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Suppression of liver allograft rejection by administration of 15-deoxyspergualin

Comparison of administration via the hepatic artery, portal vein, or systemic circulation

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Abstract In this experiment, the effect of the administration route – the hepatic artery, portal vein, or systemic circulation – of the immunosuppressive drug 15-deoxyspergualin (DSG) on the suppression of liver allograft rejection is investigated. A 3-day injection of DSG at a dose of 0.32–1.28 mg/kg per day into the systemic circulation of a rat that had received a liver transplant was not effective in prolonging liver graft survival (14.3 ± 2.9 days vs. 14.1 ± 2.5 days for controls). However, the administration of DSG into the portal vein following liver transplantation markedly prolonged survival for up to 24.9 ± 10.0 days. Survival times were prolonged even more when the DSG was administered via the hepatic artery for 3 successive days after liver grafting (30.9 ± 9.6 days). The concentration of DSG in the blood following the one-shot injection of DSG was highest when DSG was administered via the hepatic artery, intermediate when injected into the portal vein, and lowest when injected into the systemic vein. In conclusion, DSG can inhibit liver graft rejection more effectively via the hepatic arterial route than via the portal vein or systemic circulation.

Key words 15-Deoxyspergualin, liver transplantation, rat · Liver transplantation, 15-deoxyspergualin, rat · Rat, liver transplantation, 15-deoxyspergualin · Local immunosuppression, liver transplantation, rat

Introduction

Since the introduction of cyclosporin A (CyA) and other new drugs such as FK 506 and 15-deoxyspergualin (DSG), liver allograft rejection can be better managed and controlled. This contributes to the establishment of liver transplantation as a treatment for end-stage hepatic disease. However, rejection is still the limiting factor to successful transplantation, and an overdosage of these immunosuppressive drugs often results in severe viral infection and various side effects of drug toxicity. More specific immunosuppression to lessen the number of the side effects is highly desirable, and in this regard "local immunosuppression", i.e., administration of the immunosuppressive drug directly into the supplying vessel of the transplanted organ, may be an alternative approach. It allows a higher blood concentration of the drug in the transplanted tissue using a smaller dose and provides a more effective regimen for the inhibition of rejection with fewer side effects. Although the effect of local administration of drugs to renal allografts via the renal artery has been described using a pump infusion system [2, 7, 11, 20, 25], there are no reports concerning its effect on liver allograft rejection. The liver has two supplying vessels, namely, the portal vein and the hepatic artery. We felt it would be interesting to determine whether local immunosuppression is beneficial in the case of liver allografts and, if so, which vessel is most effective at delivering the drug to suppress graft rejection.

In this experiment we investigated whether a short course of DSG would be effective in suppressing liver allograft rejection. Moreover, we compared the effect of administration routes (hepatic artery, portal vein, or systemic circulation) of DSG on the inhibition of rejection.

Materials and methods

Animals

Male inbred PVG ($RT1^C$) and Lewis ($RT1^1$) rats were obtained from Seiwa Experimental Laboratory and Charles River, Japan. PVG rats and Lewis rats weighing 250–300 g were used as donors and recipients respectively.

Drug

DSG was kindly supplied by Nippon Kayaku Co., Tokyo, Japan. All the drug concentrations used were made by dissolving them in sterile normal saline and administered to the rats via the hepatic artery, portal vein or penile vein.

Liver transplantation model

The method of liver harvesting and orthotopic liver transplantation was based on the cuff technique described by Kamada and Calne [9]. Either the aorta bearing the celiac artery and hepatic artery of the donor liver (Fig. 1) or the mesenteric vein of the recipient rats (Fig. 2) was cannulated with a silicone tube (602-135 or 602-105, Silastic tubing, Dow Corning, Midland, Mich., USA), which was connected to a subcutaneously placed reservoir for intra-arterial or intraportal infusion. The rats that died within 3 days of the operation were excluded from this study. After liver transplantation, survival times were recorded and compared among the groups. The rats that were sick or died were autopsied to determine the cause of death, and all liver specimens were subjected to histological studies.

