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ORIGINAL ARTICLE

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Paclitaxel saves rat heart allografts from rejection by inhibition of the primed anti-donor humoral and cellular immune response: implications for transplant patients with cancer

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Abstract Paclitaxel is an anti-neoplastic drug that was recently shown also to have immunosuppressive properties in naïrat heart transplant recipients. Here, we tested whether paclitaxel could also effectively reverse an ongoing immune response in transplant recipients. We therefore used a model in which Lewis rat recipients receiving ACI rat heterotopic heart allografts were: (1) untreated, or treated with either (2) paclitaxel or (3) cyclosporine, starting 5 days after transplantation. Allograft survival was determined in one group, and in a second group cytotoxic T-lymphocyte (CTL) responses were determined and serum anti-donor cytotoxic antibody levels were measured. Results showed that paclitaxel was as effective as cyclosporine in saving recipients from imminent allograft rejection. Immunologically, paclitaxel reduced the allogeneic-CTL response, but most impressively, the cytotoxic antibody response was nearly eliminated in saved recipients. Therefore, paclitaxel's immunosuppressive properties, along with its known effectiveness against a wide variety of tumors, makes it potentially useful for the simultaneous treatment of rejection and neoplasms in cases of transplant-related cancer.

Keywords Paclitaxel · Post-transplant malignancy · Antibody response · Cytotoxic T lymphocytes · Immunosuppression · Heart transplantation

Introduction

Paclitaxel is a clinically approved anti-cancer drug that was originally derived from the Pacific yew tree (*Taxus brevifolia*) [15]. The value of paclitaxel stems from its remarkable cytotoxic activity against a wide variety of tumor cell lines, and most importantly, subsequent clinical cancer studies have clearly substantiated that this drug is effective in the treatment of various different types of cancer, including advanced cases of breast cancer, melanoma, lung cancer, Kaposi's sarcoma, and hepatocellular carcinoma [3, 4, 8, 10, 12, 19]. In a different context, we have recently reported that paclitaxel is an effective immunosuppressive agent capable of promoting heart allograft survival in naïrat recipients [20]. This dual anti-neoplastic and immunosuppressive activity could be useful in organ-transplant patients, since recipients with a previous tumor have a strikingly high recurrence rate of cancer, and since de novo cancer occurrence rates are also high [5, 9, 13]. In cases where this occurs, cancer growth can be slowed down—or even reversed—to some degree when conventional immunosuppression is reduced, but the transplanted organ is put at risk of rejection. Our goal in the present study was to analyze the immune response in paclitaxel-treated rats and to test if paclitaxel could be used to save an organ transplant in a rat recipient already primed to the donor antigens, cognizant of the fact that an anti-tumor effect of this drug could also be possible.

Materials and methods

Animals and heterotopic cardiac transplantation

Male Lewis and ACI rats were obtained from Harlan Sprague–Dawley (Borchen, Germany). We performed heterotopic ACI cardiac transplants on Lewis recipients weighing 200–275 g, using a modification of the technique originally described by Ono and Lindsey [11]. Graft rejection time was defined as the time at which no cardiac contractions were palpable, with verification of rejection by direct inspection of the allograft, via laparotomy. We used the Mantel–Cox log-rank test to compare allograft survival times, which are expressed as the mean ± standard deviation (SD).

All animal care and procedures were performed in accordance with the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23) and were approved by the regional authorities at the University of South Alabama, and according to German national animal-care regulations.

Drug preparation and administration

Paclitaxel was purchased in the form of Taxol (Bristol–Myers Squibb, Princeton, N.J., USA) through the hospital pharmacy. For our experiments, the paclitaxel was further diluted with saline to a total volume of 0.5 ml for each dose given. Recipients were injected intraperitoneally with 3.0 mg/kg per day on days 5 and 6 after transplantation and with 1.5 mg/kg per day for the next 5 days (final treatment given on day 11). We have reported the use of paclitaxel at a dose of 1.5 mg/kg per day for extended periods of time, but the dose of 3.0 mg/kg per day can show adverse side effects when given daily over a period of weeks [20]. Therefore, we gave a dose of 3.0 mg/kg per day *initially* and then reduced the daily dose to avoid side effects. For anti-neoplastic therapy, the drug is generally given as a larger bolus dose on a weekly or monthly basis, but for immunosuppressive therapy a continuous dosing regimen is more likely to be effective over time.

