Reperfusion Injury Following Cold Ischemia Activates Rat Liver Kupffer Cells

P.N. Rao, T. Liu, J.T. Snyder, J.L. Platt, and T.E. Starzl

LIVER transplantation is now an accepted therapy for end-stage liver disease.[1] Organ preservation, transport, and subsequent transplantation involves a period of cold ischemia followed by reperfusion. Toxic oxygen radicals liberated at reperfusion can cause severe injury to the microvascular endothelial cell.[2-6] It has recently been demonstrated that severe preservation injury to the endothelial cell results in an increased incidence of allograft rejection in liver transplantation.[7] Although the exact reasons for this phenomenon are unclear, it is possible that endothelial cell injury may activate the neighboring Kupffer cell resulting in the production of inflammatory cytokines like interleukin-1 (IL-1) and tumor necrosis factor (TNF).[8] The IL-1 and TNF thus produced can initiate the rejection cascade leading ultimately to graft failure.[9-14] An isolated perfused rat liver (IPRL) model was used to investigate whether reperfusion injury following cold preservation can activate the Kupffer cell as measured by increased production of IL-1 and TNF.

MATERIAL AND METHODS

Isolated Perfused Rat Liver Model

An IPRL model was done according to Rao et al.[15] Livers harvested from male Lewis rats (275–300 g) were preserved in cold UW solution for either (1) 24 hours, (2) 26 hours, (3) 48 hours, or (4) 50 hours (n = 6 each). At the end of the preservation period, the livers were flushed in a retrograde fashion through the infrahepatic IVC with 10 mL of cold lactated ringers, and the effluent was analyzed for content as described below. Separate experiments were done in which, following 24 and 48 hours of preservation, livers were perfused at 37°C for 2 hours (n = 6 each). Samples of the 2-hour perfusate solution as well as the washout perfusate from groups 1, 2, 3, and 4 were assayed for (1) purine nucleoside phosphorylase (PNP), an endothelial cell-specific enzyme, (2) superoxide anion (O_2^-), (3) serum glutamate pyruvate transaminase (SGPT), (4) serum glutamic oxaloacetic transaminase (SGOT), and (5) IL-1.

Assays

Endothelial cell function was assessed by measuring effluent levels of PNP.[16] Free radical generation was analyzed by measuring levels of O_2^-.[17] Hepatocyte preservation was assessed by measuring transaminase levels[18] using a Technicon RA 500 autoanalyser (Technicon Instruments, Tarrytown, NY).

Levels of IL-1 in the perfusate were assayed using a radioimmunoassay kit from Medgenix (Belgium).[19]

Statistical Analysis

Data are expressed as mean ± SD. The statistical significance of differences between group mean was analyzed by the student *t* test. The alpha level has been adjusted for multiple comparisons using Bonferronis adjustment.[20] The alpha level per comparison was $P < .05$.

RESULTS

PNP

Levels of PNP at the end of 24, 26, 48, and 50 hours of cold preservation were 46.83 ± 4.69 U/L, 45.33 ± 10.42 U/L, 49.0 ± 3.41 U/L, and 55.83 ± 2.71 U/L respectively. These values were not significantly different from each other and were similar to control values. Levels of PNP at the end of 2 hours of reperfusion following 24 hours of cold ischemia were 71.94 ± 13.32 U/L. These values were significantly higher ($P < .001$) than those observed at the end of either 24 or 26 hours of cold ischemia alone. Similarly, values at the end of reperfusion following 48 hours of cold ischemia, 149.69 ± 22.26 U/L, were significantly higher ($P < .0001$) than PNP values at the end of either 48 or 50 hours of cold ischemia alone. Endothelial cell injury was significantly greater ($P < .0001$) following reperfusion at the end of 48 hours of cold ischemia than at the end of 24 hours. Fig 1 presents values for PNP in our experiments.

O_2^-

Superoxide levels at the end of 24, 26, 48, and 50 hours of cold preservation were respectively 0.33 ± 0.31, 0.33 ± 0.19, 0.51 ± 0.05, and 0.34 ± 0.09 nmol cytochrome-c reduced/mL respectively. These values were not significantly different from each other. Reperfusion following 24 and 48 hours of cold preservation resulted in superoxide levels of 1.06 ± 0.61 and 1.85 ± 0.44 nmol cytochrome c reduced/mL respectively. These values were significantly higher ($P < .001$ each) than the corresponding values obtained at the end of cold preservation alone. Greater superoxide generation was observed following reperfusion at the end of 48 hours of cold preservation than at the end of 24 hours. Fig 2 presents the data for superoxide generation.

