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Full Papers

More on the need for circadian, circaseptan and circannual optimization of cyclosporine therapy

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Summary. Cyclosporine chronotherapy of pancreas-allotransplanted rats revealed, beyond a circadian stage-dependence of equal daily doses, further gain in graft function from doses varying from day to day with an about 7-day periodicity, the first highest dose being given on the 3rd or 5th day after surgery.
Key words. Pancreatic allograft; cyclosporine chronotherapy; circadian; circaseptan; circannual.

Introduction

Certain rat kidneys allografted across a major histocompatibility barrier function for 7 or 8 days when untreated. Slower rejections of hearts and kidneys allografted across lesser immunologic barriers tend to occur at multiples of an about 7-day period[8,11,14]. A circaseptan (about 7-day) bioperiodicity in rejection was found for human kidney transplants[5,12]. The question arises as to whether, in the case of treatment with an immunosuppressant agent such as cyclosporine (Cs), circaseptan rhythms could be exploited to optimize the prolongation of graft function. In this case, one has to determine the particular postoperative day at which the highest Cs dose should be administered. Early results with the use of a segmental pancreatic transplantation model in rats[13], suggested the importance of both circadian and circaseptan rhythms in scheduling Cs treatment. These results prompted the consideration of additional animals for further investigation. Results from all animals are presented herein and are discussed in the broader scope of a series of chronobiologic studies, all aimed at the optimization of Cs timing in transplantation.

Materials and methods

Segmental pancreatic transplantation was performed on Ma Lewis (RT-1^l) recipients with ACI (RT-1^a) donor rats, from 23 May to 29 August 1983, and again from 16 April to 16 May 1984. In both stages of this study, a total of 169 14–27-week-old male Ma Lewis rats were singly housed in cages measuring 26 × 16 × 17 cm. Rats (which did not have pneumonia and had no other obvious illness) were used as recipients. Recipients and donors were placed in 3 sound-dampened rooms kept on staggered regimens of 12 h of light (L) alternating with 12 h of darkness (D) (LD12:12), maintained at 24 ± 1 °C and ~ 50% humidity. All animals were standardized under these conditions for at least 2 weeks before study. After this time, animals are synchronized to their new LD regimen, which permits the experimenter to treat at any circadian stage, conveniently during working hours. Deionized water and Purina Rat Chow were available ad libitum. At 7–14 days prior to surgery, all recipients were made diabetic by a single i.v. injection of 50 mg/kg b.wt streptozotocin (Upjohn). All rats with a serum glucose over 400 mg% received a segmental pancreas transplant from an ACI (RT-1^a) rat standardized on the same lighting regimen.

The ACI rat was anesthetized by means of an intraperitoneal injection of Nembutal (50 mg/kg b.wt) and a segmental pancreas transplantation was completed as described earlier[16]. Cs was administered daily, starting on the day of surgery. Different animals were treated at one or another of 6 different circadian stages, 4 h apart. The timepoints, expressed as hours after light onset (HALO), were 02, 06, 10, 14, 18 and 22 HALO. A given animal was treated at the same circadian stage each day. A circaseptan-equal dose (homeostatic) group (H) received equal daily doses of 3.5 mg/kg Cs. Seven circaseptan-sinusoidal groups (S) received Cs at 1 of 6 circadian stages in a 7-day cyclically-varying schedule, the first largest dose being administered either on the day of surgery or on the 2nd, 3rd, 4th, 5th, 6th or 7th day after surgery. The average daily group-S doses equalled that of group H, with maximal departure of ± 1 mg/kg. A control group (C) included untreated rats and rats receiving the vehicle only (intralipid-ethanol solution).

Serum glucose was measured every ~ 1–2 days. The day of graft rejection was defined as the first of at least 3 consecutive days of hyperglycemia (> 200 mg%) and confirmed in most cases by laparotomy and graft histology. Exclusion criteria were technical surgical problems, respiratory arrest during anesthesia, thrombosis, hemorrhage and recurrent infection. A separate group of recipients of segmental pancreatic isografts (from Ma Lewis to Ma Lewis rats) maintained normoglycemia for > 200 days.

Data on pancreatic graft function following Cs treatment at the same six circadian stages were also available from a different study (Cavallini, unpublished results) performed in December 1982. This experiment was similarly designed and used equal daily doses of Cs. Apart from the fact that a dose of 2.5 (instead of 3.5) mg/ml was used on slightly younger animals (2.5- to 3-month-old Lewis rats), all experimental conditions were kept the same, as far as possible.

