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Effect of LS-2616 on the graft protection achieved by cyclosporin A, prednisolone, and 15-deoxyspergualin in heart-transplanted rats

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Abstract. The immunostimulator LS-2616 abolishes the effect of cyclosporin A in a rat cardiac transplantation model. The present paper compares the characteristics of rejection obtained under different immunosuppressive regimens with and without additional LS-2616 application in the same model. Cyclosporin A (CyA, 10 mg/kg daily), prednisolone (15 mg/kg daily), or 15-deoxyspergualin (2, 5, or 10 mg/kg daily), all given from the day of transplantation until day 9, protected the grafts during the treatment period. The addition of LS-2616 (160 mg/kg, day −1 until stop) resulted in a total abrogation of the immunosuppressive effect of CyA and prednisolone. However, LS-2616 could only partially or not at all reverse the effect of 15-deoxyspergualine. These results show a certain drug selectivity of LS-2616 in promoting rejection of immunosuppressed allografts. Further studies with LS-2616 may be of benefit in evaluating the mode of action of different immunosuppressive compounds and, thus, contribute to finding more effective antirejection therapies.

Key words: Immunosuppression – Cyclosporine A – Prednisolone – 15-deoxyspergualin – LS-2616

The rejection of a allograft is an immune response in which the immune system acts detrimentally. This immune reaction can to a large extent be suppressed by immunomodulating compounds. Despite the impressive graft protective effectiveness of drugs like cyclosporin A (CyA), prednisolone, or 15-deoxyspergualin (15-DSG) in experimental transplantation models in rodents [5, 7], in man graft rejection does still occur in the presence of immunosuppressive therapy. This clinical experience suggests that alternative pathways might exist in activation of the immune system which cannot be blocked by these drugs.

We described earlier LS-2616 [3, 4] as an immunomodulating compound able to induce graft rejection in the presence of CyA [2, 7, 8]. This CyA-resistant pathway might reflect such an alternative activation of the immune system. Subsequently, we tested the nature of the effect of LS-2616 on the prolongation of graft survival brought about by prednisolone or 15-DSG. In the present paper, we report that LS-2616 abrogates the effect of CyA and prednisolone while it only partially reverses the effect of 15-DSG at low doses (2 and 5 mg/kg) and was without effect when combined with a high dose of 10 mg/kg.

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Materials and methods

Cardiac allografts from male PVG donor rats were transplanted heterotopically to Wistar/Kyoto (Wi/Ky) recipients as described in detail elsewhere [8]. Rejection was defined as the absence of palpable contraction. LS-2616 (Linomide, Kabi Pharmacia, Sweden) was added to the drinking water. Treatment with a daily dose of 160 mg/kg started 1 day prior to transplantation and continued until the day of rejection.

Three different immunosuppressive protocols were used, all starting on the day of transplantation and maintained until day 9 after transplantation. (A) CyA (Sandimmune, Sandoz AG, Switzerland) was given orally in a daily dose of 10 mg/kg. (B) Prednisolone (Precortalone, Organon, The Netherlands) was given intraperitoneally at a dose of 15 mg/kg daily. (C) 15-DSG (Behringwerke AG, FRG) was given intraperitoneally at doses of 2, 5, or 10 mg/kg daily.

The Wilcoxon rank-sum test was used for the statistical analysis. The results are given as the median (and range) of the graft survival time. One cardiac graft recipient treated with 15-DSG 2 mg/kg daily which stopped 5 days after transplantation was excluded from the statistical analysis, since the pathohistological examination of this explanted heart showed no signs of necrosis and only a mild cellular infiltration within the intact normal heart muscle cells.

