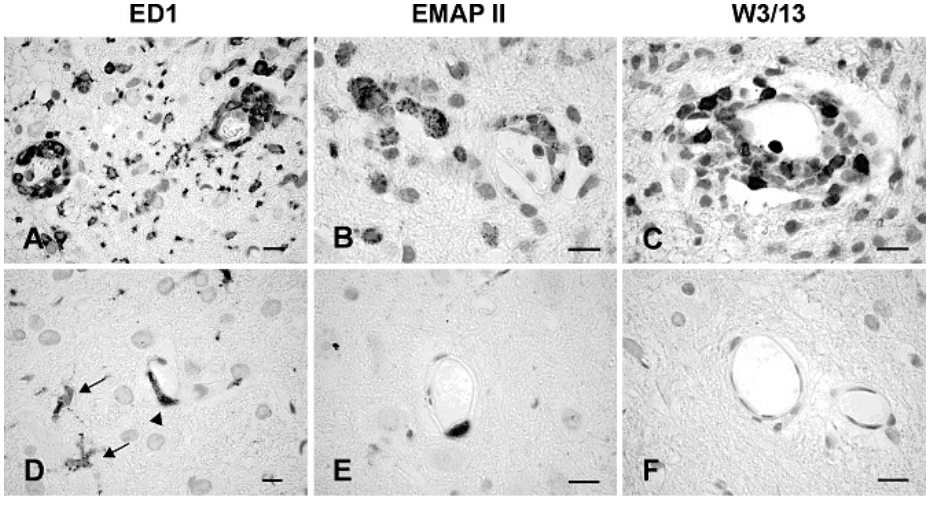

Fig. 2. Immunoreactivity in the spinal cord. In untreated EAE rats, typical inflammatory autoimmune lesions could be observed. ED1 (**A**) and EMAP II (**B**) staining of macrophages/microglia was seen within and surrounding inflammatory infiltrates. Inflammatory lesions of control EAE rats were further characterized by the presence of perivascular cuffs of W3/13$^+$ T lymphocytes (**C**). After treatment with cisplatin, no inflammatory infiltrates could be observed. The number of ED1$^+$ cells was significantly decreased and was restricted to perivascular cells (arrowhead) and some parenchymal microglial cells (arrows) showing a ramified morphology with elongated nuclei (**D**). Expression of EMAP II remained restricted to certain pervascular cells (**E**), and W3/13 immunoreactivity could not be observed in the CNS of cisplatin-treated EAE animals (**F**). Scale bars = 10 μm.

rological disease, we analyzed the brains and spinal cords of rats for inflammatory signs. A total absence of perivascular cuffs in the brains and spinal cords was seen in hematoxylin/eosin-stained sections of cisplatin-treated rats. Tissue sections were immunostained for ED1 and EMAP II to detect macrophage/microglial activation. In the spinal cords and brains of saline-treated control EAE rats (day 13, day 16), strongly stained $ED1^+$ monocytes were found in the meninges, the perivascular space of blood vessels, and in the parenchyma. $ED1^+$ mononuclear infiltrates consisted of round-shaped macrophages/microglial cells with round nuclei (see also ref. [6]; Fig. 2A). In contrast, in the spinal cords and brains of cisplatin-treated EAE rats only single, typical $ED1^+$ pervascular cells and some ramified microglia of the normal central nervous system (CNS; Fig. 2D) were seen. Further, massive immunostaining of EMAP II, a chemotactic cytokine associated with monocyte/ /microglia activation in autoimmune inflammation of the CNS [6], was seen in EAE, but not in control rats (Fig. 2E). Activated EMAP II^+ microglial cells were not seen in the brains and spinal cords of cisplatin-treated rats, but were abundant in control EAE rats (Fig. 2B). In accordance with the findings of monocytic cell activation and infiltration, no $W3/13^+$ T lymphocyte infiltration was seen in cisplatin-treated rats, while abundant $W3/13^+$ cells were seen in the perivascular cuffs of EAE control rats (Fig. 2C, F).

CONCLUSION

Cisplatin is widely prescribed against a variety of tumors, including advanced bladder carcinoma, adrenal cortex carcinoma, breast cancer, head and neck carcinoma, and lung carcinoma. Further, cisplatin is known to exert immunosuppressive effects, predominantly by inhibition of T cell activity [4, 5, 7]. Only a few studies have been concerned with the impact of cisplatin treatment on the components of the immune system, but it might have potential in the treatment of inflammatory autoimmune disease. In rat EAE we could show that cisplatin is capable of suppressing the development of neurological signs and formation of the typical inflammatory lesions. Thus, cisplatin might be considered as a candidate drug in the control of inflammatory and autoimmune processes.

Acknowledgment: This work was supported by a grant of the Deutsche Forschungsgemeinschaft (DFG).

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