Results

Among the recipients accepted for evaluation in the main study (from May 1983 to May 1984), the mean number of days (and standard deviation) elapsed until pancreas rejection in 11 controls (C), 19 rats receiving equal (H) and 49 rats receiving 7-day sinusoidally (S)-varying doses of Cs are 6.0 ± 1.4, 8.8 ± 4.0, and 11.5 ± 4.2, respectively. As compared to the average rejection time of group H,

the control rats have a 31% shorter duration of graft function ($t = 6.50$; $p < 0.01$), whereas the rats receiving sinusoidal Cs schedules have a 30% prolongation of graft function ($t = 4.41$; $p < 0.01$).

A circadian stage-dependent effect of Cs in rats receiving equal daily doses is suggested by single cosinor analysis[9] ($p = 0.077$). Longest times to rejection occur ~20 h 4 min after light onset (SE = 1 h 36 min). This result is in agreement with the 1982 data, using a constant dose of 2.5 mg/kg Cs in younger animals. Since the mean graft function of rats receiving equal daily doses of Cs does not differ significantly between the 1982 and 1983/84 studies, despite the difference of daily doses in the two studies, as shown by Student's t-test, rejection times from both studies are pooled. By so doing, the circadian rhythm in the Cs effect is shown to be highly significant ($p = 0.006$). This result is in keeping with the optimal circadian stage of Cs administration in the middle of the dark span for heart allografts[4].

The longest graft function occurs for the circaseptan Cs schedules with the first highest dose administered either on the 3rd or 5th day after surgery (12.6 ± 4.6 days). When compared to the average rejection time of rats in group H, the prolongation of graft function is statistically significant ($\Delta = 3.9$ days; $t = 2.76$; $p < 0.001$). Since a larger number of rats ($n = 14$) receive the circaseptan Cs schedule with the first highest dose administered on the 5th day after surgery, this group is also compared to group H and found to have a statistically significantly longer graft function ($\Delta = 3.7$ days; $t = 2.76$; $p < 0.05$). By comparison with rejection times noted in rats of group H pooled over the 1982 and 1983/84 studies, circaseptan schedules of Cs administration prolong graft function by 2.7 days ($t = 3.04$; $p < 0.01$). When considered alone, the circaseptan schedule with the highest Cs dose administered on the 5th day after surgery is also shown to prolong graft function ($\Delta = 3.7$ days; $t = 2.92$; $p < 0.01$) (fig. 1). It is noteworthy that a similar circaseptan timing is also found to be optimal for cisplatin administration with respect to reduced nephrotoxicity[1].

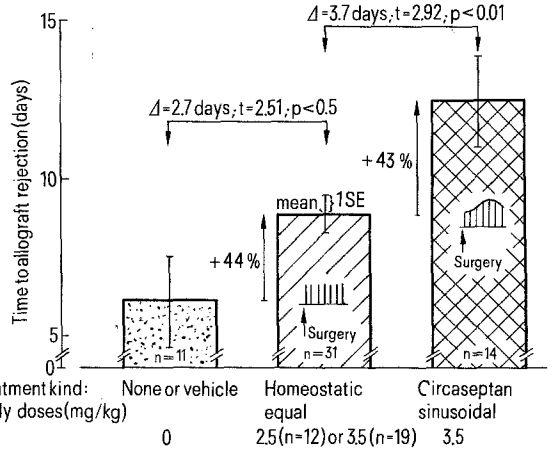

Figure 1. Comparison of segmental pancreatic graft function in diabetic male Ma Lewis (RT-1[l]) recipient rats – from donor ACI rats (RT-1[a]) – receiving no treatment or vehicle, equal daily Cs doses or the same weekly dose administered according to an optimal circaseptan-sinusoidal schedule, with the first highest dose given on the 5th day after surgery.

Discussion

Prolongation of graft function by 2.7 and 3.7 days may not look impressive. It must be kept in mind, however, that the purpose of these studies is to investigate means to optimize treatment timing. Minimal doses of Cs are to be used that merely suffice to enable the detection of any statistically significant differences in duration of graft function promptly, cost-effectively and, what is most important, as humanely as possible. The Cs doses are selected to allow for the detection of differences as a function of schedules without undue prolongation of the study, and indeed do so.

The documentation of circadian and circaseptan optimization can be complemented by the role of circannual rhythms found in islet rather than segmental pancreas transplantation across a minor immunologic barrier in male Lewis donors and male Fischer recipients. Graft function averages 54.3 (SD = 1.0) days in a study started on 24 March 1982 and 9.0 (SD = 2.5) days in a study started on 23 June 1983. The difference is statistically significant ($p < 0.001$). Circannual variation in response to Cs is also seen in data on heart allografts[3] and is shown for comparison with pancreatic islet allografts in figure 2. In both studies, the results in summer are associated with a much shorter graft function than those in winter. Circannual changes remain to be mapped by longer and denser sampling. There is already extensive evidence in different fields of biology and medicine, however, to show that circannual rhythms are ubiquitous[10].

