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Improvement in Host Immunity and a Decrease in Morbidity by the Preoperative Administration of OK432 in Cirrhotic Patients with HCC

Yoshinobu SATO[1], Kentaro TAKAGI[2] and Katsuyoshi HATAKEYAMA[1]

[1]The First Department of Surgery, Niigata University School of Medicine, [2]Department of Surgery, Niigata Prefectural Central Hospital, Niigata, Japan

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Summary. **Preoperative management in cirrhotic patients is extremely important to the liver surgeon in terms of preventing postoperative organ failure and infection. In this study we investigated the effects of OK432, a streptococcal preparation that is a well-known immunopotentiator for macrophages, in modulating the liver function of patients (9 patients) with hepatocellular carcinoma accompanied with liver cirrhosis above 20% in ICG-R15. The preoperative administration of OK432 for the patients resulted in a significant improvement in the hyaluronic acid levels (before treatment vs after treatment: 293.1±111.6 ng/ml vs 145.6±80.0 ng/ml, p=0.0008) and natural killer (NK) cell activity (before treatment vs after treatment: 24.4±21.6% vs 35.5±15.1%, p=0.0166) as well as increases in levels of HLA-DR-, CD56-, CD8-, and TCR $\gamma\ \delta$-positive lymphocytes in peripheral blood--although these data were not statistically significant. The period before the removal of the abdominal drain (10.9±16.6 days vs 26.8±11.4 days), the length of hospitalization (33.2±13.2 days vs 39.4±13.8 days, and the incidence of episodes of postoperative complications (33.3% (3/9, 3 episodes) vs 66.7% (10/15, 12 episodes)) in the OK432-treated group were all more favorable than those in the OK432-untreated group (15 patients).**

These findings suggest that the preoperative administration of OK432 in cirrhotic patients may be useful for preventing postoperative liver failure and infection through inhibition of excessive immunological reactions of Kupffer cells, the enhancement of sinusoidal endothelial cell (SEC) function, and the activation of NK cells and NKT cells.

Correspondence: Yoshinobu Sato M. D., The First Department of Surgery, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Niigata 951-8510, Japan.

Key words—sinusoidal endothelial cell, Kupffer cell, endotoxin, liver injury, tumor necrosis factor.

INTRODUCTION

Preoperative liver function in patients with hepatocellular carcinoma (HCC) has great importance for the postoperative course after liver surgery. Therefore, the preoperative management of HCC with liver cirrhosis in terms of liver function is also crucial to the postoperative course.

In this study, we investigated the effects of the preoperative administration of OK432[1,2,3], a streptococcal preparation that has been observed to elevate the capacity of hepatic macrophages to produce superoxide and tumor necrosis factor (TNF) as well as enhance the mRNA expression of interleukin-1-α, -β, and TNF-α in liver nonparenchymal cells[4]. The effects of the administration were evaluated in terms of prophylaxis from postoperative infections and liver failure in HCC patients with liver cirrhosis over 20% in indocyanine green dye retention rates for 15 min. (ICG-R15).

MATERIALS AND METHODS

Nine patients with HCC accompanied by liver cirrhosis of over 20% in ICG-R15 (from 20% to 54%, mean 32%) who were admitted from April 1997 to September 1998 were studied. The surgical procedures included 4 partial hepatectomies, 4 subsegmentectomies, and 1 lateral segmentectomy. The patients

were administered OK432 preoperatively, with a total of 4 dosages increased gradually from 0.5 KE to 2.0 KE. Immunological examination of the peripheral lymphocytes subset, natural killer (NK) activity, CH50, and serum hyaluronic acid were measured pre-or post-treatment. As a control group, fifteen patients were checked in relation to the presence of any postoperative infection, the length of hospital stay, and the period after which the abdominal drain could be removed. The patients, all of whom were assessed as having liver cirrhosis of over 17% in ICG-R15 (17%-40.7%, mean 24.5%), had undergone liver resection for HCC between May 1994 and March 1997. There were no significant differences between the two groups; however, ICG-R15 in the OK432 treated group was higher than that in the non-treated group. (Table 1)

Continuous data were analized by the t test for independent/dependent samples. The x^2 test was applied to test for differences in patient characteristics across the studies. Data were considered significant when probabilities were less than 0.05 in all figures and tables.

