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The δ_1-opioid receptor antagonist, 7-benzylspiroindanylnaltrexone, prolongs renal allograft survival in a rat model

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Abstract

In this study we demonstrate allograft survival in a rat model of renal transplantation using the δ_1-opioid receptor antagonist, 7-benzylspiroindanylnaltrexone. Treatment with 7-benzylspiroindanylnaltrexone caused 50% of the rats to survive longer than 100 days (untreated, 11 ± 3 days). Naltrindole, a δ-opioid receptor antagonist without subtype selectivity, also promoted graft survival but was substantially less effective, suggesting that antagonism at δ_1-opioid receptors is involved in allograft survival.

Keywords: Transplantation; δ_1-Opioid receptor; 7-Benzylspiroindanylnaltrexone

The enkephalins are pentapeptide neuromodulators which are selective for opioid receptors of the δ type. Originally described in the central nervous system, they are now known to be expressed in a variety of peripheral tissues, including cells of the immune system (Stefano et al., 1996). In recent years it has been shown that the enkephalins modulate immune responses. The δ-opioid receptor antagonist, naltrindole (Sofuoglu et al., 1991), which acts at both δ_1- and δ_2-opioid receptor subtypes in the central nervous system, also blocks the function of enkephalins with respect to the immune system (Shahabi and Sharp, 1995; Linner et al., 1995, 1996). Recently, Arakawa et al. (1992) used naltrindole to prolong allograft survival in a rat renal transplant model. Transplants of both Buffalo and Brown Norway donor rat kidneys into Lewis rat recipients were significantly prolonged by in vivo administration of naltrindole. In the present study we present results obtained with the δ_1-opioid receptor antagonist, 7-benzylspiroindanylnaltrexone (Ohkawa et al., 1997) which suggest that antagonism of the δ_1-opioid receptor subtype is involved in allograft survival.

Left orthotropic kidney transplantation was performed according to standard procedures (Arakawa et al., 1992). Lewis rats received either Buffalo kidneys (experimental group) or Lewis kidneys (autologous control group). Recipients were given eight post-surgical injections of either saline or the experimental drug. One injection was given at the time of transplant, and the other seven injections were given s.c. daily for the 7 days immediately following surgery. Doses of naltrindole and 7-benzylspiroindanylnaltrexone were 0.4 mg/kg and 2 mg/kg. Controls received 0.9% saline. Rats were weighed daily and monitored for their ability to urinate and as to their general health. Surgical success was measured by the ability of the rat to urinate for the first 2 days postoperatively and was also determined at necropsy. The endpoint for these studies was survival. Maximum survival time allowed in this study was 100 days. Rats surviving 100 days were indistinguishable in appearance (e.g., size, weight, coat smoothness) and behavior (e.g., food and water intake, grooming, physical activity) from either untransplanted rats or rats receiving an isologous kidney from a Lewis donor. Toxic effects of each drug alone, of which there were none, were measured in control Lewis rats receiving a Lewis kidney.

The results of the studies using 7-benzylspiroindanylnaltrexone to prolong kidney allograft survival in rats are

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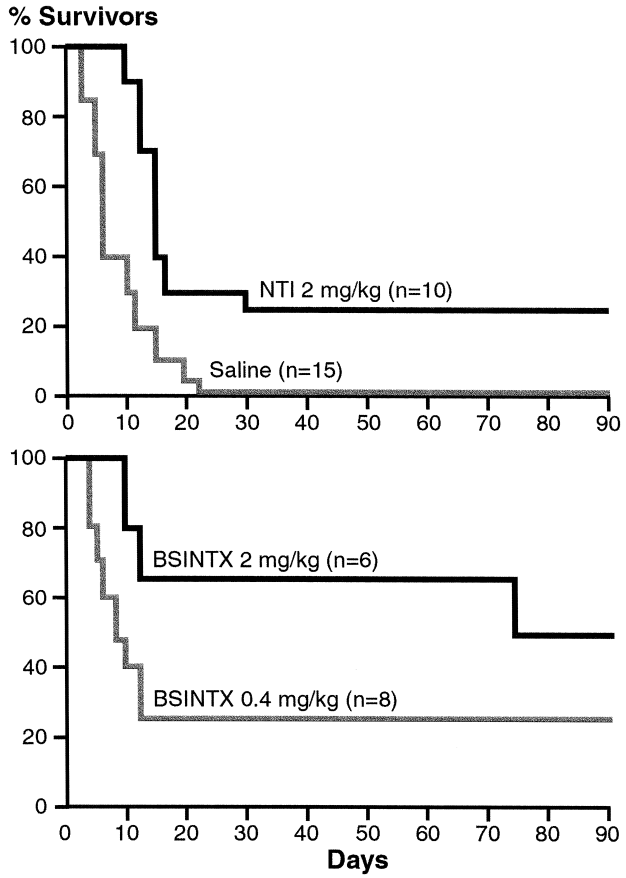

