Transplant Int (1992) 5 [Suppl 1]: S 539–S 541

TRANSPLANT
International
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Adjuvant treatment with ursodeoxycholic acid prevents acute rejection in rats receiving heart allografts

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Abstract. Adjuvant treatment with ursodeoxycholic acid (UDCA) for liver-transplant recipients has been reported to reduce the frequency of acute rejection episodes. To explore this effect further, UDCA was given to rats in an experimental heart transplantation model, with or without concomitant immunosuppressive treatment with antihymocyte globulin (ATG). UDCA was administered orally 7 days before and 14 days after transplantation. Rats treated with UDCA alone or in combination with ATG were compared with untreated controls and ATG-treated recipients. Adjuvant treatment with UDCA was found to induce prolonged graft survival and increase the amount of transplant tolerance in rats. Serum levels of bilirubin and aminotransferases were not altered irrespective of the UDCA dose given. The results indicate that UDCA has an immunomodulatory capacity that might not be restricted to the liver, but also might apply to other transplanted organs as well.

Key words: Ursodeoxycholic acid – ATG – Rat-Heart transplant – Tolerance

Medication with hydropholic bile acid ursodeoxycholic acid (UDCA) has proven to be beneficial in cholestatic conditions, such as primary biliary cirrhosis, sclerosing cholangitis, biliary atresia and chronic hepatitis [1, 5, 14, 16]. It has also been shown to have a direct protective effect on hepatocytes both in vivo and in vitro [4, 6]. We have been using UDCA in our liver transplant program since 1989 and have observed that the recipients on adjuvant UDCA treatment had significantly fewer rejection episodes than historical controls [13]. The mechanism behind this beneficial effect of UDCA is not known.

To investigate the possible immunomodulatory effect of UDCA on organs other than the liver, UDCA was tested in an experimental transplantation rat model. The mechanism and effect of UDCA on tolerance induction were studied using UDCA alone or in combination with a potent immunosuppressive agent like ATG [9].

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Materials and methods

Animals

Male inbred DA(RT1^a) rats, weighing 200–220 g, were used as recipients and female PVG/c(RT1^c) rats, weighing 110–130 g, as donors (Bantin and Kingman, Hull, UK).

Surgical technique

The rats were anesthetized intraperitoneally with 8% chloral hydrate in a dose corresponding to 3.5 ml/kg body weight. Heterotrophic heart grafts were done to the neck vessels using a nonsuture cuff technique [10]. Rejection was defined as loss of regular EKG activity.

ATG

ATG was prepared by immunization of rabbits with rat thymocytes [9]. The ATG was absorbed with rat erythrocytes to remove agglutinins and rat liver powder until free of liver and kidney-reacting antibodies, as determined by immunofluorescence. Finally, the cytotoxic titer against thymocytes was adjusted to 1:1024 with phosphate-buffered saline (PBS).

UDCA

Ursodeoxycholic acid (99% pure) and tauro-ursodeoxycholic acid (90% pure) were purchased from Sigma Chemicals, St. Louis, Mo. Unconjugated and conjugated UDCA were used in equal proportions, dissolved in PBS, and adjusted to a final concentration of 100 mg/ml. UDCA was administered orally in three different doses corresponding to 50, 100 and 200 mg/kg body weight. Since unconjugated UDCA is difficult to dissolve in PBS, the UDCA solution was vigorously shaken before administration.

Laboratory tests

Serum levels of alanine and aspartate aminotransferases (ALAT and ASAT), as well as bilirubin, were analyzed using a Reflotron (Kodak). Serum samples were collected before starting up UDCA treatment, on the day of transplantation, and 7 days post-transplantation.

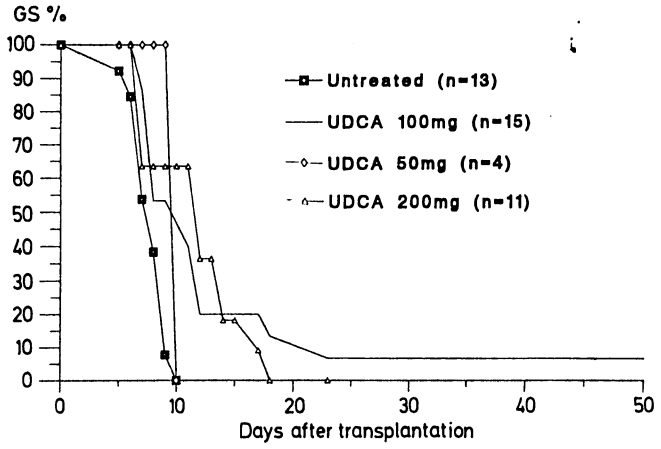

Fig. 1. Graft survival in rats with heart transplants treated with different doses of UDCA

