Transpl Int (1994) 7 [Suppl 1]: S204–S207

A. Ceriello
F. Mezza
S. Cozzolino
G. Pettinato
A. Mancini
W. Santaniello
F. Calise
O. Cuomo

Role of immunosuppression in recurrence after liver transplantation for diethylnitrosamine-induced tumors in rats

A. Ceriello · E. Mezza · S. Cozzolino
G. Pettinato · A. Mancini · W. Santaniello
F. Calise (✉) · O. Cuomo
Liver Unit P.M.P.
Research Laboratory, Ospedale Cardarelli,
Via Cardarellia, I-80131 Naples, Italy

Abstract Hepatocellular carcinoma is one of the world's most common malignant diseases, with an increasing incidence related to liver cirrhosis. The purpose of the study was to evaluate the role of immunosuppression in recurrence in rats transplanted after liver tumor induction by diethylnitrosamine (DENA), which has proved to be a reliable carcinogen. In 14-week-old Lewis rats weighing 200 g, tumors were induced by the oral administration (5 mg/100 ml in drinking water ad libitum) of DENA for 13 weeks. Orthotopic liver transplantation (OLT) was performed after 4 weeks' latency. In the Lewis/Lewis rats weighing 200 g, tumors sporin A (CsA) treatment, median survival was 199-days with no recurrence or metastasis. In the BN/Lewis group with no CsA (5 ats) median survival was 144 days. All rats died due to rejection. In the other BN/Lewis group (10 rats), OLT was followed by CsA administration (7.5 mg/kg). Median survival was 161 days. In three rats (218 days), there was liver tumor recurrence; in two rats (137.5 days), kidney and lung metastases were found. The remaining rats died of septic complications. In the Lewis/Lewis + CsA group (10 rats), median survival was 131 days with 5 recurrencies and/or metastases. Two rats are still surviving at 84 and 88 days. Our results suggest that the DENA model is reliable; it proved to have a similar carcinologic pattern to HCC in man. Moreover, immunosuppression seems to play an important role in determining recurrence. Further studies are needed to investigate the efficacy of chemotherapy agents pre- and post-transplantation.

Key words Liver transplantation
Diethylnitrosamine · Cyclosporin

Introduction

Hepatocellular carcinoma (HCC) is one of the world's most common malignant diseases, with an annual incidence of one million cases. It is widespread in the Orient and sub-Saharan Africa, and an increasing incidence in Western countries has recently been noted, mainly related to development in patients with hepatitis B virus (HBV+) and HCV+ cirrhosis [1].

Orthotopic liver transplantation (OLT) has been used in the treatment of primary liver cancer for over 30 years [2], but its role remains controversial, with high recurrence rates varying from 40 to 75% [3] and survival times rarely exceeding 2 years [4]. Several factors such as

various histologic types of malignancies or the presence of micrometastases have been advocated in order to explain the different survival rates after OLT.

The role of immunosuppression in determining recurrence either in the precyclosporine or cyclosporine era has been questioned [2, 5, 6], but no clear data so far support this hypothesis [7]. In order to estimate the influence of immunosuppression, an experimental model of OLT was devised using diethylnitrosamine (DENA) as the hepatic carcinogen in rats [8]. This report provides a more definite idea of the role of immunosuppression by cyclosporin A (CsA) after liver replacement in experimental cancer therapy.

Material and methods

Animals

Male 6-week-old Lewis rats, weighing approximately 150 g, were given 5 mg DENA/100 ml in their drinking water ad libitum for 13 weeks. OLT was performed after a 4-week latency. The rats were divided into four groups: group 1, Lewis/Lewis OLT with no immunosuppression; group 2, BN/Lewis OLT with no immunosuppression; group 3, BN/Lewis OLT followed by CsA administration (7.5 mg/kg intraperitoneally every other day); group 4, Lewis/Lewis OLT with CsA as in group 3.

Orthotopic liver transplantation

Transplantation was performed according to the technique described by Kamada [9]. Its particular characteristics include the absence of arterial reconstruction and the use of cuffs for portal vein and infrahepatic vena caval anastomosis. Shortcuts were taken to abbreviate the liver harvesting procedures: only knots were made towards the liver without cutting the main vessels.