Results

The results are summarized in Table 1. No treatment (control) resulted in a graft survival time of 8 (8–9) days, which was not altered by LS-2616 treatment alone (median 8.5, range 7–11). Rats receiving CyA or prednisolone experienced graft survival of 19 (15–27) and 12 (9–15) days, respectively. The addition of LS-2616 to both treatment groups led to graft survival times identical to that of the un-

Table 1. Graft survival times of heart-transplanted rats receiving immunosuppression alone or combined with LS-2616

Treatment	Graft survival times (days)
None	8, 8, 8, 8, 8, 8, 8, 9, 9, 9, 9, 9
LS-2616	7, 8, 8, 9, 10, 11
Prednisolone	9, 10, 10, 11, 13, 13, 14, 15
Prednisolone + LS-2616	8, 9, 9, 9, 9, 9**
Cyclosporine	15, 15, 16, 16, 17, 17, 18, 19, 19, 19, 20, 21, 21, 21, 22, 27, 27
Cyclosporine + LS-2616	8, 8, 8, 9, 9, 9***
15-DSG (2 mg/kg)	16, 17, 17, 18, 18, 20, 20, 27, 35
15-DSG (2 mg/kg) + LS-2616	6, 7, 9, 12, 13, 13, 13, 15, 17, 21**
15-DSG (5 mg/kg)	13, 15, 19, 20, 20, 31, 57, 98, > 100, > 100
15-DSG (5 mg/kg) + LS-2616	5, 9, 10, 13, 15, 17, 17, 18, 20, 21, 21, 29, 30, 30
15-DSG (10 mg/kg)	8, 18, 21, 21, 22, 23, 28, > 100, > 100, > 100
15-DSG (10 mg/kg) + LS-2616	11, 21, 24, 26, 47, > 100

15-DSG, 15-deoxy spergualin
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

treated group. Medication with 15-DSG resulted in a median graft survival of 18 (16–35) days in the group receiving a daily dose of 2 mg/kg, 25.5 (13 to > 100) days in the 5 mg/kg group, and 22.5 (8 to > 100) days in the 10 mg/kg group. The addition of LS-2616 here did not, in contrast to CyA and prednisolone, reverse the immunosuppressive effect of 15-DSG. LS-2616 only partially reversed the effect of a 2 mg/kg 15-DSG application, leading to a graft survival time of 13 (6–21) days. Three out of 10 animals rejected their heart during the treatment period with 15-DSG. In the group receiving 5 mg/kg one could see a slight and insignificant tendency towards a shortening of the graft survival time by the addition of LS-2616. Here, 2 out of 14 animals rejected their grafts in the presence of 15-DSG, and none of the LS-2616 plus 15-DSG-treated animals had grafts surviving longer than the 100 days observed in the animals given 15-DSG only. Recipients with the highest dose of 15-DSG 10 mg/kg in combination with LS-2616 had a comparable graft survival time to the animals receiving only 15-DSG.

Discussion

The results clearly indicate a certain drug-selective effect of LS-2616 on provoking rejection of rat cardiac allografts. Both CyA and prednisolone totally lose their immunosuppressive potential upon the addition of LS-2616, whereas this was only observed to a considerably lesser extent or not at all in 15-DSG-treated animals.

The mode of action of LS-2616 is believed to be due not to an induction of interleukin 2 (IL-2) [6] but to a direct or indirect stimulation of already sensitized T-cell which are prevented from becoming cytotoxic by CyA [8]. The fact that LS-2616 was capable only to a minor extent of reversing the suppression of 15-DSG could find its explanation in the hypothesis that 15-DSG prohibits the sensitization of T-cell either by directly interacting with T-cell, like CyA, or by exerting its main effect on other cells of the immune system, e.g., macrophages [1]. Concerning the relevance of our findings, it is tempting to speculate that there are signals in man which lead to CyA- and prednisolone-resistant pathways of rejection crisis and, furthermore, that these signals might be identical with those provided or induced by LS-2616. Further studies in the LS-2616 model might lead to a better understanding of the mode of action of immunosuppressive compounds such as 15-DSG and thereby contribute to optimizing antirejection therapy by prohibiting CyA/prednisolone-resistant rejections.

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