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Effect of lycopene on hepatotoxicity induced by perfluorooctane sulfonic acid in rats

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Abstract: *Objective: Intend to observe the liver injury induced by perfluorooctane sulfonic acid (PFOS) in SD rats and the protective effect of lycopene (LP) and its possible mechanism. Methods: 48 SD rats were randomly divided into 8 groups. The solvent control group of LP protection group and LP protection low, middle and high dose PFOS exposure groups were given LP suspension once a day according to their designed dose. The contents of ALT, ASP and AST in serum, the content of MDA in liver tissue and the activity of SOD and GSH-Px in liver tissue were measured after 2 months. Results: The levels of serum ALT, AST and serum ASP enzyme activity in middle and high dose PFOS groups and high dose PFOS groups were significantly higher than those in solvent control group (P < 0 05).The levels of serum ALT, ALP and AST in middle and high dose PFOS groups protected by LP were lower than those in the same dose PFOS group (P < 0 05). The content of MDA in liver tissue of rats treated with LP was significantly lower than that of rats exposed to the same dose of PFOS (P < 0.05). The contents of GSH-Px and SOD in the liver homogenate of the low, middle and high dose PFOS groups decreased compared with the solvent control group. Conclusion: PFOS can induce liver injury in rats, and lycopene can antagonize liver injury induced by PFOS through antioxidant mechanism.*

Keywords: *Lycopene, Perfluorooctane sulfonic acid, SD rats, Hepatotoxicity*

1. Introduction

In fact, Perfluorooctane sulfonic acid (PFOS) is a kind of environmental pollutants with systemic multiple organ toxicity. And lycopene (LP) is a carotenoid with strong antioxidant capacity found in nature at present. There are few reports on hepatotoxicity caused by PFOS at home and abroad at present. Besides, there are no reports on the use of natural ingredients to antagonize liver injury caused by PFOS. As a consequence, we intended to observe the liver injury and lycopene protective effects of different doses of PFOS on liver injury and lycopene in SD (Sprague dawley) rats from two aspects of liver pathological changes and functional changes. Besides, try to provide experimental basis for elucidating the hepatotoxic effect of environmental pollutant PFOS, and to make a preliminary discussion on the scientific prevention and treatment of PFOS hepatotoxic injury.

2. Materials and methods

2.1 Experimental animal

48 clean-grade 3-month-old SD rats, with half male and half female, and weighing 180.0 ±10.0g, were provided by the Experimental Animal Center of Nanhua University (certificate no: SCXK (Xiang) 2004-0009).

2.2 Reagent preparation

1% carboxymethyl cellulose sodium (CMC-Na) solution: weighed 10g CMC- Na, dissolve in fresh distilled water, and add 5 mL Tween-80 to mix, heat to dissolve, cool and add fresh distilled water to constant volume 1000 mL. Besides, LP suspension: accurately weighed 0.44 g LP dissolved in 100 mLCMC-Na solution to make 4 mg/ml LP suspension, which was now mixed and stored at 4°C.

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2.3 Grouping, feeding and handling of animals

48 clean-grade 3-month-old SD rats, with half female and half male, were randomly divided into 8 groups according to sex, with 6 rats in each group: including solvent control group, LP protection group, low, middle and high dose PFOS exposure groups, and LP protection low, medium and high dose PFOS exposure groups. The animals in the solvent control group and LP protection group were fed with 1%CMC-Na solution once a day, while the animals in the low, middle and high PFOS3 groups were fed with three doses of PFOS according to their body weight, respectively. Besides, the 1%CMC-Na solution was given intragastrically once a day. Three LP-protected animals exposed to low, medium and high PFOS were fed with 20mg/ kg bwLP suspension once a day, except for three doses of PFOS. BwLP suspension was given intragastrically at a dose of 5mL/ kg bw. Besides, the experimental period was 2 months. 24 hours after the last intragastric administration, eyeballs were removed and blood samples were collected to prepare serum. The livers of rats were isolated and the indexes were detected.

2.4 Detection index and method

The contents of ALT, ASP and AST in serum were detected by corresponding kit and automatic biochemical instrument. And liver index was calculated. Besides, liver homogenate was prepared, and MDA content, SOD, GSH-Px activity and tissue protein content were determined by corresponding kit and spectrophotometer.

2.5 Statistical analysis

SPSS21.0 statistical software was used for analysis. The experimental data were expressed in terms of statistics $\bar{x} \pm s$, and the difference was statistically significant ($P < 0.05$).