Pathological assessment

A full autopsy was performed on each animal at death in order to determine the cause and, also, the nature and extent of pathological processes eventually present in the liver and other organs. Tissues were fixed in 10% formol buffered and stained with pH 7 hematoxylin and eosin.

Statistical methods

Student's *t*-test was used to compare survival times after transplantation and chi-square to compare recurrence rates.

Results

Gross and microscopic pathology

By 13 weeks of DENA treatment and a 4-week latency period the liver of each animal had at least two nodules more than 2 mm in diameter. Compared with two previous experiences [10], DENA administration for more than 13 weeks in Lewis rats the appearance of tumors too big to be transplanted. The shorter time needed for HCC development is thought to be related to the use of inbred rats. A 4-week latency was sufficient for tumors to be confined to the liver at the time of OLT.

No evidence was found of either primary or secondary disease outside the liver. The cancer nodules often bulged beneath Glisson's capsule and were well-circumscribed, soft, and yellow-brown in color. The tumor cells simulated normal liver cells, being characterized by large, round, hyperchromatic nuclei, prominent nucleoli, abundant granular eosinophilic cytoplasm, and a tendency toward arrangement in trabeculae. Sometimes the trabecular pattern was not so obvious.

Marked proliferation of the bile ducts was always present, and in some cases there was a combination of liver cell and bile duct carcinoma, with the former predominating as a rule. Occassionally, a poorly differentiated pattern with small round cells in addition to a large polygonal cell pattern was observed. Mitotic figures, foci of necrosis, and hemorrhage were present throughout. Tumor thrombi invading the hepatic veins were often observed.

Recurrent HCC showed the same features as described above. Metastatic nodules were found in the lung, pleura, and kidney, and the histology was similar to that of the primary tumors.

Table 1 Cumulative survival of all groups

Group	OLT	CsA	*n*	Survival (days)	Mean	SD
1	Lewis/Lewis	No	5	43, 186, 365, 350, 50	198.8	155.77
2	BN/Lewis	No	5	33, 237, 35, 132, 283	144.0	114.36
3	BN/Lewis	Yes[a]	10	101, 186, 71, 75, 161 153, 200, 77, 366, 218	160.8	59.65
4	Lewis/Lewis	Yes[a]	10	87, 106, 119, 130, 67, 191, 135, 212[b]	130.9	49.25

[a] Protocol: 7.5 mg/kg intraperitoneally every other day
[b] Two recipients are still alive at 84 and 88 days

Table 2 Recurrence rates

Diagnosis	BN/Lewis + CsA			Lewis/Lewis + CsA		
	Cases	Survival (days)	Mean	Cases	Survival (days)	Mean
Liver recurrence	3	71, 366, 218	218.3	2	130, 119	124.5
Metastases	2	75, 200	137.5	4	87, 106, 119, 130	110.5

Orthotopic liver transplantation

All rats surviving fewer than 30 days after OLT were excluded from this study. The survival and recurrence of all groups are shown in Tables 1 and 2. In the Lewis/Lewis group with no CsA, mean survival was 199-days with no recurrence or metastases. In the BN/Lewis groups with no CsA, median survival was 144 days. Mortality was always due to rejection. In the other BN/Lewis group, long-term immunosuppression with CsA 7.5 mg/kg every other day kept 5 of 10 recipients alive for more than 4 months but could not provide indefinite survival. Recurrence of tumor was found in 5 rats ($P < 0.001$): in 3 rats (218 days) there was liver tumor recurrence; in 2 rats (137.5 days) lung and kidney metastases were found. The remaining rats died from septic complications.

In the Lewis/Lewis group with an identical immunosuppression regimen to group 3, four rats died from lung metastases ($P < 0.001$). In two out of the five rats, liver tumor recurrence was also found. At the time of writing, two recipients are still alive, the longest survivor alive at 88 days after transplantation.

