CHAPTER 31

ADVANCES IN THE PHARMACOLOGY OF *TREMELLA* POLYSACCHARIDES

Zhi-Bin Lin

Department of Pharmacology, Beijing Medical University, Beijing 100083, China.

1. INTRODUCTION

Tremella, also called Yin er (銀耳) or white jelly fungus, is the dried sporophore of *Tremella fuciformis* Berk. of the Basidiomycetes. It has long been valued in folk medicine as a remedy with nutritive and tonic action in treating debility and exhaustion. It is used in traditional Chinese medicine to nourish yin (陰) essence and moisten the lung, tonify the stomach and promote secretion of saliva. It is now well recognized that *Tremella* polysaccharides (TP) are bioactive components extracted from *Tremella fuciformis* by sequential hot water and ethanol extraction. TP is a protein-binding polysaccharide which consists of mannose, glucuronate, xylose, fructose and glucose with a molecular weight of 3 X 10^5 and is water-soluble (Zhang & Hong, 1984). In order to clarify the therapeutical principle of TP, we have studied the pharmacological effects of TP using modern techniques. The results indicate that TP has various pharmacological effects which are described below.

2. ANTITUMOR EFFECT

Animal experiments have shown that TP exhibited antitumor activities *in vivo*. Zhou *et al* (1987) observed antitumor activity of TP on Ehrlich ascites carcinoma in mice *in vivo* and *in vitro*. It was found that TP (100 mg/kgXd ip for 9 days) significantly decreased the ascites content and inhibited [^{3}H]TdR incorporation into DNA of tumor-bearing mice. On the other hand, TP (100 μg/ml) exerted no distinct effect on [^{3}H]TdR incorporation into DNA of tumor cells *in vitro*. These findings show that TP has antitumor activity *in vivo*, but has not cytotoxic activity *in vitro*. It is believed that the antitumor activity of TP may be related to its immunomodulating effects.

TABLE 1. Effect of TP on phagocytosis of mouse intraperitoneal macrophages.

Group	Dose (mg/kgXd,scXd)	Phagocytic percentage (%)	Phagocytic index
Control	-	30.8±3.5	0.62±0.08
TP	200X4	57.6±3.5***	1.61±0.16***

$\bar{X}$ ±SE, n=12; ***$p<0.001$ *vs* control.

3. IMMUNOMODULATING EFFECTS

3.1. Effect on Mononucleolar-Macrophage System

Lin and coworkers (1982) studied the influence of TP on the phagocytic activities of intraperitoneal macrophages in mice. The data in Table 1 show that TP (200 mg/kgXd sc for 4 days) markedly increased the phagocytosis of mouse intraperitoneal macrophages on chicken red blood cells (CRBC).

We also demonstrated that TP (100 mg/kgXd sc for 7 days) elevated the clearance rate of intravenous charcoal particles in normal mice and in mice immunosuppressed with cortisone acetate (Lin *et al.*, 1985). The results above suggest that TP can promote the function of the mononucleolar-macrophage system, which may be involved in the antitumor activity of TP.

3.2. Effects on Humoral and Cellular Immunity

Further investigation demonstrated that TP can potentiate humoral and cellular immunity. Lin *et al* (1985) observed the effect of TP on the hemolysin reaction in mice immunized with sheep red blood cells (SRBC). They showed that TP increased the production of hemolysin in SRBC - immunized mice. The same result was seen in mice immunosuppressed with cyclophosphamide(Lin

TABLE 2. Effect of TP on the plaque-forming cell (PFC) response to SRBC in mice aged 3 and 14 months.

Age (month)	Group	Dose (mg/kgXd,ipXd)	PFC/10^6 spleen cells
3	YC	-	1504±420
3	TP	50X5	2671±699**
3	TP	100X5	2734±772**
3	YC	-	1910±473
14	AC	-	995±283**
14	TP	50X5	1464±244++
14	TP	100X5	1148±491

$\bar{X}$±DF, n=6, **$p < 0.01$ *vs* young control (YC).
++ $p < 0.01$ *vs* aged control (AC).

TABLE 3. Effect of TP on the plaque forming cell (PFC) response to SRBC in stressed mice.

Group	Dose (mg/kgXd,igXd)	PFC/10^6 spleen cells
Control	-	3068±853
Stress	-	1030±265**
TP+stress	200X8	2894±473++
TP+stress	400X8	2893±538+

$\bar{X}$±SD, n=6,**$p<0.01$ *vs* control
++ $p<0.01$ *v s* stress

et al., 1985). Xia and Lin (1989) studied the effect of TP on the plaque forming cell (PFC) response to SRBC in mice aged 3 and 14 months. TP (50, 100 mg/kgXd ip for 5 days) markedly promoted the PFC response to SRBC in 3 month-old mice. Although the basal PFC response of 14 month-old mice was lower than that of 3 month-old mice, TP (50 mg/kgXd ip for 5 days) clearly enhanced the anti-SRBC PFC response to normal level in 14 month-old mice (Table 2).