3. Results

3.1 Effects of perfluorooctane sulfonic acid and lycopene on growth and liver coefficient in rats.

The body weight gain of animals in the middle and high dose PFOS exposure groups was lower than that in the solvent control group. Besides, the body weight gain was inhibited more obviously with the increase of PFOS dose. In addition, the body weight gain of animals in the low, middle and high PFOS groups protected by LP was higher than that in the same dose PFOS group, but the difference was not statistically significant ($P > 0.05$). Moreover, the liver organ coefficient of rats exposed to middle and high dose PFOS was higher than that of solvent control group ($P < 0.05$). In addition, the liver organ coefficient of middle and high dose PFOS groups was significantly lower than that of the same dose PFOS group ($P < 0.05$), which was significantly lower than that of the same dose PFOS group (see Table 1).

Table 1: Effects of PFOS and LP on body weight and liver organ coefficient of SD rats (n=48, $\bar{x} \pm s$)

Group	Weight gain (g)	Liver / body weight (%)
Solvent control group	172.6±30.3	4.26±0.37
Low PFOS group	161.8±36.4	4.40±0.50
Middle PFOS group	132.4±33.6*	6.08±0.45*
High PFOS group	123.5±31.4*	5.96±0.51*
LP protection group	190.1±34.3	4.22±0.37
LP protected low PFOS group	239.3±24.6	4.35±0.18
LP protected middle PFOS group	159.4±27.7	4.87±0.46#
LP protected high PFOS group	136.5±39.4	4.25±0.33#

*: Compared with the control group, P<0.05; #: Compared with the group exposed to the same dose of PFOS, P<0.05

3.2 Effects of perfluorooctane sulfonic acid and lycopene on liver function in rats

As shown in Table 2, the levels of serum ALT and AST in the middle and high dose PFOS groups and the serum ASP enzyme activity in the high dose PFOS group were higher than those in the solvent control group compared with the solvent control group. However, there was no significant difference

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between the low dose group and the solvent control group (P > 0.05). In Addition, the levels of serum ALT, ALP and AST in the middle and high dose PFOS groups protected by LP were significantly lower than those in the same dose PFOS group (P < 0.05).

Table 2: Effects of PFOS and LP on liver function in SD rats (n=48, $\bar{x}\pm s$)

Group	ALT(U/L)	AST(U/L)	ASP(U/L)
Solvent control group	51.26±3.15	116.27±12.15	315.60±11.94
Low PFOS group	53.11±5.36	122.00±13.16	322.36±12.45
Middle PFOS group	68.70±7.22*	137.15±23.27*	349.18±14.10*
High PFOS group	70.25±4.18*	146.88±12.95*	355.30±11.82*
LP protection group	50.00±2.27	108.50±11.06	311.07±13.25
LP protected low PFOS group	52.13±3.68	121.57±19.63	320.39±17.13
LP protected middle PFOS group	55.36±7.19#	122.17±12.06#	326.81±15.89#
LP protected high PFOS group	55.13±5.98#	125.16±15.96#	325.27±16.81#

∗: Compared with the control group, P<0.05; #: Compared with the group exposed to the same dose of PFOS, P<0.05

3.3 Effects of perfluorooctane sulfonic acid and lycopene on MDA content and SOD and GSH-Px activities in rat liver tissue

As we can see from Table 3, it showed that the content of MDA in liver tissue homogenate of rats exposed to low, middle and high dose PFOS was higher than that of solvent control group(P<0.05) , showing a dose-effect relationship. Besides, the content of MDA in liver tissue of rats exposed to PFOS protected by LP was significantly lower than that of rats exposed to the same dose of PFOS (P < 0.05).

The contents of GSH-Px and SOD in liver homogenate of rats in low, middle and high dose PFOS groups were significantly lower than those in solvent control group(P<0.05). Besides, there was a dose-effect relationship, and the activities of GSH-Px and SOD in liver tissue of rats in each PFOS group protected by LP were significantly higher than those in the same dose PFOS group (P < 0.05).

Table 3: Effects of PFOS and LP on MDASOD and GSH-Px in liver of SD rats (n=48, $\bar{x}\pm s$)

Group	MDA(nmol/gPro)	SOD(U/mgPro)	GSH-Px(U/mgPro)
Solvent control group	67.67±5.36	158.81±14.78	0.67±0.07
Low PFOS group	75.60±5.93*	133.61±13.29*	0.35±0.09*
Middle PFOS group	103.62±7.89*	100.85±11.02*	0.28±0.05*
High PFOS group	118.25±4.66*	92.22±6.57*	0.25±0.06*
LP protection group	62.75±1.37	169.50±10.95*	0.75±0.11
LP protected low PFOS group	66.22±4.40#	143.09±15.52	0.61±0.03#
LP protected middle PFOS group	91.87±5.56#	135.51±12.18#	0.57±0.09#
LP protected high PFOS group	95.08±7.86#	121.97±15.36#	0.45±0.10#