Experimental protocols

In order to know the effect of a 3-day course of DSG on the survival of liver allografts, various doses of DSG (0.32 mg/kg, 1.28 mg/kg, 2.56 mg/kg, 5.12 mg/kg) were given daily to the recipient rats for 3 days after the operation. The rats were divided into four groups according to the method of administration: group 1 – no immunosuppression was administered (control group, $n = 8$), group 2 – DSG (0.32 mg/kg per day) was administered via the penile vein for 3 days (systemic administration group, $n = 8$), group 3 – DSG (0.32 mg/kg per day) was administered via the hepatic artery for 3 days (arterial administration group, $n = 8$), and group 4 – DSG (0.32 mg/kg per day) was administered via the portal vein for 3 days (portal administration group, $n = 7$).

Graft function and body weight

Rats were bled on the 4th, 7th, and 14th postoperative days and weekly thereafter for 1 month, then triweekly for another 2 months for the measurement of liver functions including serum glutamine oxaloacetic transaminase (GOT) and total bilirubin. The rats' weight was also monitored for 2 months and compared among groups.

Pharmacokinetic studies

In order to know whether there is some difference in the declining rate of the peripheral or portal blood concentration of DSG after the one-shot injection of DSG into either the hepatic artery, portal vein, or penile vein (systemic circulation), 4.0 mg/kg DSG was administered to normal rats that were then bled by puncture of the infrahepatic vena cava or portal vein from 15 min to 240 min after injection to measure the blood concentration of DSG.

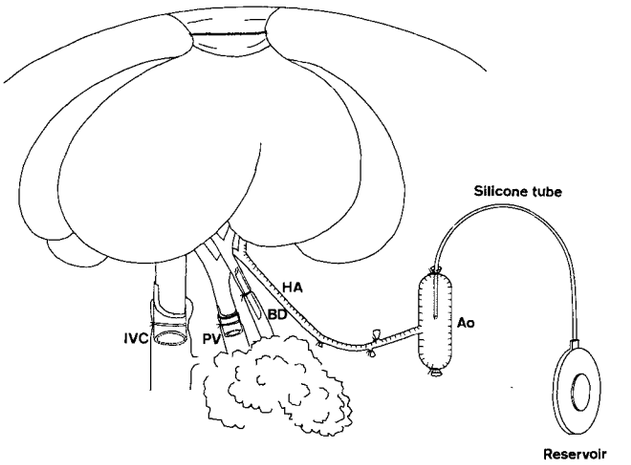

Fig. 1 Schematic representation of orthotopic liver transplantation using drug administration via the hepatic artery of the grafted liver. A silicone tube was introduced into the aorta bearing the hepatic artery of the transplanted liver. The other end of the tube was connected to a reservoir placed in a subcutaneous pocket. *IVC* Infrahepatic inferior vena cava; *PV* portal vein; *BD* bile duct; *HA* hepatic artery; *Ao* aorta

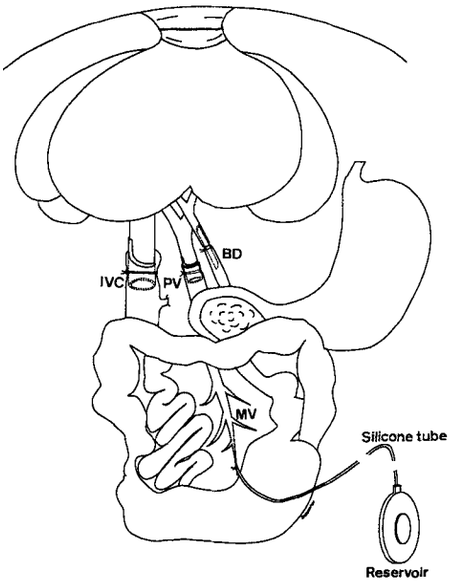

Fig. 2 Schematic representation of orthotopic liver transplantation using drug administration via the portal vein of the grafted liver. A silicone tube was introduced into the mesenteric vein of the recipient rat and ligated with silk suture. The other end of the tube was connected to the reservoir. *IVC* Infrahepatic inferior vena cava; *PV* portal vein; *BD* bile duct; *MV* mesenteric vein

Histology

Liver biopsies from each group were obtained 4 days after transplantation and subjected to histological study. Liver biopsies were also taken from the long-term (122 days) surviving rats treated via hepatic artery administration.