RESULTS

1. Changes in serum hyaluronic acid, CH50, and serum endotoxin levels after administration of OK432

All patients were Child B or C and showed abnormalities in their serum hyaluronic acid levels (normal: ≦ 50 ng/ml). After the administration of OK432, there was a statistically significant increase in the levels of hyaluronic acid from 293.1±111.6 ng/ml to 145.6± 80.0 ng/ml (p=0.0008) (Fig. 1). Serum CH50 levels (normal: 35-45 U/ml) reached a lower limit in almost all patients. These levels increased in a few patients following treatment with OK432, but the difference was not significant (Fig. 2).

There was no statistical difference in the serum endotoxin levels before and after the treatment with OK432 (Fig. 3).

2. Changes in natural killer activity after the administration of OK432

Almost all patients showed an elevation of NK activity levels with a statistical significance from 24.4± 21.6% to 35.5±15.1% (p=0.0166) after the administration of OK432, except for one case (67% to 54%) (Fig. 4).

3. Changes in peripheral lymphocyte subsets after administration of OK432

There were no significant differences in the subsets of peripheral lymphocyte levels, including those of CD 3-, CD4-, CD8-, CD56-, T cell receptor (TCR) γ δ-, and HLA-DR-positive lymphocytes. However, the levels of HLA-DR-, CD56-, CD8-, and TCRγ δ-positive lymphocytes tended to increase following treatment with OK432 (Figs. 5 and 6).

Table 1. Comparison of preoperative laboratory data between the OK432 treated group and non-treated group

	OK432 Treated group	OK432 non Treated group	p-value
Number	9	15	
Age	69±6 (62−78)	66.7±6.0 (59−78)	0.382
F/M	3/6	4/11	0.727
Alb (g/dl)	3.26±0.4	3.19±0.17	0.683
T. Bil (mg/dl)	1.13±0.46	0.88±0.36	0.156
PT (%)	74.6±6.16	68.4±16.2	0.287
ICG-R15 (%)	31.9±13.4	24.5±7.24	0.091

There were no significant differences between the two groups; however, ICG-R15 in the OK432 treated group was higher than that in the OK432 non-treated group. PT, prothrombin time; ICG-R15, indocyanine green dye retention rate for 15 min.

Data expressed as mean±SD.

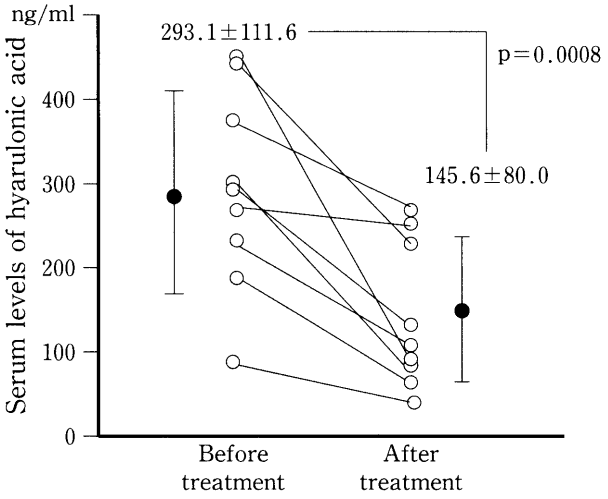

Fig. 1. Changes in serum hyaluronic acid levels after the administration of OK432. Serum levels of hyarulonic acid decreased in all patients with a statistically significant difference. Data expressed as mean±SD.

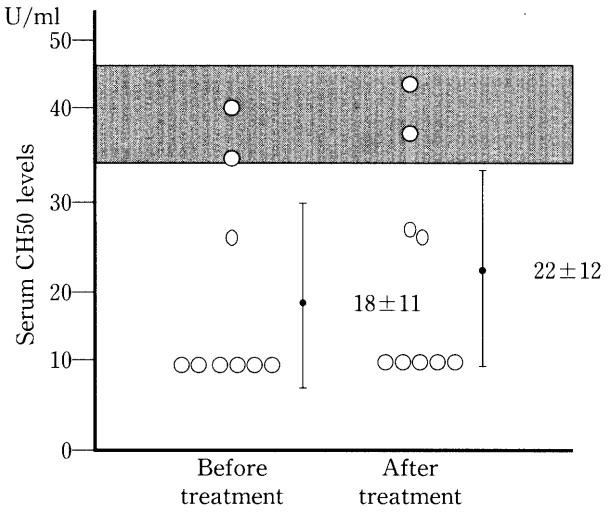

Fig. 2. Changes in serum CH50 levels after the administration of OK432. Serum CH50 levels reached a lower limit in almost all patients. These levels increased in a few patients following treatment with OK432, but the difference was not significant. Data expressed as mean± SD.