∗: Compared with the control group, P<0.05; #: Compared with the group exposed to the same dose of PFOS, P<0.05

4. Discussion

In fact, organ coefficient is a sensitive index in toxicological subchronic toxicity test. The organ weight / body weight ratio of experimental animals will be kept in a certain normal range after the body matures. The increase of organ coefficient indicates that the cell of the organ is enlarged in the case of excluding the effect of the change of body weight on the change of organ coefficient, which can generally estimate the nature and degree of visceral organ disease. As a result, it showed that PFOS could increase the liver organ coefficient of rats, indicating that PFOS could lead to liver damage in rats.

In addition, the liver is not only the most important detoxification organ in the human body, but also the main organ to accumulate and degrade pollutants. Generally speaking, high-dose chemicals with accumulation will cause liver damage after entering the body. The main mechanism is liver parenchyma cell injury and necrosis. Moreover, the study of liver enzyme activity released into blood by damaged hepatocytes is a common method to study liver damage at present. We chosen ALT, AST and ALP as observation indexes to comprehensively analyze the liver injury of rats exposed to different doses of PFOS in this experiment. As a result, it showed that the indexes of ALT, AST and ALP in the middle

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and high dose PFOS groups were significantly higher than those in the solvent control group compared with the solvent control group, indicating that the hepatocytes of rats were damaged.

Besides, oxidation is the normal physiological activity of all organisms in the process of aerobic respiration, which will produce reactive oxygen species (ROS). In fact, a certain concentration of ROS is also necessary to maintain the normal physiological function of organisms. Reactive oxygen species are continuously produced in the body, and they are constantly removed to maintain dynamic balance under the action of antioxidant enzymes. However, it will trigger a chain reaction on unsaturated fatty acids on the biofilm if the ROS produced in the body exceeds the scavenging capacity , resulting in the formation of lipid peroxides (LPO) that are toxic to cells. In other words, it is called oxidative stress reaction. Malondialdehyde (MDA) is the final decomposition product of lipid peroxides induced by free radicals and one of the markers of oxidative damage. The determination of its content can directly reflect the level of LPO and the degree of cell damage.

The main antioxidant enzymes in the body are superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). In fact, SOD is the key enzyme for scavenging oxygen free radicals, which can increase the disproportionation scavenging rate of superoxide free radicals by 1010 times. Besides, it is an important quantitative index of oxidative damage. GSH-Px is a selenium-containing antioxidant enzyme that exists widely in the body. It converts H2O2 and many organic peroxides into water or corresponding alcohols, thus preventing H2O2 from reacting with iron to form a more toxic hydroxyl radical (OH). The level of free radicals in the body continues to increase with the stimulation of exogenous compounds, and the activity of GSH-Px will decrease. As a consequence, it can be used as an important basis for evaluating the level of tissue oxidative stress.

In this research, the content of MDA in liver homogenate of middle and high dose PFOS groups increased significantly, indicating that oxidative stress plays an important role in liver injury induced by PFOS. However, the activities of SOD and GSH-Px in liver tissue homogenate decreased significantly in PFOS group, reflecting that PFOS led to a significant decrease in the ability of scavenging free radicals in liver tissue. At the same time, a large number of lipid peroxidation products accumulated, which will cause damage to liver function.

The results showed that lycopene could significantly antagonize the oxidative stress induced by fluorooctane sulfonic acid. We consider the following ways to antagonize oxidative stress: (1) Restoring the activity of SOD and GSH-Px: PFOS can significantly inhibit the activity of SOD and GSH-Px in liver tissue, while the addition of LP can antagonize the inhibition of SOD activity of liver tissue by PFOS, indicating that LP improves liver function by restoring SOD activity and scavenging free radicals produced by PFOS oxidative damage. (2) The direct antioxidation of LP: there are 11 conjugated double bonds and 2 non-conjugated carbon-carbon double bonds in the LP structure, which scavenges reactive oxygen species in two ways. The physical way is to transfer the activation energy from singlet oxygen to LP, to produce ground state oxygen and activated triplet LP, to release excess energy in the form of heat, and then generate ground state LP to join the next cycle. It is the main way for LP to directly quench singlet oxygen. Besides, in chemical, lycopene can react directly with hydrogen peroxide, nitrogen dioxide and other active oxygen fragments, thus scavenging oxygen free radicals.

Generally speaking, subchronic PFOS exposure can lead to liver injury in rats. However, lycopene (LP) has a good protective effect on the injury, and antioxidation is the main mechanism.

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