Statistical analysis

Statistical analysis was performed using the Wilcoxon rank test and differences were considered to be statistically significant when P was less than 0.05 in two-tailed test.

Results

Operation time

The total operation time for harvesting the donor liver in groups 1–4 was 22.1 ± 0.8 min, 23.0 ± 1.3 min, 36.5 ± 1.2 min, and 22.1 ± 1.1 min, respectively. It took 14 min more in group 3 due to the separation of the hepatic artery from the aorta. Portal crossclamping time was 20 min on the average and similar among the four groups. The total operation time for the recipient operation was 39.9 ± 1.6 min in group 1, 42.4 ± 3.2 min in group 2, 51.3 ± 2.4 min in group 3, and 51.4 ± 2.8 min in group 4. It took approximately 10 min more in groups 3 and 4 because of the cannulation of the hepatic artery or mesenteric vein.

Survival

Lewis rats that received PVG livers without any immunosuppression died between 9 and 21 days postoperatively due to acute graft rejection (control group 14.1 ± 2.5 days, $n = 8$). This combination of donor and recipient in this experiment is a rejection combination and is different from the nonrejection combination of DA as donor and PVG as recipient. The administration of 0.32 mg/kg per day DSG in the recipient rat via the penile vein for 3 consecutive days did not result in any prolongation of survival: all rats died within 15 days due to rejection (systemic administration group 12.0 ± 1.8 days, $n = 8$). When the dose was increased to 1.28 mg/kg for 3 days, survival was not extended (14.3 ± 2.9 days, $n = 3$); however, when it was increased to 2.56 or 5.12 mg/kg per day, survival of the liver allograft was 32.0 ± 10.5 days and 31.0 ± 16.6 days, respectively (Table 1).

When DSG was administered at the concentration of 0.32 mg/kg for 3 days via the hepatic artery, survival was markedly prolonged (30.9 ± 9.6 days), and two of the eight recipients survived over 60 days. This survival was comparable to or better than that of rats treated with 15 times the dose of DSG via the systemic route. Intraportal administration of DSG also extended survival for up to 24.9 ± 10.0 days, and one rat out of eight survived for more than 60 days (Table 2).

Table 1 Survival times of liver allografts with various doses of DSG administration. I.V., Intravenous injection; MST, mean survival time ± SD

Group	n	Treatment	Survival times (days)	MST ± SD (days)
1	8	None	9, 9, 13, 13, 14, 17, 17, 21	14.1 ± 2.5
2	8	DSG 0.32 mg/kg for 3 days, I.V.	9, 10, 12, 12, 12, 13, 13, 15	12.0 ± 1.8[a]
3	3	DSG 1.28 mg/kg for 3 days, I.V.	11, 16, 16	14.3 ± 2.9[a]
4	3	DSG 2.56 mg/kg for 3 days, I.V.	21, 31, 43	32.0 ± 10.5[a]
5	3	DSG 5.12 mg/kg for 3 days, I.V.	19, 24, 50	31.0 ± 16.6[a]

[a] Wilcoxon rank test for difference between group 4 and groups 1, 2 and 3 ($P < 0.01$), group 5 and groups 1, 2 and 3 ($P < 0.05$), and groups 2, 3 and 1 (NS)

Table 2 Survival times of liver allografts with various modes of DSG administration. MST, Mean survival time ± SD

Group[a]	n	Treatment	Survival times (days)	MST ± SD (days)
1	8	None	9, 9, 13, 13, 14, 17, 17, 21	14.1 ± 2.5
2	8	DSG 0.32 mg/kg for 3 days	9, 10, 12, 12, 12, 13, 13, 15	12.0 ± 1.8[b]
3	8	DSG 0.32 mg/kg for 3 days	18, 19, 20, 20, 21, 29, > 60, > 60	30.9 ± 9.6[b]
4	7	DSG 0.32 mg/kg for 3 days	13, 13, 14, 16, 17, 41, > 60	24.9 ± 10.0[b]

[a] 1: No treatment (control group), 2: Intravenous injection (systemic administration group), 3: Intrahepatic arterial injection (arterial administration group), 4: Intraportal venous injection (portal administration group)

[b] Wilcoxon rank test for difference between group 3 and groups 1 and 2 ($P < 0.01$), group 4 and groups 1 and 2 ($P < 0.05$), and groups 2 and 1 (NS)

Liver function and body weight change

The results of the serial measurement of serum GOT, total bilirubin, and body weight are shown in Fig. 3–5. Serum GOT gradually rose until death in groups 1 and 2. Serum GOT in group 4 reached its peak at 7 days post-transplantation, decreased by 3 weeks post-transplantation, but gradually increased thereafter until death. Serum enzyme release in group 3 was clearly suppressed throughout the observation as compared with that in groups 1, 2, and 4. The comparison of serum total bilirubin among groups showed that that of group 3 was lower throughout the observation than that of groups 1, 2, and 4.

