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The influence of dose interval on the immunosuppressive potency of methylprednisolone: an experimental organ transplant study in isogenous rats

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Abstract. Groups of isogenous Brown-Norway rats received heterotopic heart transplants from (Brown-Norway/Wistar Furth) x F_1 hybrid rats. Methylprednisolone was administered IV in a daily dose of 5–40 mg/kg, using a dose interval of 24, 12, or 0 h (continuous infusion). Continuous infusion proved to be superior when small daily doses were used and then caused a more than threefold increase in graft survival. High daily doses created a substantial mortality with all dose intervals. Moreover, the equipment used for continuous drug administrations was unreliable beyond 4 weeks of infusion.

Key words: Heart transplantation, experimental, rat – Prednisolone, in experimental heart transplantation

Glucocorticoid agents have a profound suppressive effect on every tested part of the immune response. Moreover, they are the only agents that are effective in every tested species with the capacity to evoke an adaptive immune response [1]. Obviously, the turnover rate, absorption from the GI tract, and excretion strongly influence the glucocorticoid effects.

Methylprednisolone is a glucocorticoid agent with widespread use in clinical transplantation because of its nonsignificant mineralocorticoid effect. The influence of different dose intervals on its immunosuppressive efficiency has not been studied systematically. The present investigation was carried out to evaluate whether identical daily IV doses had different efficiency and toxicity in the rat when given once a day, twice a day, or continuously.

Materials and methods

Groups of heart-transplanted isogenous Brown-Norway rats received methylprednisolone in a dose of 5, 10, 20, or 40 mg/kg per day. The drug was administered intravenously once a day, twice a day, or by continuous infusion. The functional survival times (FST) of the transplanted hearts were recorded.

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Animals

A total of 101 isogenous Brown-Norway (BN) rats received (Brown-Norway/Wistar Furth) x F_1 hybrid hearts. Male and female rats weighing 220–280 g were used. They were fed pellets and water ad libitum.

Transplant technique

The heterotopic heart transplant model described by Ono and Lindsay [6] was used. In our hands, the model has a more than 95% technical success rate, defined as the presence of a beating heart transplant 24 h after surgery. FST was registered by palpation of unanesthetized animals twice a day. When cardiac arrest was suspected, an exploration of the graft was carried out to ascertain FST. In two cases, the previously estimated FST was prolonged by .5 days after the exploration.

Drug administration

Methylprednisolone was administered intermittently through a rubber membrane-equipped catheter protruding through the interscapular skin. The catheter tips were placed in an iliac vein. Continuous steroid infusions were given into a jugular vein through a catheter entering the interscapular skin. The extracorporeal portion of the catheter was protected by a steel tube sutured to the skin. A swivel connected the tube to the infusion pump, allowing the rat to move freely in its cage (Harward swivel tethering system 56-7461, 20-gauge). The rats were sacrificed by IV chloral hydrate injection through the catheters at the end of transplant FST. By this method, the patency of the catheters at the end of the experiments also could be ascertained. (Only rats experiencing "sudden death" were considered to have patent catheters.) Methylprednisolone (Solu-medrol Upjohn, Kalamazoo, Mich, USA) was mixed daily with sterile water and was fed into the infusion pump chambers or the bolus infusion catheters.

Statistical analysis

The results were analyzed using a one-way analysis of variance to compare functional survival times of daily doses for each dosing interval and of dosing intervals for each daily dose. Follow-up comparisons of means were performed using the Bonferrone multiple comparison procedure in which the alpha equals the 0.05 level of significance.

Table 1. Functional survival time (FST) in days of heterotopic (BN/WF) x F_1 hearts transplanted to BN rats given methylprednisolone intravenously in different doses and intervals, expressed as mean ± SD

Daily dose (mg/kg)	Dose interval		
	24 h	12 h	Continuous
5	–	10.3 ± 0.6 (10) [9.5–11.5]	20.6 ± 2.1 (10) [17.5–33]
10	–	14.6 ± 1.0 (10) [13–16]	29.6 ± 1.9 (10) [27–32]
20	8.2 ± 0.5 (10) [7.5–9]	38.9 ± 5.8 (10) [31–46]	[25–51] (5)[a]
40	25.6 ± 7.1 (8) [20–42]†††	69.4 ± 7.6 (10) [59–87]††	[20–42] (8)[a] †††††
	Control FST = 8.5 ± 0.4 (10) [7.5–9]		

[a] Catheter clogging days 27–40
† Number of animals which died with functioning graft
Figures in parentheses indicate number; figures in brackets show the range