With the advent of implantable programmable pumps, automatic Cs chronotherapy with multiple frequencies becomes possible for the scheduling of chronotherapy. Elsewhere, we have summarized tests of the effects of timing Cs administration on nephrectomized dogs bearing an allografted kidney[2]. Doses of 1.5 mg/kg Cs per day were given via a programmed implanted Medtronic pump (Minneapolis, Minnesota, USA) at a continuous injection rate or with one of six sinusoidally varying

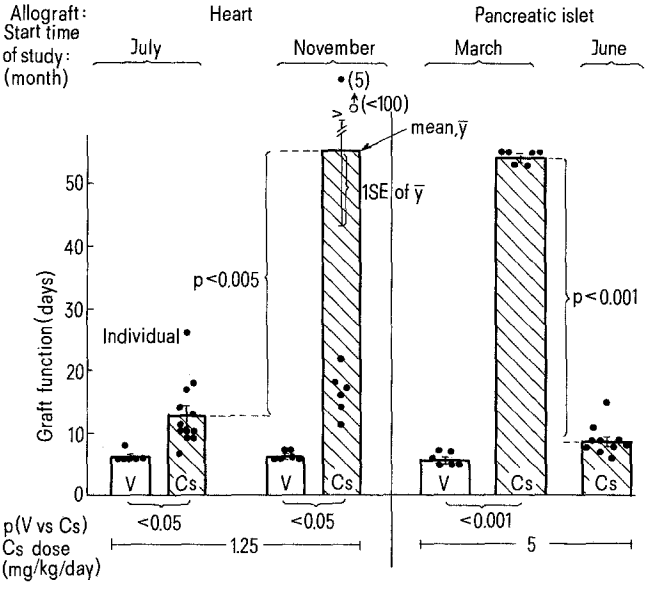

Figure 2. Circannual variation in immunosuppressive effect of cyclosporine in a study with heart as well as pancreatic islet transplant in rats. V, vehicle; Cs, cyclosporine.

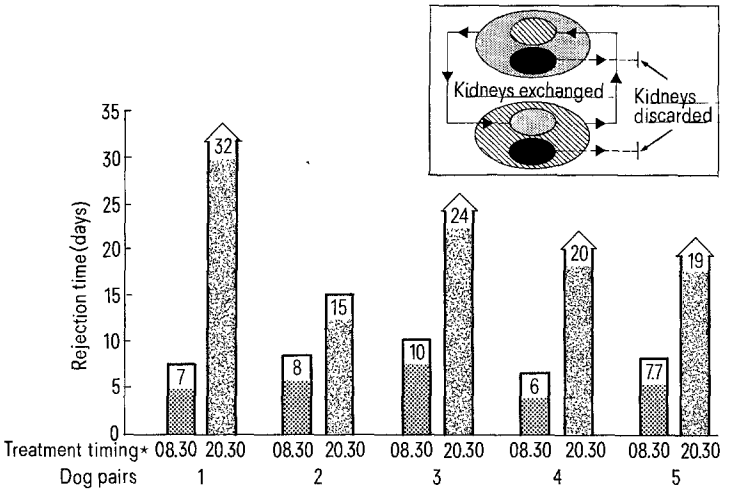

Figure 3. In dogs kept in light from 06.00 to 18.00 h, treated at 08.30 and at 20.30 h with a single daily oral dose of 12.5 mg/kg cyclosporine, in the absence of a pharmacokinetic difference, the evening dose is over twice as effective as the morning dose in prolonging kidney allograft function (arrow corresponds to functioning graft at time of summary, while bar corresponds to death from rejection).

rates, simulating a circadian rhythm. Each sinusoidal schedule involved eight different steps of 3 h each, the total dose/day being equal to that in dogs infused at a constant rate. (Checks at the time of replenishing Cs, of the reservoir of all but two pumps, revealed that the intended volume was injected within about 6%. Two of the pumps grossly ($\sim$ 50%) underinfused.) With both the constant and the sinusoidal rate, Cs, on the average, prolongs graft function with statistical significance. Moreover, some sinusoidal infusion schedules were better than others. The fit of a 24-h cosine function demonstrated a statistically significant circadian rhythm in response to sinusoidal Cs treatment (whether or not data from a single underinfused dog were included). The optimal schedule of Cs administration is also found to correspond to higher rates in the middle of the dark span. Chronotherapy by sinusoidal and/or other timed schedules with multiple frequencies can be readily implemented by an approach including, with the use of a programmable implantable pump, marker rhythmometry with results analyzed by cosinor (and related procedures, in conjunction with diagnostic regression tests) for a variety of drugs[6,7,15]. Most recently, results with the modern drug administration device also served to optimize the circadian timing of a conventional treatment mode: the oral administration of cyclosporine (fig. 3).

