ELSEVIER

2043

Tacrolimus Enhances the Immunosuppressive Effect of Cyclophosphamide But Not That of Leflunomide or Mycophenolate mofetil in a Model of Discordant Liver Xenotransplantation

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LEFLUNOMIDE (LEF), which suppresses both T and B cells, prolongs survival of heart xenografts in the concordant hamster to rat animal model.[1] However, xenotransplantation across discordant barriers is the most likely approach to solve the problem of organ shortage. By perfusing guinea pig livers with University of Wisconsin solution containing sodium nitroprusside, we have solved the problem of poor reperfusion of this organ when transplanted into rats.[2] Accordingly, we tested the effect of LEF alone or in combination with tacrolimus (FK) on survival of guinea pig to rat liver xenografts. Results were compared with those obtained following the use of cyclophosphamide (CyP) and mycophenolate mofetil (MMF).

MATERIALS AND METHODS

Animals

Hartley guinea pigs weighing 200 to 250 g were used as liver donors and Lewis rats weighing 200 to 270 g were the recipients.

Liver Transplantation

The cuff technique was used for the orthotopic procedure with one difference; the cuff for the infrahepatic inferior vena cava (IVC) anastomosis was placed in the rat recipient and afterward inserted into the infrahepatic IVC of the donor liver.

Immunosuppression

Rat recipients received 80 U/kg cobra venom factor (CVF) from day −1 to day +2 post-Tx. LEF was given at a dose of 10, 15, and 30 mg/kg per day from day −1 until rejection. Eighty mg/kg CyP was given as a single intravenous injection 10 days before Tx. MMF and FK were used at a dose of 10 and 1 mg/kg per day, respectively, from day −1 onwards. Antibody formation and total hemolytic complement (CH50) were measured in xenograft recipients.

RESULTS AND DISCUSSION

As shown in Table 1, untreated liver xenografts underwent hyperacute rejection within 4 h (group I). CVF alone prolonged animal survival to a mean of 26 h (group II); an effect that was doubled by MMF (group V) and LEF (group VI). It was interesting that although CyP did not enhance the effect of CVF (group IV), the addition of FK resulted in 4 of 10 recipients surviving for up to 1 week post-Tx (group VII). Contrarily, the addition of FK to MMF (group VIII) or LEF (group IX–XI) did not result in further prolongation of recipient survival. Needless to say, the absence of CVF in any immunosuppressive cocktail resulted in hyperacute rejection in a few hours. Hemorrhagic gastritis was

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Aided by Project Grant No. DK29961 from the National Institutes of Health, Bethesda, Maryland.

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Table 1. Survival of Guinea Pig-to-Rat Liver Xenograft Recipients Immunosuppressed With CVF, Tacrolimus, CyP, LEF, or MMF

Group	N	Treatment	Recipient Survival (h) (Mean ± SD)	P
I	10	None	3.1 ± 1.7	
II	5	CVF	26.2 ± 12.2	<.001 vs GI
III	4	CVF + FK 1 mg	20.7 ± 1.2	
IV	5	CVF + CyP 80 mg	28.6 ± 14.3	NS vs GII
V	5	CVF + MMF 10 mg	49.4 ± 2.7	<.01 vs GII
VI	4	CVF + LEF 15 mg	59.7 ± 13.1	<.01 vs GII
VII	10	CVF + FK + CyP 80 mg	110.7 ± 48.3	<.0001 vs all
VIII	5	CVF + FK + MMF 10 mg	55.8 ± 8.8	NS vs GV
IX	4	CVF + FK + LEF 10 mg	36.0 ± 16.9	
X	5	CVF + FK + LEF 15 mg	62.5 ± 31.3	NS vs GVI, IX
XI	5	CVF + FK + LEF 30 mg	35.6 ± 9.8	

0041-1345/98/$19.00
PII S0041-1345(98)00166-3

universally observed in animals receiving LEF. It is possible that the guinea pig liver, as has been described in the dog, may have a genetic deficiency to acetylate, an aniline metabolite of LEF, leading to the appearance of toxic effects.[3] While xenoreactive natural antibodies became undetectable following Tx, and despite the use of immunosuppression, elevated titers of circulating antibodies were observed from post-Tx day 2; at the time when most xenografts were rejected. Liver rejection occurred in the absence of complement, which returned to normal levels by post-Tx day 7. Liver function tests in long-surviving recipients were relatively normal and histopathologic analysis of their livers revealed minimal or no signs of vascular injury.

In conclusion, tacrolimus enhances the immunosuppressive effect of CyP and prolongs survival of guinea pig to rat liver xenograft recipients receiving CVF. This effect of tacrolimus is not evident with LEF or MMF.

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