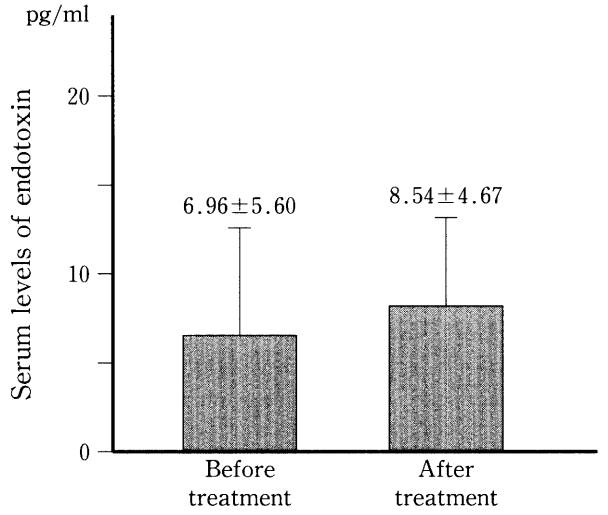

Fig. 3. Changes in serum endotoxin levels after the administration of OK432. There was no statistical difference in the serum endotoxin levels before and after the treatment with OK432. Data expressed as mean±SD.

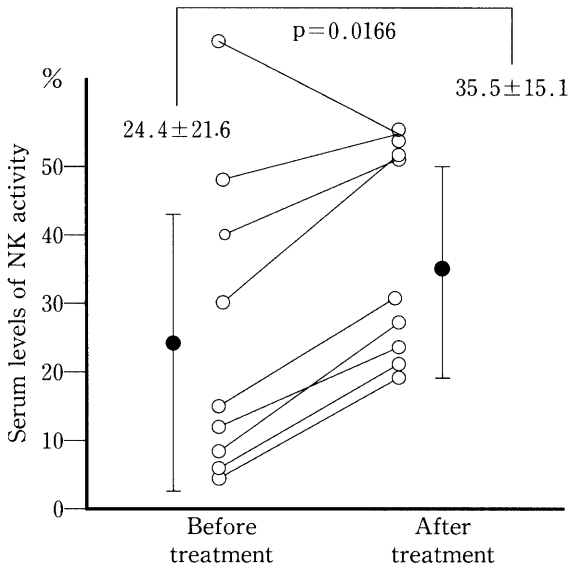

Fig. 4. Changes in natural killer activity after the administration of OK432. Almost all patients showed an elevation of NK activity levels with a statistical significance after the administration of OK432, except for one case. Data expressed as mean±SD.

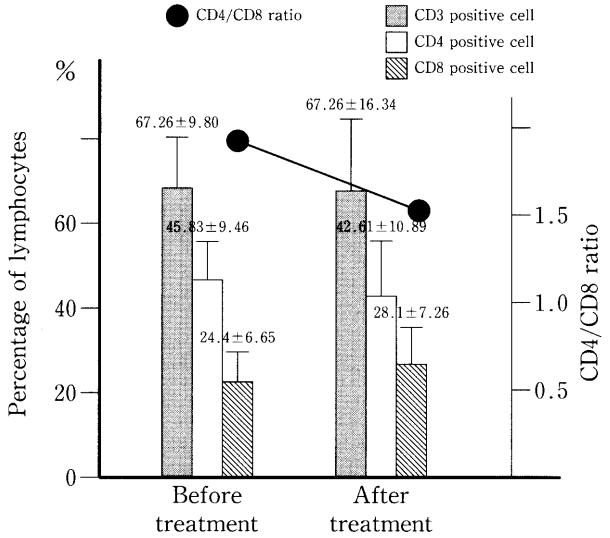

Fig. 5. Changes in CD3, CD4, and CD8 lymphocytes in the peripheral blood after the administration of OK432. There were no significant diferences in CD3-, CD4-, and CD8-lymphocyte levels before and after the treatment with OK432. However, CD4-lymphocytes tended to decrease and CD8-lymphocytes tended to increase following treatment with OK432. Data expressed as mean±SD.