Fig. 1. Survival curves for high dose naltrindole- and low and high dose 7-benzylspiroindanylnaltrexone-treated rats. The results presented in this figure are graphed as percent survivors over the 100 days of the study. Lewis recipients received a Buffalo kidney. Each rat was treated with eight daily s.c. injections of either saline, naltrindole or 7-benzylspiroindanylnaltrexone following the transplant surgery. Naltrindole and 7-benzylspiroindanylnaltrexone were synthesized as reported (Sofuoglu et al., 1991; Ohkawa et al., 1997). Naltrindole was dissolved in water. 7-Benzylspiroindanylnaltrexone was dissolved in 20% ethanol, dried under nitrogen and reconstituted in water. Rats showing signs of acute graft rejection (i.e., hunched posture, ruffled fur, no food or water intake) were immediately euthanized. The upper graph shows the results obtained with 2 mg/kg naltrindole (NTI) and saline. The lower graph shows the results obtained with 0.4 and 2 mg/kg 7-benzylspiroindanylnaltrexone (BSINTX). Not shown are the results obtained with 0.4 mg/kg naltrindole, which were similar to those obtained with saline. Statistical significance was determined by the Wilcoxin Paired Ranks Test. All animals were given the highest standard of care as mandated by the National Institutes of Health Guidelines and approved by our Institutional Animal Care and Use Committee.

shown in Fig. 1 and are summarized as follows: 7-benzylspiroindanylnaltrexone at 0.4 mg/kg allowed 25% of the rats to survive greater than 100 days, which was similar to the results obtained with a 5-fold higher dose of naltrindole. In fact, there was no statistical difference in the efficacy of the low dose of 7-benzylspiroindanylnaltrexone and the 5-fold higher dose of naltrindole ($P = 0.5$). The higher dose of 7-benzylspiroindanylnaltrexone (2 mg/kg) caused 66% of the rats to survive longer than 75 days and 50% to survive longer than 100 days. Mean survival at this dose of 7-benzylspiroindanylnaltrexone was significant with respect to all other treatment groups ($P < 0.05$). The group receiving the lowest dose of naltrindole (0.4 mg/kg) had one rat surviving greater than 100 days (data not shown); however, there was no statistical difference in survival between this group and the control group.

Histological examination of transplanted kidney tissue suggested that naltrindole and 7-benzylspiroindanylnaltrexone act via different mechanisms (data not shown). Naltrindole prevented cellular infiltration and subsequent tissue damage, whereas 7-benzylspiroindanylnaltrexone did not affect cellular infiltration, but did prevent subsequent rejection of the grafted kidney. Thus, naltrindole may affect the ability of cells to access the grafted site, whereas 7-benzylspiroindanylnaltrexone may affect their ability to function in the grafted site.

It has been reported that naltrindole is at least an order of magnitude more potent ($K_e = 0.13$ nM; Portoghese et al., 1990) than 7-benzylspiroindanylnaltrexone ($K_e = 2.1$ nM; Ohkawa et al., 1997). The present results which show 7-benzylspiroindanylnaltrexone to be 5-fold more potent than naltrindole in prolonging kidney allograft survival suggest that δ_1-opioid receptor antagonism is responsible for its greater effectiveness. Also, the finding that naltrindole and 7-benzylspiroindanylnaltrexone differentially affect infiltration by immune cells is consistent with this interpretation. The results of this study raise the possibility that the design of superior, selective δ_1-opioid receptor antagonists may afford therapeutically effective immunosuppressive agents.

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