All rats in the four groups initially lost weight after the operation. The rats in group 4 lost weight progressively, while those in group 3 only gained weight after 21 days.

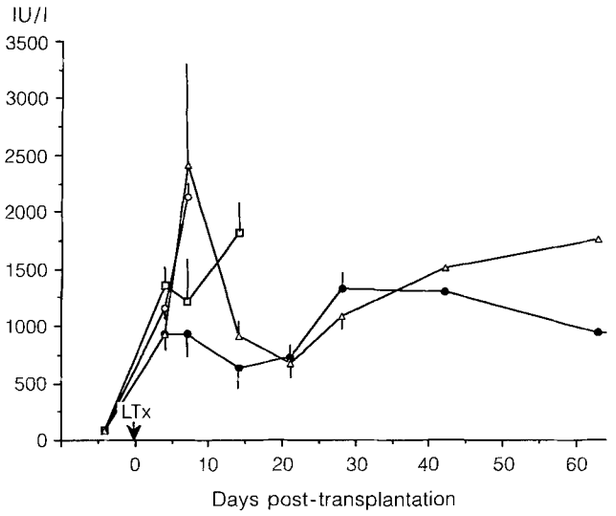

Fig. 3 Postoperative serum GOT in rats after liver transplantation using various modes of DSG administration. -□- Group 1 (no treatment), -○- group 2 (intravenous injection), -●- group 3 (intrahepatic arterial injection), -△- group 4 (intraportal venous injection)

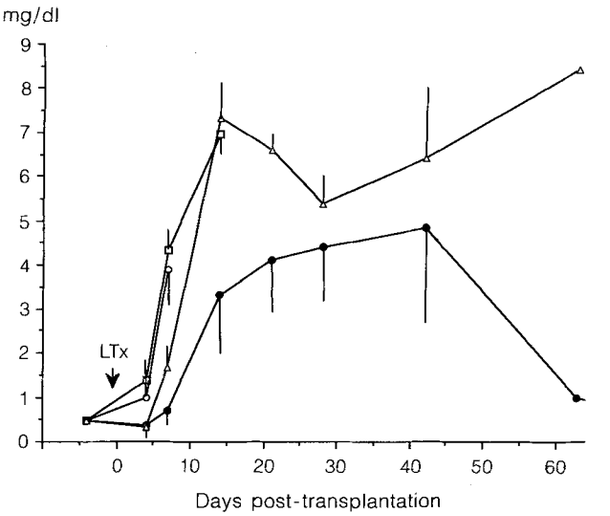

Fig. 4 Postoperative total bilirubin concentration in rats after liver transplantation using various modes of DSG administration. -□- Group 1 (no treatment), -○- group 2 (intravenous injection), -●- group 3 (intrahepatic arterial injection), -△- group 4 (intraportal venous injection)

Histological findings

Table 3 summarizes the histopathological findings in the allografted livers on day 4 after transplantation. A histopathological distinction was apparent between groups 1 and 2 and groups 3 and 4. The allografted livers in groups 1 and 2 showed almost the same findings. Marked portal inflammation was predominated by the presence of lymphocytes admixed with variable numbers of polymorphonuclear leukocytes (Fig. 6 A) that infiltrated into the liver parenchyma, corresponding to "spillover", and also into the sinusoids. Attachment of lymphocytes to the endothelium and subendothelial infiltration were conspicuous in both the portal and central veins (endothelialitis). The bile duct demonstrated degenerative or reactive changes including nuclear enlargement, pleomorphism, and overlapping of nuclei, but there was no apparent pyknosis or necrosis. A few single necrotic cells and vacuolar degeneration of hepatocytes were also noted, especially in the periportal area. Cholestasis was minimal. The findings as a whole were diagnosed as moderate to marked acute rejection. Although portal infiltration, endothelialitis and bile duct damage were also found in groups 3 and 4 (Fig. 6 B, C), they were less prominent than in groups 1 and 2. A few single hepatocytes showed necrosis, but no vacuolar degeneration or cholestasis was evident. The allografted livers in groups 3 and 4 were diagnosed histologically as showing mild acute rejection. One rat each in groups 3 and 4 demonstrated no signs of rejection in the grafted liver according to histology. On day 10 after transplantation, the portal infiltrate became