Discussion

The DENA model for hepatic tumor induction has proven to be simple and reliable, providing a similar carcinologic pattern to the one observed in man. Previous reports [11] have confirmed the association of DENA-induced liver tumors with lung metastasis. In this case, metastases arise when the liver tumors are too big for OLT; until now no elucidation has been given of the natural history of the tumor after liver transplantation, with a prolonged follow-up.

Isogenic liver transplantation in male Lewis rats provides the longest survival with no evidence of recurrent disease. In the allogeneic combination, death occurred because of liver rejection, with rats being free of tumors. In both groups with CsA immunosuppression, (3 and 4), the mean survival was not significantly different from that of group 2, but in half of the rats recurrence and/or metastases were found.

The most important factor determining survival is the presence of extrahepatic metastases at the time of transplantation; notably, in all rats of this study, there was no gross or microscopic evidence of extrahepatic disease at that time.

In the study from the Pittsburgh group [12], which utilised the same DENA model, no recurrence was found. The median survival time of 18 and 54 days in the groups treated with immunosuppression is, however, too short to draw any conclusion about the recurrence rate. In the Pittsburgh study, repeated laparotomy to assess the size of the tumors to be transplanted may have influenced the poor survival rate reported.

Whether or not the immunosuppression necessary to prevent graft rejection accelerates the growth of residual tumor [13, 14] also by influencing some host factors in the elimination of circulating cancer cells [5, 6] remains to be clarified. The data obtained confirm the hypothesis that immunosuppression plays a main role in the determination of recurrence after OLT. Further studies are warranted to investigate the efficacy of chemotherapy agents pre- and posttransplantation.

Acknowledgements We thank Domenico Capone, biologist, for statistical tests and Armando Coppola for technical assistance.

References

1. Wright T (1989) Cancer of the liver. Curr Opin Gastroenterol 5:405–410
2. Starzl TE, Demetris AJ (1990) Liver transplantation – a 31-year perspective. Year book medical, Chicago, pp 121–124
3. Penn I (1991) Hepatic transplantation for primary and metastatic cancers of the liver. Surgery 110:726–735
4. Pichlmayr R, Brolsch CH, Woigeit K (1984) Experiences with liver transplantation in Hannover. Hepatology 4:56–60
5. Alexander P, Eccles SA (1983) Host mediated mechanisms in the elimination of circulating cancer cell. Symp Fundam Cancer Res 36:293–328
6. Milas L, Peters TJ (1983) Conditioning of tissues for metastasis formation by radiation and cytotoxic drugs. Symp Fundam Cancer Res 36:321–336
7. Ringe B, Wittekind C, Bechstein WD, Bunzendhal H, Pichlmayr R (1989) The role of liver transplantation in hepato-biliary malignancy. Ann Surg 209:88–98
8. Argus MF, Mochligeti C (1961) Comparative study of the carcinogenic activity of nitrosamines. JNCI 27:695–709
9. Kamada A (1979) Orthotopic liver transplantation in the rat: techniques using cuff for portal vein anastomosis and biliary drainage. Transplantation 28:47
10. Ostrowsky JL, Ingleton PM, Underwood JCE, Parsons MA (1988) Increased hepatic androgen receptor expression in female rats during diethylnitrosamine liver carcinogenesis. Gastroenterology 94:1193–1200
11. Druckrey H, Schilldbach A, Schmahl D, Preussmann R, Ivankovic S (1963) Quantitative Analyse der Carcinogenwirkung von Diäthylnitrosamin. Arzneimittelforschung 13:841–851
12. Matsuzaki T, Murase N, Yagihashi A, Shinozuka H, Shimizu Y, Furuya T, Burrell N, Iwatsuki S, Starzl E (1992) Liver transplantation for diethylnitrosamine-induced hepatocellular carcinoma in rats. Transplant Proc 24:748–751
13. Iwatsuki S, Gordon RD, Shaw BW Jr, Starzl TE (1985) Role of liver transplantation in cancer therapy. Ann Surg 202:401–407
14. Starzl TE, Koep LJ (1978) Surgical approaches for primary and metastatic liver neoplasms, including total hepatectomy with orthotopic liver transplantation. Prog Clin Cancer 7:181–193