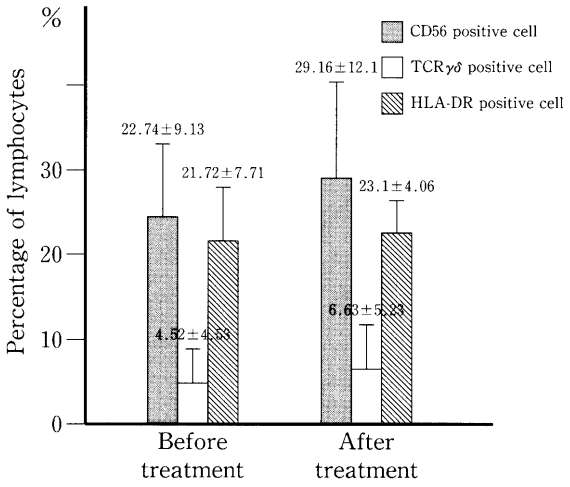

Fig. 6. Changes in CD56-, TCR γ δ-, and HLA-DR lymphocytes in peripheral blood after the administration of OK432. CD56-, TCR γ δ-, and HLA-DR lymphocytes tended to increase following treatment with OK432, but the difference was not significant.
Data expressed as mean±SD.

4. Operative procedure, period before the removal of the abdominal drain, hospital stay, and postoperaive complications after surgery in the patients with or without treatment of OK432

The period before the removal of the abdominal drain in the OK432 treated group was significantly shorter than that of the untreated group (16.6±10.9 days vs 26.8±11.4 days: p=0.0446). The hospital stay of the treated group was also shorter than that of the untreated group, but not significant (33.2±13.2 days vs 39.4±13.8 days: p=0.293). The OK432-treated group exhibited three complications in three patients (3/9: 33.3%): one of these was postoperative bleeding, which did not require laparotomy; and the two others involved infections of the liver cut margin. The non-treated group exhibited twelve complications in ten patients (10/15: 66.7% vs treated group: p=0.112): 6 infections of the liver cut margin, two wound infections, two cases of MRSA enteritis, one case of adult respiratory distress syndrome (ARDS), and one case of catheter infection (Table 2 and 3). Moderate fever was shown in all patients treated with OK432; however, all responded to symptomatic treatment without any problem.

DISCUSSION

The fact that major hepatic resection is increasingly common in the treatment of malignant disease is largely due to the decreasing morbidity and mortality arising from the procedure. The mortality of liver surgery has been reported as 2.8-20%. Despite these reductions in mortality, however, the morbidity of the procedure remains high, with a 27% to 47% postoperative complication rate.[5-9] In particular, liver cirrhotic patients[10] are compromised hosts, suffering reduced liver function, immunity, metabolic function, renal function (hepato-renal syndrome), and respiratory function (hepato-pulmonary syndrome). In these patients, liver failure, infections, ARDS, and multiple organ failure (MOF) more likely occurred after hepatic resection. Therefore, preoperative management in cirrhotic patients is extremely important for preventing these complications. This study employed OK432, a streptococcal preparation that is a well-known immunopotentiator for macrophages, to modulate the function of hepatic macrophages[1-3,11,12]. Moreover, we have examined the efficacy of OK432 in sinusoidal endothelial cell function. There have been no earlier reports of pretreatment with OK432 in HCC patients with liver cirrhosis similar to our

Table 2. Operative procedure, removal periods of the abdominal drain, hospital stay, and postoperative complications in the OK432 treated group

Case	Operative procedure	Removal of drain	Hospital stay	Complication
1	S5 subseg.	9 days	21 days	–
2	S8 part. S5 MCT	12 days	24 days	hemolytic jaundice
3	Lat. Seg. S4 part.	12 days	20 days	–
4	Ext. S8 subseg.	45 days	47 days	infection of cut margin
5	S6 part.	12 days	31 days	–
6	S3 part. S58 MCT	14 days	32 days	–
7	S5 seg.	12 days	26 days	–
8	S6 part.	18 days	60 days	infection of cut margin
9	S8 subseg.	16 days	38 days	–
		16.6±10.9	33.2±13.2	33.3% (3/9, 3 episodes)

subseg, subsegmentectomy; part, partial hepatectomy; MCT, microcoagulation therapy; Lat seg, lateral segmentectomy; Ext subseg, extended subsegmentectomy

Data expressed as mean±SD.

Table 3. Operative procedure, removal periods of the abdominal drain, hospital stay, and postoperative complications in the OK432 non treated group

Case	Operative procedure	Removal of drain	Hospital stay	Complication
1	S7 subseg.	21 days	29 days	–
2	S8 subseg.	14 days	24 days	–
3	S5 subseg. S2 part.	36 days	42 days	infection of cut margin
4	S8 part.	18 days	30 days	wound infection
5	S4,5,6 part.	28 days	32 days	infection of cut margin
6	S8 part	20 days	35 days	–
7	Extended medialseg	38 days	38 days	infection of cut margin
8	rt. hepatectomy	35 days	54 days	massive ascites
9	S5 subseg. S8 part	48 days	52 days	infection of cut margin
10	S8 part	16 days	26 days	–
11	S4, S8 subseg.	28 days	51 days	infection of cut margin
12	S7, S8 part	15 days	28 days	cathetel infection
13	S7 part, S8EI	25 days	72 days	ARDS, MRSA enteritis
14	Anterior seg.	46 days	51 days	MRS Aenteritis, wound infection
15	S3 subseg.	14 days	27 days	–
		26.8±11.4	39.4±13.8	66.7% (10/15, 12 episodes)

subseg, subsegmentectomy; part, partial hepatectomy; seg, segmentectomy; rt, right; medialseg, medial segmentectomy; EI, ethanol injection.