Recently, Cui and Lin (1992) observed the effects of TP on immune function in physically-stressed mice. Mice were stressed by swimming in cold water (14±1°C). The maximal decrease of PFC was reached in 9-12 days after stress. The data in Table 3 show that TP (200, 400 mg/kgXd ig for 8 days) can restore the suppressed PFC response in stressed mice.

Xia and Lin (1989) reported the effect of TP on lymphocyte proliferation induced by concanavalin A (Con A) in mice. ConA-induced proliferation was measured by [^{3}H]TdR incorporation into mouse spleen cells from C57BL/6j mice. Table 4 shows that TP (50,100, 150 and 200 μg/ml) significantly increased the ConA-induced lymphocyte proliferation in a concentration-dependent manner. Hydrocortisone can significantly inhibit mouse lymphocyte proliferation induced by ConA. At doses of 25, 50, 100 and 150 μg/ml, TP did not antagonize the suppressive effect of hydrocortisone. It was found that lymphocyte proliferation in 14 month-old mice was lower than that of 3 month-old mice. TP (50 μg/ml) can partly restore the lymphocyte proliferation induced by ConA in 14 month-old mice. TP (100 μg/ml) had no significant effect, but TP (150 and 200 μg/ml) inhibited the lymphocyte proliferation response in 14 month-old mice. This suggests that the optimal dose of TP for lymphocyte proliferation in aged mice was less than young mice.

Cui and Lin (1992) also demonstrated that, at doses of 200 and 400 mg/kgXd ig for 14 days, TP can antagonize the suppressive effect of physical stress on delayed cutaneous hypersensitivity

TABLE 4. Effect of TP on lymphocyte proliferation induced by ConA in mice.

Dose of TP (μg/ml)	[^{3}H]TdR incorporation (cpm)
0	34997±8990
50	58846±8217***
100	64488±11785***
150	72943±10859***
200	81361±11499***

$\bar{X}$±SD, n=12, ***$p<0.001$ *vs* control

TABLE 5. Effect of TP on lymphocyte proliferation stimulated by ConA in physically stressed mice.

Group	Dose (mg/kgXd, igXd)	Uptake of [^{3}H]TdR (dpm)
Control	-	93235±27857
Stress	-	57465±6460*
TP+Stress	200X8	97971±34055+
TP+Stress	400X8	89773±35962

$\bar{X}$±SD, n=6, *p<0.05 *vs* control.
+ p<0.05 *vs* stress control.

(DCH) induced by 2,4-dinitrochlorobenzene (DNCB). As shown in Table 5, lymphocyte proliferation stimulated by ConA was decreased by 38.4% in physically stressed mice. However, TP (200, 400 mg/kgXd, ig for 8 days) can restore the depressed lymphocyte proliferation to normal levels (Cui & Lin, 1992).

3.3. Effect on Interleukin 2 (IL-2) Production and Intracellular Calcium

Ma and Lin (1992) investigated the effect of TP on IL-2 production by mouse splenocytes. TP (1, 5, 10 and 50 μg/ml) significantly increased IL-2 production by mouse splenocytes. In aged mice, the level of IL-2 production was much lower than that in young controls. TP (0.05, 0.5 and 5 μg/ml) restored IL-2 production to the normal levels of young controls (Table 6).

Recently, we examined the effect of TP on intracellular calcium of mouse splenocytes (Cui *et al.*, 1992). The intracellular calcium ($[Ca^{2+}]i$) was measured by the fluorescence probe method with fura-2. Preliminary results revealed that, at concentrations of 8, 24 and 80 μg/ml, TP can increase $[Ca^{2+}]i$ of mouse splenocytes. In the absence of extracellular calcium ($[Ca^{2+}]ex$), TP did not increase $[Ca^{2+}]i$. Therefore, TP may promote transport of $[Ca^{2+}]ex$ into the mouse splenocytes.

4. PROTECTIVE EFFECT ON ARREST OF BONE MARROW

TABLE 6. Effect of TP on IL-2 production by aged mouse splenocytes.

Group	Age (month)	Dose (μg/ml)	[^{3}H]TdR incorporation (cpm)	
YC	3	0	38335±3517	(n=6)
AC	19	0	30572±4394++	(n=6)
AC+TP	19	0.05	39123±1840**	(n=5)
AC+TP	19	0.5	35239±1293*	(n=6)
AC+TP	19	5	35646±2597*	(n=5)
AC+TP	19	50	33682±2137	(n=6)

$\bar{X}$=SD, ++ p<0.01 *vs* young control (YC).,
*p<0.05, **p<0.01 *vs* aged control (AC).

TABLE 7. Effect of TP on arrest of bone marrow caused by ^{60}Co γ-ray irradiation and cyclophosphamide.

