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Electrochemotherapy with CDDP on LPB sarcoma: comparison of the anti-tumor effectiveness in immunocompetent and immunodeficient mice

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Abstract

Electrochemotherapy combines chemotherapy with electric pulses in order to potentiate anti-tumor effectiveness of chemotherapeutic drugs. Electrochemotherapy with drugs that normally do not accumulate in the tumor cells in sufficient quantities, such as bleomycin and, to some extent, *cis*-platin, has already been well elaborated. For complete tumor eradication, the immune system of the organism is essential. In this study, LPB sarcoma was treated in immunocompetent C57Bl/6 and immunodeficient nude mice, with the aim to determine the differences in anti-tumor effectiveness of electrochemotherapy with *cis*-platin. Differences observed in anti-tumor effectiveness were evaluated by tumor growth delay and the percentage of tumor cures obtained. The tumor growth delay in immunocompetent mice was approximately twice longer than in immunodeficient mice. Furthermore, a high percentage of tumor cures was achieved in immunocompetent mice, but none in immunodeficient mice. The results of our study clearly demonstrate that the host immune system is essential for obtaining cures after electrochemotherapy with *cis*-platin. © 1997 Elsevier Science S.A.

Keywords: Electrochemotherapy; CDDP; Immunocompetent mice; Immunodeficient mice

1. Introduction

Electrochemotherapy is an antitumor treatment that utilizes electric pulses to increase drug delivery into the tumors [1]. The application of short intense electric pulses to the tumor results in tumor cell electropermeabilization and therefore in the increased internalization of chemotherapeutic drugs, that otherwise do not freely diffuse into the cells. Consequently, a potentiation of anti-tumor effects of such drugs is easily obtained. Electrochemotherapy with chemotherapeutic drugs bleomycin and *cis*-platin (CDDP), has already been elaborated in preclinical studies [2–6] and some clinical trials have been performed [7–11]. The results demonstrated that electrochemotherapy with bleomycin and CDDP offers a promising approach to the treatment of cutaneous and subcutaneous tumor lesions.

It is known that the immune system of the organism is involved in the defense against tumor, thus contributing to the anti-tumor effectiveness of cytotoxic therapies by eradicating the remaining viable tumor cells. The role of the immune response in the anti-tumor effectiveness of electrochemotherapy with bleomycin has already been established [2,12,13]. However, it is not known to what extent immune response is involved in the anti-tumor effectiveness of electrochemotherapy with CDDP. Therefore, the aim of this study was to determine the differences in anti-tumor effectiveness of electrochemotherapy with CDDP on LPB sarcoma in immunocompetent and immunodeficient mice.

2. Materials and methods

2.1. Animals and tumors

Inbred female immunocompetent C57Bl/6 and immunodeficient Swiss *nu/nu* mice (nude) maintained at the Institut Gustave Roussy were used in the experiments. C57Bl/6 mice were kept in a conventional animal colony whereas nude mice were kept in a specific pathogen-free colony. Subcutaneous tumors in the animals were obtained by inoculation of LPB tumor cells. The highly tumorigenic

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murine LPB cell line is a clonal derivative of TBL.Cl2, a methylcholanthrene-induced C57Bl/6 mouse sarcoma cell line [14]. The cells were routinely maintained in vitro in Eagle minimal essential medium (EMEM) (Sigma, USA) supplemented with 8% fetal calf serum (Sigma) and antibiotics. Solid subcutaneous tumors, located dorsolaterally in mice, were initiated by injection of 8×10^5 cells into C57Bl/6 mice and 10×10^5 cells into nude mice. When the tumors reached approximately 25 mm^3 in volume, the mice were marked individually, divided randomly into experimental groups consisting of 5–9 mice, and subjected to a specific experimental protocol on day 0. The experiments were repeated twice.

2.2. Electrochemotherapy

cis-Diamminedichloroplatinum(II) (CDDP) (Cisplatyl, Bellon Rhone–Poulenc Rorer, France) was dissolved in sterile H_20 to a concentration of 1 mg ml^{-1}. The final concentration was prepared in physiological saline. Fresh solutions were prepared for each experiment. CDDP was injected intravenously in bolus into the retroorbital sinus of the mice. The CDDP treatment (1, 4, 6 and 8 mg kg^{-1}) was well tolerated by the mice.

Electric pulses were delivered by two parallel stainless steel plate electrodes 6 mm apart (two stainless steel strips with rounded tips) placed at the opposite margins of the tumor. Electrocardiographic paste was used to assure good contact between the electrodes and the skin. Eight square-wave high-voltage dc pulses (amplitude 780 V, pulse width 100 μs, repetition frequency 1 Hz) were generated by an electropulsator Jouan GHT 1287 (Jouan, France). The treatment with electric pulses was performed without anesthesia and was well tolerated by the animals.