Data expressed as mean±SD.

protocol with OK432.

We can consider two reasons for liver injury after hepatectomy. The first is that the obligatory increase in portal blood through the small remnant may be central to the pathogenesis because of SEC injury and Kupffer cell activation, which induces an increased production of TNF, platelet activating factor (PAF), and superoxide[13-18]. We have reported that Kupffer cells and sinusoidal endothelial cell (SEC) are activated after partial hepatectomy based on the expression of class I and class II antigens and an increased production of PAF[19,20,21]. Moreover, the excessive activation of Kupffer cells has resulted in a massive liver necrosis after a 70% partial hepatectomy in the rat[22]. In that study, a remarkable increase was demonstrated between the expression of class II antigens in Kupffer cells and the production of TNF α. Several investigations have indicated the impact of OK432 in the prevention of such forms of liver failure as galactosamine-induced hepatic failure. It has also been speculated that the mechanism responsible for the OK432 ability to decrease the incidence of liver injury involves the enhancement of the reticuloendothelial system, which probably increases the clearance capacity of endogenous endotoxin[17,23]. Blair et al. also reported that pretreatment with IFN-gamma decreases infectious complications after partial hepatectomy because of the diminution of bacterial translocation. Macropharges from animals treated with IFN-gamma had higher *in vitro* tumoricidal activity and production of O^{2-} [24]. Fujita et al. have reported that the administration of gadlinium chloride, which is a specific inhibitor of hepatic macrophages, decreases the severity of endotoxin-induced liver injury and reduces the mortality of rats, and that intravenous preoperative administration of OK432 reduces the mRNA expression of TNF α in liver NPC enhanced by the endotoxin-injection[4,25]. Some investigations have also shown that repeated stimulation of hepatic macrophages decreases their response to each new stimulation[26,27]. Therefore, the Kupffer cell is a double-edged sword for the host. In this clinical study, the effect of the preoperative administration of OK432 appears to be two fold, as it enhances the reticuloendothelial system—as can be seen in the enhancement of phagocytic function—but inhibits the excessive immunological reaction of Kupffer cells. We have mentioned that dynamic immunological changes, including the activation of both extrathymic T cells and NKT cells, have been demonstrated during the early period after partial hepatectomy[28,29,30]. In this study, a significant increase in NK activity occurred as a result of the preoperative treatment with OK432; moreover, CD 56-, TcR γ δ-, and HLA-DR-positive lymphocyte levels were higher than those of the pretreatment period, although these differences were not statistically significant. It has been reported that extrathymic T cells, NK cells, and TcR γ δ lymphocytes play important roles against infection. These findings may help to explain the reduction in the incidences of infection in the OK432-treated group. In fact, the complications of patients given OK432 preoperatively were fewer and less severe than those of the untreated group, which showed serious complications such as MRSA enteritis and ARDS.

Second, we have already proposed[21,31] that the surplus portal pressure, reflecting the shear stress[32] in response to portal flow after hepatectomy, induces the SEC and hepatocyte injuries that result in microcirculation failure in the liver. We have now confirmed that a portosystemic shunt by subcutaneous splenic transposition decreases the incidence of liver injury after 90% partial hepatectomy, as assessed by the levels of serum GOT and serum hyaluronic acid, and reduces the mortality of 95% partial hepatectomy in rats. Serum hyaluronic acid is an indication of SEC damage. Preoperative treatment with OK432 resulted in a significant improvement in serum hyaluronic acid levels. The activation of SEC as a result of preoperative treatment with OK432 might prevent liver failure due to excessive portal pressure. The period before the removal of the abdominal drain in the OK432-treated group was shorter than that of the untreated group, which suggested that the ascites of the OK432-treated group were smaller than those of the untreated group.

In conclusion, the preoperative administration of OK432 in cirrhotic patients may be useful in preventing postoperative liver failure and infection through inhibition of the excessive immunological reaction of Kupffer cells, the enhancement of SEC function, and the activation of NK cells and NKT cells.

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