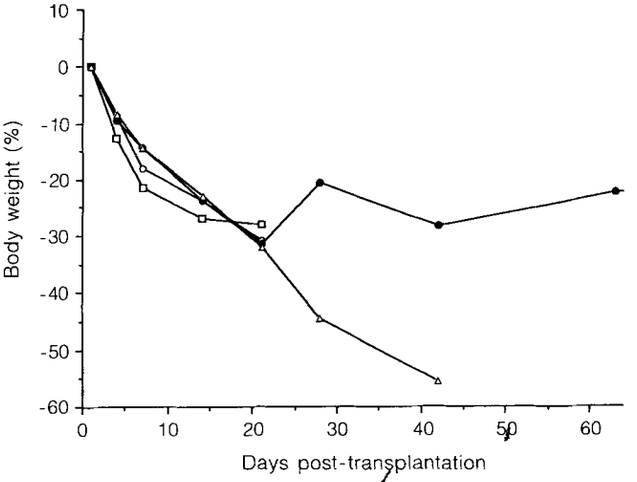

Fig. 5 Postoperative body weight curve in rats after liver transplantation using various modes of DSG administration. - □ - Group 1 (no treatment), -○- group 2 (intravenous injection), -●- group 3 (intrahepatic arterial injection), -△- group 4 (intraportal venous injection)

progressively associated with an increasing number of sinusoidal inflammatory cells in all of the grafted livers in the four groups. Bile duct damage was apparent with focal epithelial necrosis and inter- and intraepithelial granulocytic infiltrates. Endothelialitis was more conspicuous. In addition to a few single necrotic hepatocytes, recent zonal coagulation necrosis was observed in groups 1 and 2, but not in groups 3 and 4. The zonal liver cell ne-

Table 3 Histopathological findings in the allografted liver on the 4th day after transplantation

[a] 1: No treatment (control group), 2: Intravenous injection (systemic administration group), 3: Intrahepatic arterial injection (arterial administration group), 4: Intraportal venous injection (portal administration group)

Group[a]	Portal infiltrate	Endothelialitis	Bile duct damage	Liver cell necrosis	Other
1	+ + +	+ + +	+	+	Vacuolar degeneration
2	+ + + (spillover)	+ + +	+	+	Vacuolar degeneration
3	+ / + +	+	+	+	
4	+ / + + (spillover)	+	+	+	

crosis was estimated as affecting 30%–60% of the total parenchyma.

An open biopsy was performed in a rat that survived until 122 days after transplantation with DSG via the hepatic artery. The grafted liver showed mild portal inflammatory infiltration and fibrosis (Fig. 7 A) but did not show loss of bile ducts, which is one of the characteristic findings of chronic rejection in the human grafted liver. On the other hand, a large number of small bile ducts had proliferated in some Glisson's sheaths (Fig. 7 B). Endothelialitis was not evident. Hepatocytes were well preserved without cholestasis, fatty degeneration, or zonal necrosis.

Necropsy was performed on all rats after death. All of the grafted livers showed various degrees of zonal necrosis with granulocytic and lymphocytic infiltration. Granulation tissue replaced the liver parenchyma in some cases. There were no significant changes in other organs except for acute congestion. All of the rats were considered to have died of grafted liver dysfunction.

Discussion

15-Deoxyspergualin has been shown to be an effective immunosuppressive agent in experimental heart, kidney, pancreas, and liver transplantation and in clinical kidney transplantation. However, documentation of the effect of DSG on the suppression of liver allograft rejection is rare. To the best of our knowledge, Engemann and Inagaki have demonstrated that 14-day administration of DSG (1.0–2.5 mg/kg per day) can induce a transplantation tolerance in the orthotopic rat liver transplant model (ACI into LEW or PVG into LEW combination) [4, 5, 8].