Group	Dose (mg/kgXd, scXd)	Myeloid granulocytesX10^{20}	
		^{60}Co γ-ray radiation	Cyclophosphamide
Control	-	10.68±0.48	8.27±0.71
Arrest	-	2.21±0.25**	2.36±0.42***
TP	200X4	6.32±1.37+	4.18±0.62+

$\bar{X}$±SE, n=10, ***p<0.001 *vs* control,
+ p<0.05 *vs* arrest.

Lin, *et al* (1982) have also shown that TP exerted a protective effect on arrest of bone marrow caused by ^{60}Co γ-ray irradiation or by cyclophosphamide. As shown in Table 7, both ^{60}Co γ-ray irradiation and adminstration of cyclophosphamide can decrease myeloid granulocytes. TP (200 mg/kgXd, sc for 3~5 days) can partially improve the arrestment effect on bone marrow induced by ^{60}Co γ-ray irradiation and by cyclophosphamide. These findings indicate that TP may be able to promote hematogenesis in the bone marrow.

5. EFFECT ON THE LIVER

Lin and coworkers (1982) observed the effect of TP on [^{3}H] uridine incorporation into protein, DNA and RNA in mouse liver. This study revealed that TP (200 mg/kgXd, sc for 4 days) increased [^{3}H] leucine incroporation into protein and [^{3}H] uridine incorporation into mouse RNA liver, which suggests that TP can promote the synthesis of protein and RNA in mouse liver cells. However, TP does not exert any influence on DNA synthesis in mouse liver cells (Table 8)

Yue and Cong (1986) reported that TP (200 mg/kgXd, ip or sc for 5 days) increased [^{3}H] leucine incorporation into liver protein and [^{3}H] orotic acid incorporation into RNA in normal and partially hepatectomized mice. But, in both normal and injured liver, TP showed no significant effect on the incorporation of [^{3}H] thymidine into liver DNA. This suggests that promotion of transcription may be the chief mechanism underlying the effect of TP on the synthesis of protein in the liver. However, TP did not alter the glycogen content of liver and did not prevent the decrease in glycogen content induced by CCl_4 or by partial hepatectomy in mice (Yue & Cong, 1986). Cheng *et al* (1984) assessed

TABLE 8. Effect of TP on [^{3}H] leucine, [^{3}H] thymidine and [^{3}H] uridine incorporation into protein, DNA and RNA in mouse liver.

Group	Incorporation of radioactivity (cpm/100 mg liver weight)		
	[^{3}H]Leu	[^{3}H]UR	[^{3}H]TdR
Control	8446±273 (n=10)	4019±241 (n=14)	6087±822 (n=8)
TP	9574±296* (n=10)	5201±458* (n=14)	6236±581 (n=8)

$\bar{X}$±SE, *p<0.05 *vs* control.

the effect of TP on the ultrastructure of liver under the electron microscope using the method of quantitative analysis. The results demonstrated that, after administration of TP (100 mg/kgXd, Sc for 4 days), the rough endoplasmic reticulum (RER) of liver cells was increased from 17% to 26% and liver glycogen increased from 5% to 13%. In contrast, TP decreased the cytoplasmic matrix of the liver cells from 48% to 30%. It is generally considered that the RER can synthesize and secrete protein in liver cells. Therefore, the increase in RER activated by TP may be related to the promoting effect of TP on protein synthesis.

Further evidence revealed that TP (100 mg/kgXd, sc for 7 days) showed no significant effect on the content of cytochrome p-450 in liver homogenates from normal mice but antagonized the decrease in spleen weight and the increase in the content of liver cytochrome p-450 caused by cortisone acetate. At a dose of 200 mg/kgXd, TP remarkably increased spleen weight and reduced the content of cytochrome p-450 in liver homogenates from normal mice. Take together, these findings suggest that the promoting effect of TP on the biosynthesis of nucleic acid and protein is probably an essential mechanism in its liver-protective effect. The effect of TP on the biosynthesis of nucleic acid and protein may also involve the effect of TP on other organs or tissues in the organism (Lin *et al.*, 1985).

6. CONCLUSIONS

The studies reviewed in this paper demonstrate the variety of pharmacological effects of TP and provide a theoretical basis for the clinical use of TP. It is believed that TP exerts antitumor effects through its immunomodulating action. TP not only exhibits immunomodulating effects on the humoral and cellular immune functions in normal mice, but also possesses immunorestorative effects on the suppressed immune function in immunosuppressed mice induced by immunosuppressive drugs, antitumor drugs, stress and aging. These results suggest that TP may be used as an immuno-chemotherapeutical or immuno-radiotherapeutical drug to treat cancers, and as an immunorestorative drug to improve immunodeficiency induced by immune deficient disease, including acquired immune deficiency syndrome (AIDS), by stress or by aging. These possibilities need further investigation through clinical trials. Moreover, since nucleic acid and protein play an important role in biological processes, the promoting effect of TP on the biosynthesis of nucleic acid and protein may contribute to the pharmacological mechanism at the cellular and molecular levels.

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