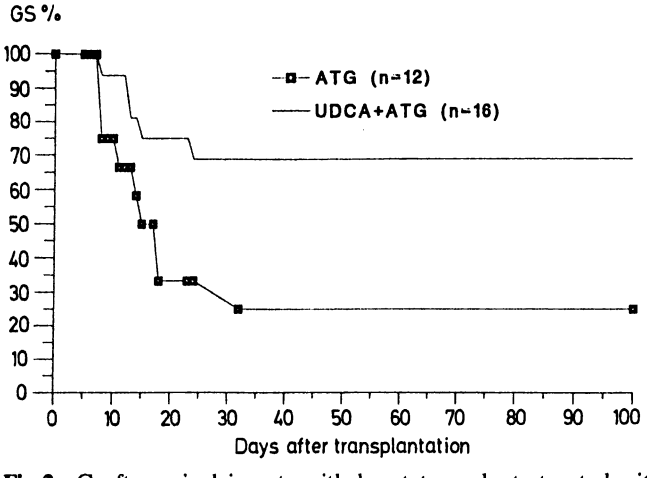

Fig. 2. Graft survival in rats with heart transplants treated with UDCA and ATG

Test protocol

The rats were divided into six groups and given the following treatment; I: 1 ml PBS 1 week prior to transplantation of an allogeneic graft. PBS treatment was continued until rejection was complete ($n = 13$). II: UDCA 100 mg/kg per day for 1 week prior and 2 weeks after transplantation ($n = 15$). III: As in group II and in addition 0.01 ml ATG intravenously (IV) 2 days before transplantation ($n = 16$). IV: 0.01 ml ATG IV 2 days before transplantation as the only treatment ($n = 12$). V: As in group II but 50 mg/kg per day of UDCA ($n = 4$). VI: As in group II but 200 mg/kg per day of UDCA ($n = 11$).

Statistical methods

The Wilcoxon rank sum nonparametric test for independent samples was used for analyses of statistical differences in graft survival between groups. Fisher's exact two-tailed test was used to compare differences in ratios of tolerant rats [3].

Results

UDCA alone improved graft survival significantly, irrespective of the dose tested, compared with untreated controls (Fig. 1). One of the 15 rats in the 100 mg UDCA group did not reject the transplant during an observation time of > 100 days; the remaining rejected their grafts 2–23 days after transplantation ($P < 0.05$). The 200 mg UDCA-treated rats rejected all grafts 7–18 days post-transplantation ($P < 0.05$) and the 4 rats in the 50 mg UDCA group at day 10 ($P < 0.01$). Rats given UDCA in addition to ATG had a significantly better graft survival rate than rats treated with ATG alone ($P < 0.05$) and 68% of the grafts functioned over the long term compared to 25% in the group treated with ATG alone (Fig. 2). The proportion of tolerant rats in the combined UDCA and ATG group was higher than in the ATG-treated group, but the difference did not reach statistical significance ($P = 0.056$).

The different groups were also tested for variations in aminotransferases and bilirubin levels before and after UDCA treatment and transplantation. No statistical differences between groups given different doses of UDCA, with or without ATG, were registered before or after transplantation.

Discussion

Our study suggests that UDCA has an immunomodulatory effect in rat recipients of heart transplants and also confirms the clinical observations in liver-transplant recipients that there is a reduced frequency of acute rejection episodes the first year after transplantation [13]. The exact mechanism behind the immunomodulatory effect of UDCA is unknown, but Poupon and coworkers have shown that UDCA is capable of reducing class-I antigen expression on hepatocytes in patients with primary biliary cirrhosis [2]. In a more recent study from a German group, UDCA has similarly been shown to down-regulate class-I/II antigen expression on biliary duct cells [8]. Since HLA antigen expression in the liver is normally induced by cholestasis, a non-immunological improvement in the disease as a result of UDCA administration could indirectly reduce the up-regulation of HLA antigens [15]. A direct effect on hepatocytes and antigen expression could, on the other hand, also be one explanation for the reduced frequency of rejection episodes seen in liver transplant patients receiving adjuvant UDCA treatment [13].

UDCA administration to transplanted rats did not result in any pathological values of bilirubin or aminotransferases, even with a high dose of 200 mg/kg, which is in agreement with the data reported earlier on this bile acid, which has been described as being safe and atoxic in man [7]. In the experimental transplantation model used, ATG has earlier been shown to induce a tolerant state by a suppressor cell mechanism [11] rather than through reduced graft antigenicity, although this possibility could not be completely excluded [12]. Whether UDCA also acts directly on peripheral immunocompetent T cells, or via down-regulation of class-I/II antigens in the graft, or via some other unknown mechanism has not been clarified by our results. Our study suggests that the effect of UDCA treatment is not completely specific for liver transplants, and further studies are needed to elucidate the exact mechanism behind this effect on the immune system.

Acknowledgements. This study was supported by grants from the Medical Faculty of the University of Göteborg, The Professor L-E Gelin Memorial Foundation, Fresenius AG, Federal Republic of Germany, Svenska Läkaresällskapet, Göteborgs Kungl vetenskaps och vitterhets samhälle, Riksförbundet för Njursjuka. We thank Ms. Rovena Eriksson for skillful technical assistance.

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