However, it has also been suggested that DSG can cause severe gastrointestinal problems such as diarrhea, vomiting, or gastrointestinal bleeding, even with small doses. In this regard, it is a key point to reduce the total dose of DSG in order to avoid side effects while still retaining its beneficial effect.

The concept of "local administration" via the feeding artery into the affected organ was first described by Klopp et al. in 1950 and originally applied in cancer chemotherapy [10]. This method maintains a high blood concentration of the anticancer drug in tumor tissue with a reduced peripheral blood concentration and is regarded as being ideal treatment in terms of increasing the pharmacological benefit and decreasing the systemic drug toxicity. It would be very exciting if this method would also work in the treatment of suppression of rejection after organ transplantation. There is evidence that rejection can be prevented by controlling immunological events occurring at the graft site. Ascher et al. demonstrated that donor-specific T cells are recruited from the circulation to the graft and locally expanded in the transplanted graft where they mediate graft rejection [1]. Gergely and Coles [6] and Wolf et al. [27] have also shown that local irradiation of an allograft delayed the onset of rejection. These studies support the rationale of local immunosuppression after organ transplantation.

It is particularly interesting to note that the effect of the administration route for the delivery of the immunosuppressive drug (arterial route > portal route > intravenous route) decreases with regard to survival and postoperative liver function. The reason why the effect differs among the routes is still controversial. One reason is presumably due to the mechanisms of liver rejection. Biliary epithelial cells and sinusoidal lining cells normally display both class I and class II MHC antigens only weakly, if at all [12], and become an initial immune target for recognition by host T cells when livers are transplanted. However, during rejection, the expression of MHC antigens is markedly increased on all types of cells by the cytokines [21, 26]. Class I antigens in particular, are expressed strongly not only by hepatocytes but also by bile duct cells [16, 22], and class II antigens are massively induced on bile duct epithelial cells [3, 23]. Rejection thus develops more vigorously. As the vanishing bile duct is regarded as a final stage of the rejected liver, bile duct epithelial cells are the principal target of immune attack in the mechanisms of liver rejection [13]. Considering the fact that the bile duct system is nourished by arterial blood flow [15, 18, 19], it seems obvious that direct delivery of immunosuppressive drugs into the hepatic artery after liver transplantation is a more effective regimen for the suppression of bile duct epithelial cell damage by immune attack due to liver rejection. This notion of "local immunosuppression" makes it possible to have a higher blood concentration of the immunosuppressive drug in the afferent ves-