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1 Bixby, E.K., Levi, F., Haus, R., Sackett, L., Haus, E., Halberg, F., and Hrushesky, W., Circadian aspects of murine nephrotoxicity of cisdiamminedichloroplatinum (II), in: Toward Chronopharmacology, pp. 339–347. Eds R. Takahashi, F. Halberg and C. Walker. Pergamon Press, Oxford/New York 1982.

2 Cavallini, M., Halberg, F., Cornélissen, G., Enrichens, F., and Margarit, C., Organ transplantation and broader chronotherapy with implantable pump and computer programs for marker rhythm assessment. J. contr. Release (in press).

3 Cavallini, M., Magnus, G., Halberg, F., Florack, G., and Sutherland, D.E.R., Murine model for a chronoimmunotherapy of allotransplantation: circadian and circannual variation in cyclosporine effects. Eur. Surg. Res. *15*, suppl. 1 (1983) 19–20.

4 Cavallini, M., Magnus, G., Halberg, F., Liu, T., Field, M.Y., Sibley, R., Najarian, J.S., and Sutherland, D.E.R., Benefit from circadian timing of cyclosporine revealed by delay of rejection of murine heart allograft. Transpl. Proc. *15*, suppl. 1 (1983) 2960–2966.

5 DeVecchi, A., Halberg, F., Sothern, R.B., Cantaluppi, A., and Ponticelli, C., Circaseptan rhythmic aspects of rejection in treated patients with kidney transplant, in: Chronopharmacology and Chronotherapeutics, pp. 339–353. Eds C.A. Walker, C.M. Winget and K.F.A. Soliman. Florida A & M University Foundation, Tallahassee 1981.

6 Halberg, F., Chronopharmacology and chronotherapy, in: Cellular Pacemakers, pp. 261–297. Ed. D.O. Carpenter. John Wiley & Sons Inc., New York 1982.

7 Halberg, F., and Halberg, E., Chronopharmacology and further steps toward chronotherapy, in: Pharmacokinetic Basis for Drug Treatment, pp. 221–248. Eds L.Z. Benet, N. Massoud and J.G. Gambertoglio. Raven Press, New York 1984.

8 Halberg, F., Halberg, E., Herold, M., Vecsei, P., Günther, R., and Reinberg, A., Toward a clinospectrometry of conventional and novel effects of ACTH 1-17 – Synchrodyn® – in rodents and human beings, in: Toward Chronopharmacology, Proc. 8th IUPHAR Conf. and Sat. Symp., Nagasaki, 27–28 July 1981, pp. 119–161. Eds R. Takahashi, F. Halberg and C. Walker. Pergamon Press, Oxford/New York 1982.

9 Halberg, F., Johnson, E.A., Nelson, W., Runge, W., and Sothern, R., Autorhythmometry – procedures for physiologic self-measurements and their analysis. Physiol. Tchr. *1* (1972) 1–11.

10 Halberg, F., Lagoguey, J.M., and Reinberg, A., Human circannual rhythms in a broad spectral structure. Int. J. Chronobiol. *8* (1983) 225–268.

11 Kawahara, K., Levi, F., Halberg, F., Rynasiewicz, J., and Sutherland, D.E.R., Circaseptan bioperiodicity in rat allograft rejection, in: Toward Chronopharmacology, Proc. 8th IUPHAR Conf. and Sat. Symp., Nagasaki, 27–28 July 1981, pp. 273–280. Eds R. Takahashi, F. Halberg and C. Walker. Pergamon Press, Oxford/New York 1982.

12 Knapp, M.S., Cove-Smith, J.R., Dugdale, R., Mackenzie, N., and Pownall, R., Possible effect of time on renal allograft rejection. Br. med. J. *1* (1979) 75–77.

13 Liu, T., Cavallini, M., Halberg, F., Cornélissen, G., Field, J., and Sutherland, D.E.R., Multifrequency chronotherapy with circaseptan and eventually circannual optimization may follow early circadian-sinusoidal pump-implemented infusions of cyclosporine, in: Annual Review of Chronopharmacology, Proc. 1st Int. Montreux Conf. of Biological Rhythms and Medications, Montreux, Switzerland, 26–30 March 1984, pp. 133–136. Eds A. Reinberg, M. Smolensky and G. Labrecque. Pergamon Press, Oxford 1984.

14 Ratte, J., Halberg, F., Kühl, J.F.W., and Najarian, J.S., Variations circadiennes du rejet de l'allogreffe rénale chez le rat. Union méd. Canada *102* (1973) 289–293.

15 Reinberg, A., and Smolensky, M.H., Circadian changes of drug disposition in man. Clin. Pharmacokinet. *7* (1982) 401–420.

16 Squifflet, J.P., Sutherland, D.E.R., Rynasiewicz, J.J., Bentley, F.R., Florack, G., and Najarian, J.S., Technical aspects of segmental pancreatic grafting in rats. J. Microsurg. *4* (1983) 61–66.

0014-4754/86/010020-03$1.50 + 0.20/0