In the electrochemotherapy protocol mice were treated with electric pulses 3 min after a CDDP injection.

2.3. Response evaluation and statistical analysis

Tumor growth was followed by measuring three mutually orthogonal tumor diameters with a caliper. From the measured tumor diameters, tumor volumes as well as tumor doubling times (DT) and tumor growth delays were calculated [6]. The data were expressed as arithmetic mean (AM) and standard error of the mean (SE). In the tumor growth curves and specific tumor growth delay curves, only mice with tumor recurrence after the treatment were included. The mice which were tumor free 100 days after the treatment were considered cured and were excluded from tumor growth curves and specific tumor growth delay curves.

In order to compare anti-tumor effectiveness of electrochemotherapy in the two strains of mice where the LPB tumors grew with different growth rate, specific growth delay (SGD) of the tumors was calculated. SGD more closely reflects the amount of cell killing after the therapy in tumors with different growth rate, providing more accurate comparison of anti-tumor effectiveness of electrochemotherapy on C57Bl/6 and nude mice. SGD was calculated by subtracting DT of the control group from the DT of the treated group and dividing it by the DT of the control group (growth delay/DT of the control group), thus normalizing tumor growth rate [15].

The significance of the differences between the mean values of the DT, growth delay and SGD of the experimental groups was evaluated by Newman–Keuls test after one way analysis of variance was performed and fulfilled.

3. Results

To determine the differences in response to electrochemotherapy immunocompetent C57Bl/6 and immunodeficient nude mice bearing LPB sarcoma were treated with the same treatment protocol, as described in materials and methods. Anti-tumor effectiveness was evaluated by tumor growth delay and curability of tumors in mice. Anti-tumor effectiveness of electrochemotherapy with CDDP was demonstrated in both immunocompetent and immunodeficient mice. (Fig. 1, Table 1). Treatment with electric pulses alone or CDDP alone, given in different doses, had moderate anti-tumor effect, but when both treatments were combined their effect was more than additive. However, this more than additive effect was observed only when the CDDP dose used for electrochemotherapy exceeded the 1 mg kg^{-1} dose. Treatment of tumors with electrochemotherapy using CDDP doses of 4 mg kg^{-1} induced 15.0 ± 3.0 days tumor growth delay in C57Bl/6 mice and 5.3 ± 0.4 days in nude mice. When escalating the CDDP dose used for electrochemotherapy up to 8 mg kg^{-1} even better anti-tumor effects were observed, tumor growth delay being 31.0 ± 4.0 days in C57Bl/6 and 13.1 ± 0.6 days in nude mice (Fig. 1). This displacement of tumor growth curves also reveals the differences in anti-tumor effectiveness of electrochemotherapy in the two strains of mice: anti-tumor effectiveness was much more pronounced in immunocompetent than in immunodeficient mice.

Due to differences in the growth rate of untreated LPB tumors in C57Bl/6 and nude mice (Table 1), specific tumor growth delay (SGD) was calculated. Treatment with CDDP given alone had almost the same anti-tumor effectiveness in both strains of mice (Fig. 2). Treatment with electric pulses alone had 0.1 SGD in C57Bl/6 mice and 0.2 SGD in nude mice. Also, CDDP treatment in the range of all the doses tested had no dose dependent effect. In contrast, electrochemotherapy had pronounced anti-tumor effect on both strains of mice, but with great differences between both strains. To determine the difference in tumor response to electrochemotherapy, SGD regression lines were constructed. The regression lines for SGD in immunocompetent C57Bl/6 and immunodeficient nude mice