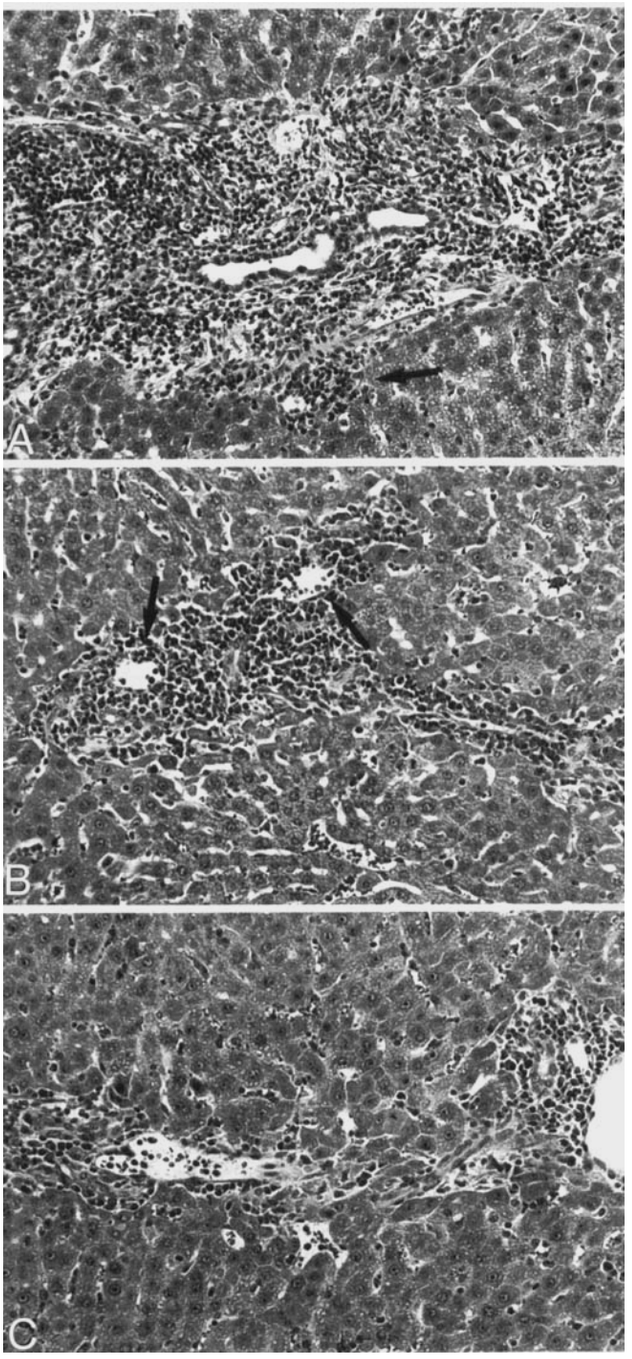

Fig. 6 A–C Histological findings of the grafted livers on the 4th day after transplantation using intravenous, intraportal, and intrahepatic arterial DSG (0.32 mg/kg) administration: **A** intravenous administration shows marked portal infiltrate composed predominantly of lymphocytes, endothelialitis with bile duct damage including pleomorphism, anisocytosis, and overlapping of nuclei, corresponding to moderate to marked acute rejection. Lymphocytes infiltrate into lobule ("spillover"; *arrow*); **B** allografted liver receiving intraportal DSG reveals less marked portal infiltrate, endothelialitis *(arrows)*; **C** administration via the hepatic artery results in minimal portal infiltrate without conspicuous damage of bile ducts (H & E, × 150)

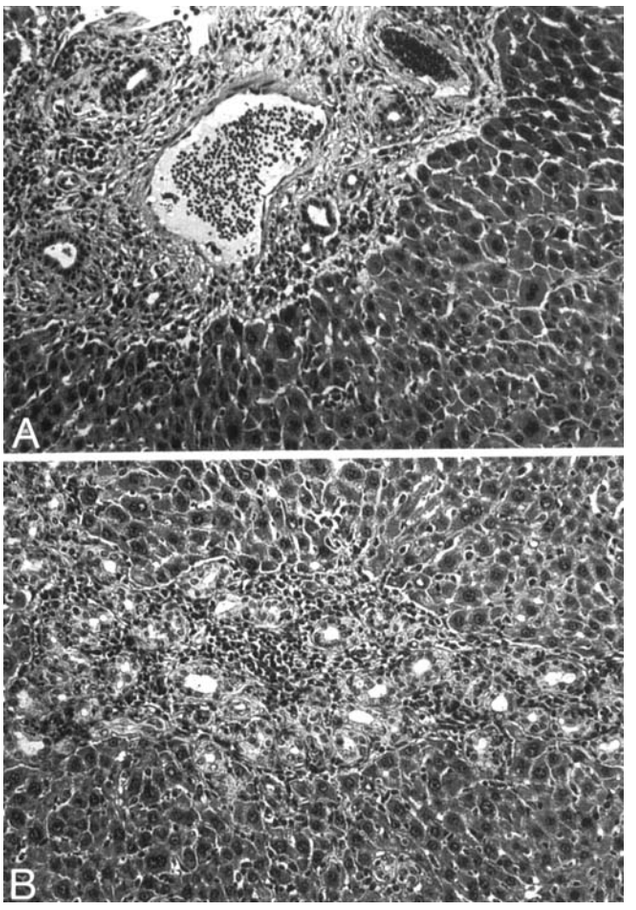

Fig. 7 A, B Histopathological findings of grafted livers surviving more than 122 days after transplantation receiving DSG via the hepatic artery: **A** portal area shows apparent fibrosis with few lymphocytic infiltrates. Loss of bile duct and obliterative endarteritis are not observed; **B** small bile ducts proliferate in some portal tracts These findings are not consistent with chronic rejection. (H & E × 150)

sels of transplanted organs with a reduced total dosage and also decreases toxicity of the drugs in the peripheral vessels.