Results

The FST of a (BN/WF) x F_1 heterotopic heart transplant in nonimmunosuppressed BN rats is 8.5 ± 0.4 days. The survival times during different methylprednisolone treatment protocols are shown in Table 1. On the 40 mg/kg per day level, an immunosuppressive effect of the once-a-day dose schedule was recorded, but a twice-a-day regimen had a stronger effect. However, this daily dose of methylprednisolone proved to be highly toxic. Altogether, in the three groups of rats that received 40 mg/kg per day with different dose intervals, 10 out of 26 rats died with beating heart transplants. The infusion catheters clogged during week 5–6 postoperatively, making FST assessment in the continuous steroid delivery group of little interest. Intravenous methylprednisolone, 20 mg/kg per day, created a prolongation of FST (38.9 ± 5.8 days) when given twice a day but not when given once a day (8.2 ± 0.5 days). During continuous infusion all grafts were functioning when the infusion catheters ceased to be patent on postoperative days 27–40. The immunosuppressive efficiency of continuous versus twice-a-day methylprednisolone treatment could be compared without mortality or catheter cloggings on the 5 and 10 mg/kg daily dose levels. As seen in Table 1, a significantly better transplant survival was obtained with the continuous steroid infusion protocol in both instances. The 5 and 10 mg/kg once a day methylprednisolone treatments were not tested.

Results from an analysis of variance comparing different doses and dose intervals in the groups without mortality or catheter clogging were highly significant in every case except when the 20 mg/kg once-a-day dose was compared to controls.

Discussion

The pharmacokinetics of methylprednisolone have been studied extensively in humans [5] and in animals [3], and various administration routes and intervals have been used clinically, both after organ transplantation [2] and in inflammatory/autoimmune diseases [5]. However, as will be discussed here, few studies exist that focus on the influence of dose intervals on organ transplant survival.

The marked differences in the immunosuppressive effect of methylprednisolone when given once a day, twice a day, and by continuous infusion are remarkable and somewhat unexpected. The 40 mg/kg per day dose was too toxic with all dose intervals to yield precise information. The 20 mg/kg dose given once a day did not result in prolonged transplant survival. Therefore, smaller once-a-day doses were not felt to be of interest in the study. The FSTs in the higher dose, continuous infusion groups might have been even longer if an infusion system that was reliable beyond 4 weeks had been available. As the infusion catheters clogged to an unacceptable degree when used longer than 4 weeks, statistically usable survival data from methylprednisolone administration by continuous infusion could only be obtained with smaller, less immunosuppressive doses. A dose of 10 mg/kg per day given every 12 h was moderately effective in prolonging transplant survival. However, despite a strong histocompatibility barrier, a continuous infusion of this daily methylprednisolone dose created a threefold increase in FST. A dose of 5 mg/kg per day had a barely noticeable immunosuppressive effect when given IV twice a day but created a marked prolongation of graft survival when given continuously. Thus, in rats, a given amount of methylprednisolone seems to have the strongest protective effect for a transplant when administered by continuous infusion.

The differences in immunosuppressive potency after changes in dose intervals are most likely to be seen with the IV route of drug administration. Early researchers demonstrated a clear immunosuppressive effect from once-a-day (mostly intraperitoneal) glucocorticoid administration given in doses equivalent to those found to be nonimmunosuppressive in the experiments reported here [11, 12]. Continuous intravascular steroid infusion after organ transplantation has been reported both clinically [4] and experimentally [8, 10]. However, the aim of those experiments has generally been to prove the superiority of local steriod administration into a transplant-supplying artery, and control groups with continuous IV steroid administration are often lacking [4, 9]. Also, continuous steroid administrations have often been given by implanted devices with no proof of maintenance of the drug activity at normothermia after weeks of infusion [8–10]. In the experiments reported here, a fresh steroid preparation was added daily. In one recent study, prednisolone was administered continuously into rat kidney transplant-feeding arteries [8]. A control group was treated with continuous IV prednisolone. No immunosuppressive effect was obtained with 4 mg/kg per day given IV through implanted pumps. In another rat study, heart allograft survival was superior when prednisolone was given by continuous subcutaneous infusion as compared to intermittent subcutaneous boluses [7].

The issue of effect versus side effect – whether a steroid regimen with a certain immunosuppressive potency has more or fewer side effects – has been addressed only by mortality figures in our study. Ruers and coworkers

[10] have addressed this issue in an elegant way, transplanting heterotopic rat hearts with venous drainage into the portal vein and using a steroid product with rapid clearance by the liver. Comparing IV and transplant arterial infusion of identical steroid doses, identical transplant-protective effects, but fewer side effects, were documented by the intra-arterial route.

The heterotopic heart transplant model in isogenous rats was selected in our studies because of its reproducibility and easily definable rejection time (FST). However, the results that were obtained should be applicable for all vascularized organ transplantation in the rat. The data seem to encourage clinical trials using continuous methylprednisolone infusion during the early postoperative course after an organ transplant. However, our results obviously must be interpreted with caution, as rats and humans have markedly different glucocorticoid turnover rates. With regard to steroid metabolism, rats, like most rodents, belong to a group of "steroid-sensitive" species, while humans belong to the "steroid-resistant" ones [1].

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