Transaminases

SGOT. Levels of SGOT at the end of 24, 26, 48, and 50 hours of cold preservation were 68.42 ± 24.30 U, 68.33 ±

From the Department of Surgery, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania.

Address reprint requests to Prakash N. Rao, PhD, University of Pittsburgh, 203 DeSoto Street, Room 8200, Pittsburgh, PA 15261.

0041-1345/91/$3.00/+0

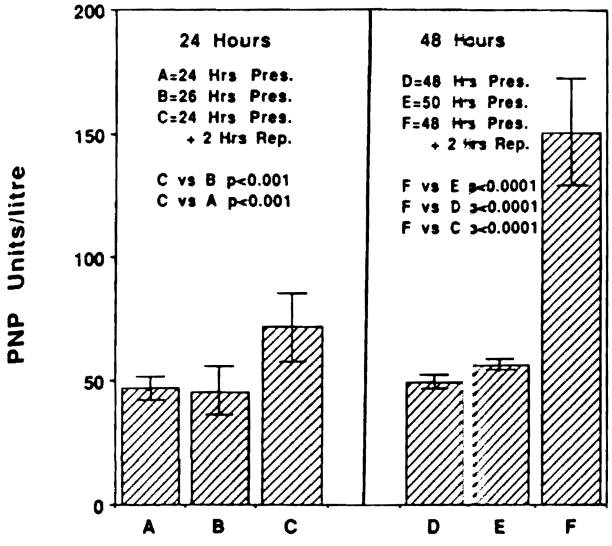

Fig 1. Levels of PNP at the end of 24, 26, 48, and 50 hours of cold preservation were similar to control levels and not different from each other. Reperfusion following either 24 hours (C) or 48 hours of cold preservation (F) resulted in significant injury to the microvascular endothelial cell ($P < .001$). Injury was more severe following 48 hours of cold preservation (F) as compared with 24 hours ($P < .0001$).

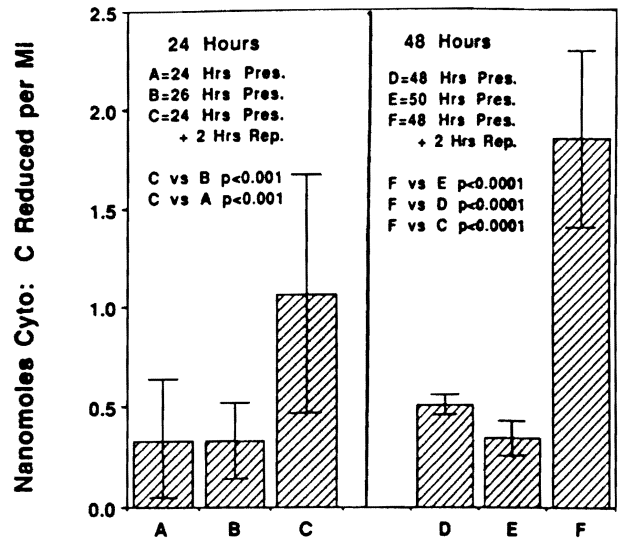

Fig 2. Reperfusion following 24 or 48 hours of cold preservation resulted in a significant burst of superoxide generation (C and F). Levels of superoxide were significantly greater ($P < .001$, $P < .0001$) than at the end of cold ischemia alone. Significantly higher superoxide levels were observed on reperfusion following 48 hours (F) of preservation, than the levels found after 24 hours (C; $P < .001$).

20.11 U, 88.83 ± 51.62 U, and 127.5 ± 19.15 U respectively. Reperfusion following 24 and 48 hours of cold preservation resulted in significantly higher ($P < .001$) levels of SGOT, of 221.31 ± 146.26 U, and 436.25 ± 248.87 U respectively. Hepatocellular injury was also significantly higher ($P < .007$) due to reperfusion at the end of 48 hours of cold preservation. Fig 3 presents the data for SGOT.

SGPT. Levels of SGPT at the end of 24, 26, 48, and 50 hours of cold preservation were 56.33 ± 24.41 U, 61.33 ± 21.52 U, 82.83 ± 69.27 U, and 182.17 ± 14.48 U respectively. Reperfusion at the end of 24 and 48 hours of cold preservation resulted in SGPT levels of 118.56 ± 74.33 U and 414.31 ± 221.02 U respectively, which were higher than those obtained at the end of cold preservation alone. Fig 4 shows the data for SGPT.