Another interesting finding is that administration of DSG via the hepatic artery resulted in a higher venous plasma concentration of DSG than administration via the portal vein, which in turn resulted in a higher venous plasma concentration than systemic administration of the drug. Thus, administration of the same dose via different routes results in a hierarchy of venous plasma concentrations (Figs. 8, 9). These results were not anticipated and may suggest that the total administered dose can be reduced in the case of hepatic arterial administration as compared to either the portal or venous route if similar peripheral blood concentrations of DSG can be obtained.

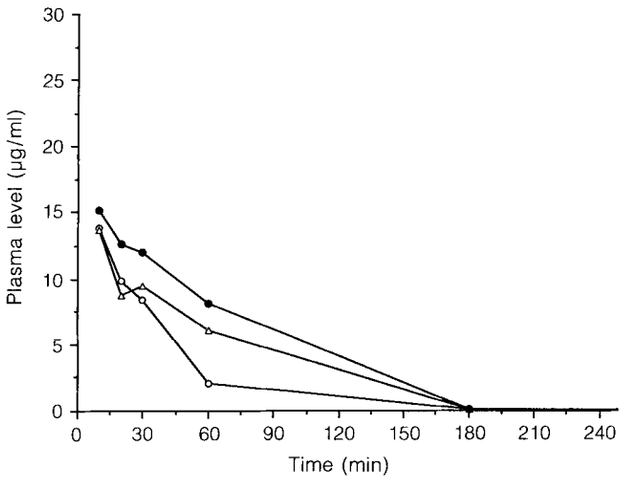

Fig. 8 Venous plasma concentration of DSG versus time using various modes of DSG administration (4.0 mg/kg dosage). -○- Intravenous injection, -●- intrahepatic arterial injection, -△- intraportal venous injection

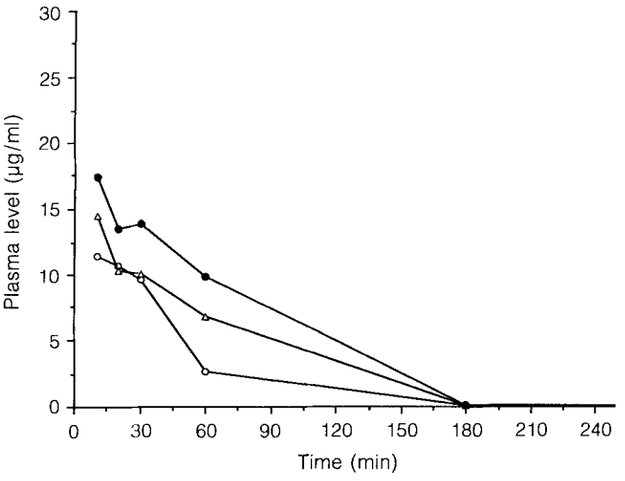

Fig. 9 Portal plasma concentration of DSG versus time using various modes of DSG administration (4.0 mg/kg dosage). -○- Intravenous injection, -●- intrahepatic arterial injection, -△- intraportal venous injection

There is no need to explain this phenomenon; however, it may well be said that the intra-arterial injection of DSG produces its high blood concentration during the initial circulation in the bile duct tissue of transplanted livers where rejection is occurring. DSG is not a drug [17, 24] that is metabolized in the liver like FK 506 [14], and the data in Fig. 8 and 9 show that DSG blood concentrations are maintained for hours after intra-arterial administration. DSG may bind to a receptor in bile duct tissue without metabolism or become deposited among graft tissue and is slowly released into the systemic circulation. These hypotheses should be investigated further.

In conclusion, DSG can be immunosuppressive in the rat liver transplant model and higher venous plasma concentrations of DSG are associated with more effective immunosuppression. Furthermore, administration of the same dose of DSG via the hepatic artery, portal vein, and systemic vein results in a hierarchy of venous plasma concentrations. These results suggest that intra-arterial infusion of the immunosuppressive drug intermittently to the transplanted liver post-operatively is an effective regimen for preventing rejection while reducing the total dosage and subsequently eliminating side effects. This method is now open to clinical application.

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