Cyclosporine (CyA, Sandimmune; Novartis, Basel, Switzerland) was purchased, diluted in saline, and injected intraperitoneally according to the same schedule used for paclitaxel, except that the first two doses were given at 10 mg/kg per day and the following five doses at 5 mg/kg per day.

Cytotoxic T-lymphocyte assay

On day 7 after transplantation, cervical lymph nodes were removed from Lewis rats and the lymphocytes freed with a sterile wire mesh. T cells were enriched by passage over degalan beads coated with rabbit anti-rat IgG (Cappel Laboratories, Cochranville, Pa., USA), as previously described [6]. A limiting dilution assay for anti-ACI cytotoxic T-lymphocyte (CTL) precursors was set up exactly as previously described [6]. Development of CTL in these cultures was promoted by the addition of rat IL-2 (Becton Dickinson, Heidelberg, Germany) at a final concentration of 10 U/ml in each well. Seven days after setting up the assay, we determined CTL killing against ACI, Con-A-stimulated, ^{51}Cr-labeled target cells. The anti-ACI CTL precursor frequencies were calculated by the maximum likelihood method as described by Derry and Miller [2].

Cytotoxic antibody assay

We performed a complement-mediated antibody cytotoxicity assay to assess the titer of anti-ACI antibody in Lewis serum [16]. All blood samples for serum were collected via the tail vein. In this assay, increasing dilutions of serum were pipetted into a 96-well plate with ^{51}Cr-labeled Con-A-activated ACI lymphoblasts. The mixture was incubated for 30 min at 37 °C. Following incubation, Low-Tox-H complement (Cedarlane Laboratories, Ontario, Canada) was added to each well at a final ratio of 1:24 and incubated for 1 h at 37 °C. We used Skatron filters (Sterling, Va., USA) to absorb the culture supernatants, and ^{51}Cr-release was measured with a gamma counter. We determined background ^{51}Cr release by mixing labeled lymphoblasts with normal Lewis serum plus complement. The percentage of target-cell lysis was calculated as follows: (unknown cpm–background cpm) × 100/(maximum cpm–background cpm).

Grouping of treated animals for experiments

Table 1 shows the grouping of animals as tested in the described heart transplantation, antibody assay, and CTL experiments. The animals treated in group 1 were used only for determination of ACI allograft survival. Animals in group 2 were used either for the antibody assay or for CTL determinations, but not for both. Because of sample-collection procedures, the results of allograft survival from the animals in group 2 were not added to the data in group 1.

Results

In the first group of experiments, we tested the potential of paclitaxel to save allografts in a situation where heart transplants were already undergoing rigorous immunological rejection. For these experiments ACI-to-Lewis heart transplantation was performed as usual, but immunosuppression was not initiated until day 5 after transplantation. At this time the heart beat is typically diminished, and complete rejection of non-treated controls normally occurs within 24–48 h. In this situation,

Table 1 Study design. All treated ACI heart allograft recipients received either paclitaxel or cyclosporine starting on day 5 after transplantation. Controls (*no treatment*) received no drug therapy. Note that rats that were required for the immunological assays (group 2) were used for either the antibody assay or CTL assay, but not both (*n* number of rats tested within the group)

Treatment	Group 1 ACI allograft survival (*n*)	Group 2 Total (*n*)	Antibody assay (*n*)	CTL assay (lymph node tissue) (*n*)
No treatment	6	6	3	3
Paclitaxel	5	7	4	3
Cyclosporine	5	7	4	3

Table 2 Effect of short-course, delayed, paclitaxel or cyclosporine treatment on saving of ACI heart allografts in Lewis recipients (*GST* graft survival time). Treatment with paclitaxelor CyA was initiated on day 5 after heart transplantation.There was no significant difference between the paclitaxel-treated and cyclosporine-treated groups ($P=0.13$)

Treatment	Individual GST (days)	Mean GST (days)	*P* value vs control
None (control)	6, 6, 6, 6, 7, 7	6.3±0.5	–
Paclitaxel	8, 13, 14, 14, 17	13.2±3.3	0.0014
Cyclosporine	6, 16, 17, 18, 18	15.0±5.1	0.013

doses of 3.0 mg/kg paclitaxel were administered on days 5 and 6, and a dose of 1.5 mg/kg per day for the following 5 days; similarly, CyA was given at 10 mg/kg per day on days 5 and 6 and 5 mg/kg per day during the next 5 days. The results show that paclitaxel saved four out of five animals from imminent heart allograft rejection, as did CyA treatment (Table 2). There was no significant difference in graft survival between paclitaxel and CyA treatment of ongoing rejection.