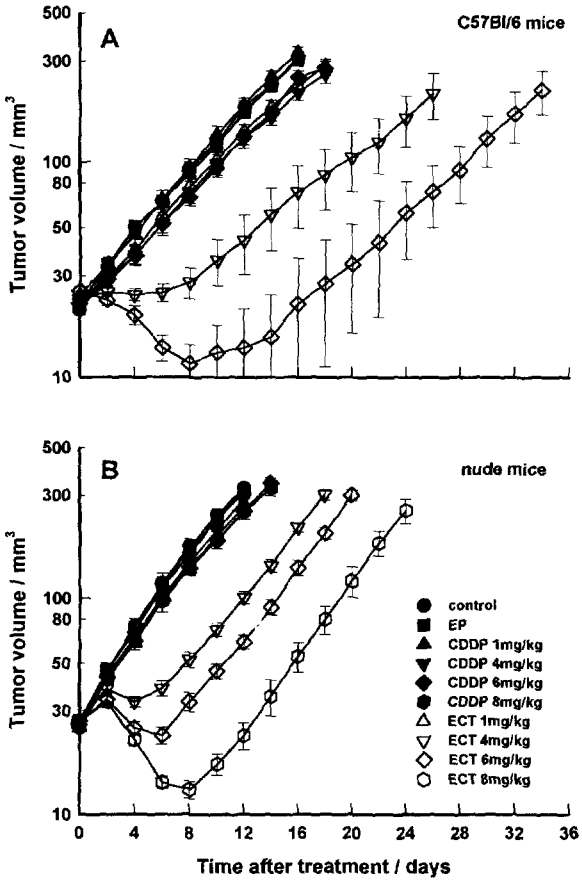

Fig. 1. The anti-tumor effectiveness of eletrochemotherapy with CDDP in LPB sarcoma in immunocompetent C57Bl/6 (A) and immunodeficient nude mice (B). Mice were treated with different CDDP doses intravenously and 3 min thereafter tumors were exposed to electric pulses (EP). Tumor growth curves represent the AM ± SE of the tumor volumes measured every second day. Included were only the animals in which tumors regrew; those that were tumor-free 100 days after the treatment were excluded. Due to the small number (3) of tumors that regrew in C57Bl/6 mice treated with 8 mg kg^{-1} electrochemotherapy, this growth curve is not shown.

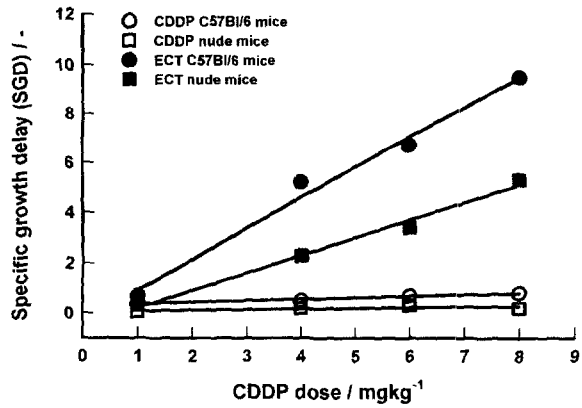

Fig. 2. Specific growth delay (SGD) of the LPB sarcoma tumors treated with electrochemotherapy in immunocompetent C57Bl/6 and immunodeficient nude mice. Mice were treated with different CDDP doses intravenously and 3 min thereafter tumors were exposed to electric pulses (EP). SGD was calculated by subtracting tumor doubling time of the control group from the tumor doubling time of the treated group and dividing it by tumor doubling time of the control group [15]. SGD of the tumors treated with electric pulses was 0.1 and 0.2 in C57Bl/6 and nude mice, respectively. Included were only the animals in which tumors regrew; those that were tumor-free 100 days after the treatment were excluded.

were 1.22 × CDDP dose−0.29 and 0.70 × CDDP dose−0.48, respectively. The ratio obtained in response to electrochemotherapy treatment was approximately two. Thus, for the same anti-tumor effectiveness of electrochemotherapy approximately a twice higher CDDP dose was needed in immunodeficient compared to immunocompetent mice. Probably this factor indicates the contribution of the immune system to the anti-tumor effectiveness of electrochemotherapy in immunocompetent mice.

The difference in response to electrochemotherapy was also determined by comparing the induction of long term complete responses (cures) in immunocompetent and im-

Table 1
Anti-tumor effectiveness of electrochemotherapy (ECT) with CDDP in LPB sarcoma in mice. Comparison of the effects on immunocompetent (C57Bl/6) and immunodeficient (nude) mice

Experimental groups	C57BL/6 mice			Nude mice		
	n	DT [a]	Cures (%; *n*) [b]	*n*	DT [a]	Cures (%; *n*) [b]
Control	15	3.2 ± 0.4	0	17	2.5 ± 0.2	0
EP 8 pulses	11	3.5 ± 0.3	0	12	3.0 ± 0.3	0
CDDP 1 mg kg^{-1}	12	4.6 ± 0.5	0	12	2.6 ± 0.3	0
CDDP 4 mg kg^{-1}	12	5.3 ± 0.6	0	13	3.0 ± 0.2	0
CDDP 6 mg kg^{-1}	12	5.4 ± 0.6	0	13	3.2 ± 0.3	0
CDDP 8 mg kg^{-1}	12	5.7 ± 0.4	0	13	3.1 ± 0.3	0
ECT [c] 1 mg kg^{-1}	18	5.5 ± 0.5	0	15	3.3 ± 0.3	0
ECT 4 mg kg^{-1}	18	19.7 ± 3.3	29; 5	16	8.3 ± 0.4	0
ECT 6 mg kg^{-1}	18	24.6 ± 2.5	56; 10	17	10.9 ± 0.5	0
ECT 8 mg kg^{-1}	17	33.5 ± 4.0	82; 14	18	15.7 ± 0.5	0

[a] Tumor doubling time (AM ± SE).
[b] Percentage and number of complete responses 100 days after the treatment.
[c] Electrochemotherapy.