IL-1. Levels of IL-1 following 24, 26, 48, and 50 hours of cold preservation were respectively 0.81 ± 0.19 ng/mL, 0.77 ± 0.14 ng/mL, 0.55 ± 0.16 ng/mL, and 0.61 ± 0.09 ng/mL respectively. Reperfusion following 24 and 48 hours of cold preservation resulted in IL-1 levels of 1.04 ± 0.18 ng/mL and 0.89 ± 0.18 ng/mL respectively. These values were significantly higher ($P < .05$) than IL-1 values at the end of cold preservation alone. Fig 5 presents the data for IL-1.

DISCUSSION

Reperfusion following cold preservation resulted in significant injury to the microvascular endothelial cell as reflected by elevated levels of PNP, an endothelial cell-specific enzyme[16] (Fig 1). This injury was more severe on reperfusion following 48 hours of cold preservation as compared with 24 hours of preservation, and was not evident after cold preservation alone. Our data is in agreement with that of Caldwell-Kenkel et al[21] and Thurman et al[22] and contrary to that of McKeown et al and

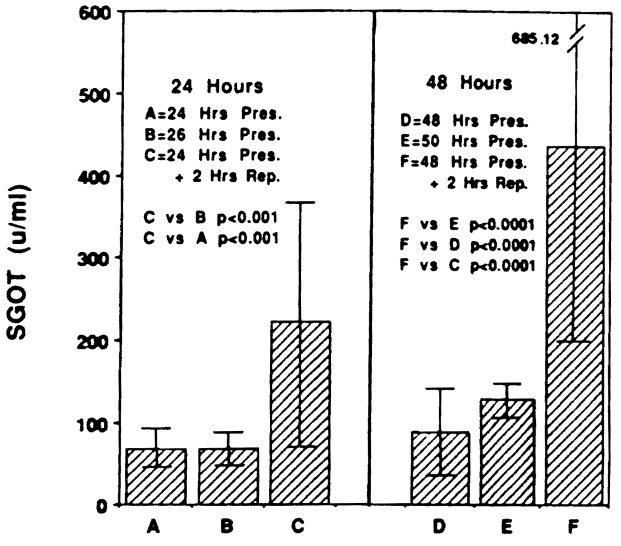

Fig 3. Reperfusion injury to the endothelial cell eventually resulted in hepatocellular injury. Hepatocellular injury as indicated by SGOT levels was more severe following 48 hours of cold preservation (F) as compared with 24 hours (C; $P < .007$).

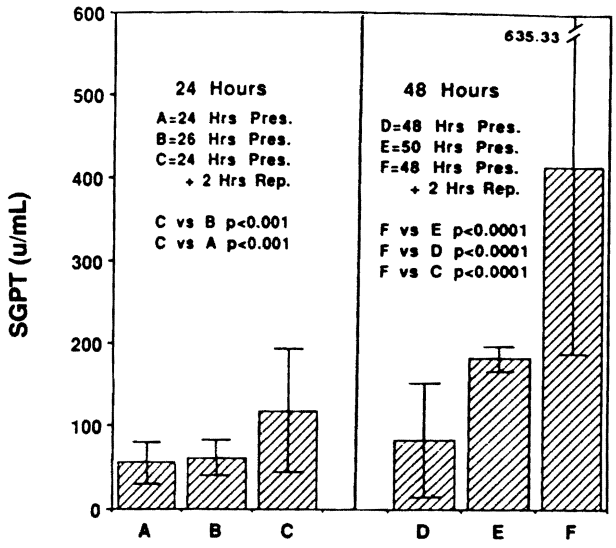

Fig 4. Levels of SGPT following 24 and 48 hours of cold preservation and reperfusion.

Holloway et al[23,24] who demonstrated injury to the sinusoidal endothelial cell following cold preservation alone. Toxic oxygen radicals liberated at reperfusion have been implicated in the injury to the endothelial cell.[4-6,15] In our experiments endothelial cell injury was accompanied by the generation of toxic oxygen radicals (Fig 2). Irreversible endothelial injury eventually resulted in severe injury to the hepatocyte (Figs 3 and 4).