The effect of paclitaxel on the development of anti-ACI CTL in Lewis animals undergoing rejection was determined on day 7, which was 2 days after drug treatment was initiated. Results show that although the number of CTL precursors was diminished by paclitaxel treatment, a significant anti-ACI response remained after stimulation with IL-2 in the assay, compared with controls that did not receive the drug (Fig. 1).

Finally, we tested the effect of delayed paclitaxel treatment on the development of cytotoxic antibodies in the same model. Lewis recipients either received no treatment or were given paclitaxel or cyclosporine as usual, which was begun on day 5 after transplantation. Serum samples were collected for analysis on days 7 and 10 relative to organ transplantation and were subsequently tested for anti-ACI reactive antibodies, by complement-mediated antibody cytotoxicity assay. Results show that control recipients that were not receiving paclitaxel had very high cytotoxic antibody levels by the normal day of complete allograft rejection (day 7, Table 3). Cytotoxic antibody titers remained very high in these animals through day 10 after transplantation. In contrast, three of the four recipients treated with paclitaxel in this group of experiments showed either non-detectable or low levels of cytotoxic anti-ACI antibodies on days 7 and 10 after transplantation. One of the four paclitaxel-treated recipients showed a high antibody titer on day 7; however, we noted that this animal rejected its ACI allograft on day 7. Interestingly, although the cytotoxic antibody titer was high in this rat on day 7, the antibody titer did show a decrease by day 10. In contrast to paclitaxel, recipients treated with CyA had high anti-donor antibody titers similar to controls at 7 days. However, titers in the CyA-treated recipients had decreased slightly by the 10th day.

Fig. 1 Effect of delayed paclitaxel treatment on donor-reactive CTLs in Lewis recipients of ACI heart allografts. Allografts were placed in Lewis recipients, and paclitaxel treatment was initiated, as usual, on day 5 after transplantation. Cervical lymph nodes were removed on day 7 after transplantation and were tested in a limiting dilution assay for anti-ACI CTL precursors. The results given are from a representative animal either not treated with or treated with paclitaxel. The relative amount of ACI target-cell killing is shown for each of the limiting dilution cultures as well as the calculated CTL precursor frequency (*upper left corner of each graph*). Similar results were obtained in two additional animals for each group

Discussion

In the present study we show that the anti-neoplastic drug paclitaxel may have potential usefulness in a transplant situation where recipients have developed cancer under conventional immunosuppression. Normally, in this situation, conventional immunosuppression must be reduced so that tumor growth can be slowed down or reversed, although transplant rejection is risked. To save or prevent these transplants from rejection without promoting cancer progression, drugs such as paclitaxel may prove beneficial. Data from our experiments show that paclitaxel is indeed capable of preventing imminent heart allograft rejection in rats, even at a late stage of rejection.

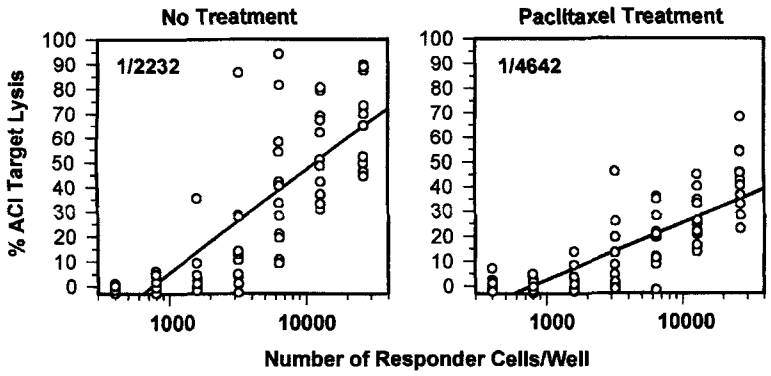

Table 3 Effect of paclitaxel and CyA on the antibody response in Lewis recipients already undergoing acute rejection of ACI heart allografts. The titer represents the first dilution of serum where killing of ^{51}Cr-labeled ACI targets did not exceed 2% above background in the complement-mediated antibody cytotoxicity assay. The serum dilutions tested in the assay were 1:10, 1:50, 1:250, and 1:1250