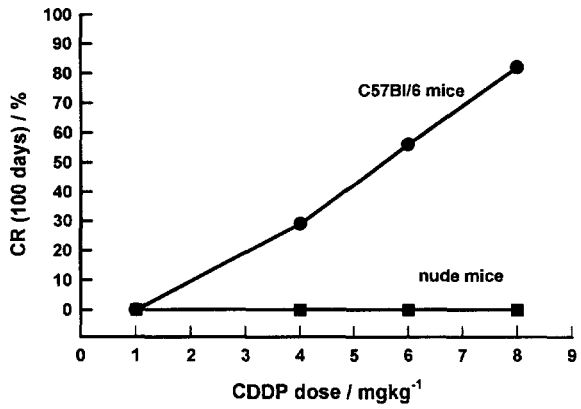

Fig. 3. Percentage of animals cured after electrochemotherapy with CDDP. Animals 100 days after the treatment that were tumor-free (complete response-CR) were considered as cured.

munodeficient mice. In immunocompetent C57Bl/6 mice, a higher percentage of cures was obtained. With the increasing CDDP doses used for electrochemotherapy, proportionally higher percentages of the tumors were cured, starting at 4 mg kg^{-1} where 29% of the tumors were cured, up to 82% cures obtained at 8 mg kg^{-1} treatment (Table 1, Fig. 3). In contrast, no cures were observed in immunodeficient nude mice regardless the CDDP dose used. Since the amount of the LPB tumor cells killed after electrochemotherapy at a particular CDDP dose should be similar in both, C57Bl/6 and nude mice, the high curability of the tumors in C57Bl/6 mice could be ascribed to the contribution of the immune system.

The C57Bl/6 mice, cured after electrochemotherapy, were challenged with the same LPB tumor cells to determine the level of specific immunity in the mice evoked by electrochemotherapy. Of the mice cured after electrochemotherapy, 75% rejected tumor challenge, whereas in the control mice 100% tumor take was observed. These results indicate that specific memory for the LPB tumor cells developed in the majority of immunocompetent C57Bl/6 mice that were cured.

4. Discussion

This study shows that a much better anti-tumor effect of electrochemotherapy with CDDP is obtained in immunocompetent than in immunodeficient mice. We found that tumor growth delay was approximately twice longer in immunocompetent mice than in immunodeficient mice, and that high curability was achieved in immunocompetent mice compared to the absence of cures in immunodeficient mice.

The data from this study are in agreement with the results obtained on electrochemotherapy with bleomycin on the same type of tumors, the LPB sarcoma. With bleomycin and electric pulses, no curability of the tumors and only partial responses were obtained in immunodeficient nude mice whereas high curability of the tumors was achieved in immunocompetent mice [2]. The results of both studies demonstrate that immune response is essential for complete eradication of all clonogenic tumor cells. With increasing drug dose less clonogenic cells remain after electrochemotherapy. The contribution of the immune response in immunocompetent C57Bl/6 mice was higher after electrochemotherapy with higher CDDP doses, as demonstrated by increasing curability rate of these tumors (Fig. 3).

From previous studies, it is known that the host's immune response involving T-lymphocytes activity is involved in obtaining cures after electrochemotherapy with bleomycin. This was demonstrated in mice by transient depletion of mature T-lymphocytes [12], and in mice treated with IL-2 after electrochemotherapy [12,13]. In addition, histological examinations proved the involvement of $CD4^+$ and $CD8^+$ lymphocytes in the host's immune response after electrochemotherapy with bleomycin [13]. The same effector cells could be involved in electrochemotherapy with CDDP.

In our study, it has been demonstrated that anti-tumor effectiveness of electrochemotherapy with CDDP is dependent on immune response of the organism. A substantial difference in anti-tumor effectiveness was demonstrated using immunocompetent and immunodeficient mice: in the former, longer tumor growth delays and a higher curability rate of the tumors were obtained.

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