Reperfusion injury following cold preservation resulted in significant activation of Kupffer cells as reflected by

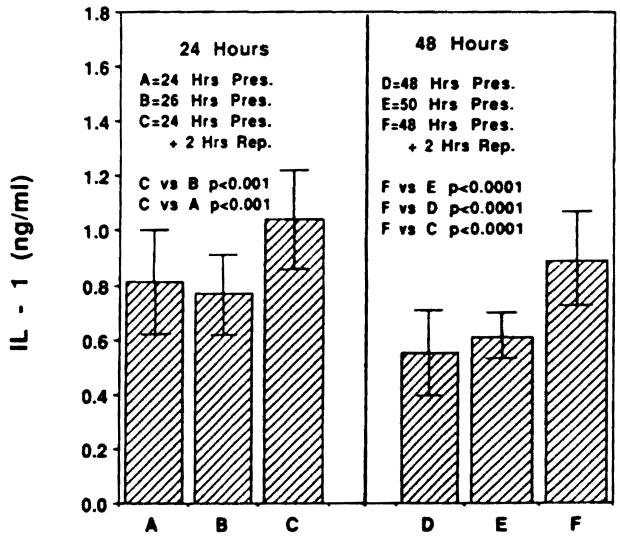

Fig 5. Reperfusion injury to the endothelial cell was accompanied by a significant degree of Kupffer cell activation as measured by IL-1 levels. Levels of IL-1 at the end of cold preservation alone were significantly lower ($P < .05$) than those following reperfusion.

elevated levels of IL-1 (Fig 5). Levels of TNF were also significantly elevated (data not shown). Again, elevated levels of IL-1 and TNF were observed only after reperfusion following cold ischemia. Our data is in complete agreement with that of LeMasters et al[25] and Colleti et al.[26] Howard et al[7] have recently demonstrated that severe preservation injury results in an increased incidence of cellular rejection. Activated Kupffer cells following reperfusion can further release toxic oxygen radicals, perpetuating endothelial cell injury. This injury can result in the recruitment of inflammatory cells into the allograft to clean up the injury. Inflammatory cells thus attracted, including lymphocytes and macrophages, could activate the immune response with synchronous expression of histocompatibility antigens in the allograft, increasing its antigenicity.

In conclusion, our data suggest that reperfusion injury following cold ischemia injures the endothelial cell and activates the Kupffer cell with a self-perpetuating injury. It is attractive to speculate that this injury may upregulate the antigenicity of the allograft.

REFERENCES

1. Van Thiel DH, Schade RR, Hakala TR, et al: Hepatology 4:66, 1984

2. Parks D. Bulkley GB, Granger DN, et al: Gastroenterology 82:9, 1982

3. Granger DN, Rutili G, McCord JM: Gastroenterology 81:22, 1987

4. Adkison D, Hollwart ME, Benoit JN, et al: Acta Physiol Scand 126:101, 1986

5. McCord JM: N Engl J Med 312:159, 1985

6. Marubayashi S, Dohi K, Ochi K, et al: Surgery 184:191, 1986

7. Howard TK, Klintmalm GBG, Cofer JB, et al: Transplantation 49:103, 1990

8. Wardle EN: Liver 7:63, 1987

9. Pober JS, Gimbrone MA: Proc Natl Acad Sci USA 79:6641, 1982

10. Pober JS, Gimbrone MA, Cotran RS, et al: J Exp Med 157:1339, 1983

11. Bevilacqua MP, Pober JS, Wheeler ME, et al: J Clin Invest 76:2003, 1985

12. Collins T, Lapierre LA, Fiers W, et al: Proc Natl Acad Sci USA 83:446, 1986

13. Bevilacqua MO, Pober JS, Mendrick DL, et al: Proc Natl Acad Sci USA 84:9238, 1987

14. Pober JS, Gimbrone MA, Lapierre LA: J Immunol 137: 1893, 1986

15. Rao PN, Walsh TP, Makowka L, et al: Transplantation 49:193, 1990

16. Rao PN, Walsh TR, Makowka L, et al: Hepatology 11:193, 1990

17. McCord JM, Fridovich I: J Biol Chem 244:6049, 1969

18. Ontell SJ, Makowka LM, Ove P, et al: Gastroenterology 95:1617, 1988

19. Cannon JG, Dinarello CA: Science 227:1247, 1985

20. Milliken GA, Johnson DE: Analysis of Messy Data. Vol 1, Designed Experiments. Belmont, CA, Wadsworth, 1981

21. Caldwell-Kenkel J, Thurman RG, LeMasters JJ: Transplantation 45:834, 1988

22. Thurman RG, Marzi I, Seiz G, et al: Transplantation 46:502, 1988

23. McKeown CMB, Edwards V, Phillips MJ, et al: Transplantation 48:178, 1989

24. Holloway CMB, Harvey PRC, Mullen JBM: Transplantation 48:178, 1989

25. LeMasters JJ, Caldwell Kenkel J, Currin RT, et al: In: Wisse E, Knook DL, eds. Cells of the Hepatic Sinusoid, Vol 2 277. Kupffer Cell Foundation, Rijswijk, The Netherlands, 1989

26. Colleti LM, Remick DG, Burtch GD, et al: J Clin Invest 85:1936, 1990