Treatment	Individual animals in each group	Titer	
		Day 7	Day 10
No treatment	1	1,250	1,250
	2	>1,250	>1,250
	3	>1,250	>1,250
Paclitaxel treatment (days 5–11)	1	<10	50
	2	<10	<10
	3	<10	250
	4[a]	1,250	50
Cyclosporine treatment (days 5–11)	1	>1,250	250
	2	>1,250	250
	3	>1,250	250
	4	>1,250	>1,250

[a]This recipient was not saved from allograft rejection (rejected on day 7)

Investigation of the immunological effects of paclitaxel in this clinically relevant situation revealed that the CTL response was inhibited by drug treatment, albeit to a moderate degree. However, with respect to our experiments it is important to note that the CTL limiting dilution assay is an in vitro assay that is set up in the presence of exogenously added IL-2. Therefore, it is possible that paclitaxel does effectively kill effector CTL in vivo, while relatively quiescent CTL precursors are not eliminated at this phase of the rejection response. CTL precursors that are not destroyed could then be propagated to form new effector cells when stimulated with IL-2 in our assay system. Indeed, our previous data [20] and data from others [1, 17] indicate that paclitaxel can induce apoptosis of activated, but not unactivated, T cells. This theory is consistent with previous reports, indicating that at least part of the anti-tumor paclitaxel effect is mediated by apoptotis mechanisms [14, 21]. Interestingly, apoptosis is mediated by paclitaxel via different intracellular signaling systems, including the calcineurin-NFAT/CD95 ligand [17], Bcl-2/Bax [18], and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) [22] pathways of apoptosis. Further experiments will be required if the more long-term effects of paclitaxel on CTL precursors and on effector CTL are to be studied.

In the present study, the most dramatic effect of paclitaxel was its potent inhibition of the pre-activated anti-donor humoral immune response. The development of cytotoxic antibodies in recipients being saved from rejection was either eliminated or was severely curtailed by initiation of paclitaxel treatment. Notably, this effect was likely an important component of the acute rejection response, since the one recipient not saved from normal rejection by paclitaxel showed a significant cytotoxic antibody response. However, paclitaxel did have a delayed effect on the humoral response in this same animal, as evidenced by a substantial decrease in antibody titer by day 10, despite the lack of a positive impact on graft survival in this recipient. Although the effects of paclitaxel on antibody production in an organ transplant situation had not been reported previous to our recent study [20], there is evidence of B-cell-proliferation inhibition [7] and apoptosis promotion [1] after treatment with this drug. The potency of this paclitaxel effect can be measured by the fact that it was more effective than CyA in our studies at lowering early anti-donor antibody formation. Therefore, besides the possible usefulness of paclitaxel treatment in organ transplant recipients with tumors, its effects on the humoral immune response warrants its being tested in xenogeneic models and in animal models where recipients have been sensitized over a prolonged period of time. The controlling of these strong and well-established humoral responses remains a significant problem in organ transplantation.

In summary, in rats, paclitaxel can provide a level of immunosuppressive activity to block ongoing heart allograft rejection. Furthermore, at least part of the immunosuppressive paclitaxel effect is related to potent blockage of the primed humoral anti-donor immune response. It is notable that we have recently shown that paclitaxel works synergistically with low-dose CyA to inhibit allograft rejection [20]. Therefore, the concurrent use of paclitaxel may prove to be an option in situations where CyA or other immunosuppressive treatment is likely to contribute to cancer growth in transplant recipients and must be reduced. In general, the immunosuppressive properties of paclitaxel, along with its known effectiveness against a wide variety of tumors including those typical in organ transplant patients, makes it a candidate drug for simultaneously treating rejection and neoplasms in cases of transplant-related cancer.

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