Protective effects of medical ozone combined with traditional Chinese medicine against chemically-induced hepatic injury in dogs

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Abstract

AIM: To investigate the protective effect of medical ozone (O₃) combined with Traditional Chinese Medicine (TCM) Yigan Fuzheng Paidu Capsules (YC) against carbon tetrachloride (CCl₄)-induced hepatic injury in dogs.

METHODS: Thirty healthy dogs were divided randomly into five groups ($n = 6$ in each group), namely control, oleanolic acid tablet (OAT), O₃, YC and O₃ + YC, given either no particular pre-treatment, oral OAT, medical ozone rectal insufflation every other day, oral YC, or oral YC plus medical ozone rectal insufflation every other day, respectively, for 30 consecutive days. After pre-treatment, acute hepatic injury was induced in all dogs with a single-dose intraperitoneal injection of CCl₄. General condition and survival time were recorded. The biochemical and hematological indexes of alanine aminotransferase (ALT), aspartate aminotransferase/alanine aminotransferase (AST/ALT), serum total bilirubin (TBIL), prothrombin time (PT), blood ammonia (AMMO), and blood urea nitrogen (BUN) were measured after CCl₄ injection. Hepatic pathological changes were also observed.

RESULTS: Compared to the other four groups, the changes of group O₃ + YC dogs' general conditions (motoricity, mental state, eating, urination and defecation) could be better controlled. In group O₃ + YC the survival rates were higher ($P < 0.05$ vs group control). AST/ALT values were kept within a normal level in group O₃ + YC. Hepatic histopathology showed that hepatic injury in group O₃ + YC was less serious than those in the other four groups.

CONCLUSION: Medical ozone combined with TCM YC could exert a protective effect on acute liver injury induced by CCl₄.

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Key words: Carbon tetrachloride; Ozone; Traditional Chinese Medicine

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INTRODUCTION

Medical ozone, a mixture gas of ozone and oxygen, has been used for several decades in the therapy of diabetic foot, arthritis, arterial angiopathy and ulcerative colitis^[1-9]. It has been used in an empirical fashion in recent years for therapy of viral hepatitis. Rectal insufflation with ozone can reduce hepatic and renal ischemia-reperfusion injury^[10-15].

Yigan Fuzheng Paidu Capsules (YC), an empirical formula, is a compound preparation of traditional Chinese medicine (TCM) for treating chronic viral hepatitis. It has been demonstrated experimentally that YC can protect the liver and decrease transaminase level, has anti-lipid peroxidation activity, and *ex vivo* antiviral activity.

This study investigated the effect of medical ozone combined with YC affect on carbon tetrachloride (CCl₄)-induced acute liver injury in order to establish a reliable baseline for clinical application.

MATERIALS AND METHODS

Animals

Thirty healthy mongrel dogs aged 1-2 years, weighing 12-15 kg, were used in the experiments. These dogs received regular feeding, were inspected medically, treated with helminthicide, and acclimatized for at least 1 month prior to the experiments in the Experimental Animal Center of Nanfang Hospital, Southern Medical University. The experiments were carried out in accordance with the animal experiment regulations of the university.

Drugs and reagents

YC was manufactured in the Department of Traditional Chinese Medicine, Southern Medical University (Guanglian-zhi-zi 2004, No. FPGZ150) with Batch No. 050715. Oleanolic acid tablets (OAT) were produced by Nanguo Biological Pharmacy Co., Guangdong Province, China (Guo-yao-zhun-zi No. H44023537) with Batch No. 040701. CCl₄ was produced by the Chemical Plant of Guangzhou with Batch No. 050317, and mixed with the same amount of peanut oil before use.

Equipment

The medical ozone generator (OZONOSAN alpha Plus 1107, Germany) was registered and licensed for medical therapy by SFDA (registration No. 1570177).

Groups and administration

Thirty healthy dogs were divided randomly into five groups ($n = 6$ in each group): group control, which received no preconditioning treatment; group OAT, treated with oral OAT at 10 mg/d; group O₃, treated with 8.1 mL/kg medical ozone at 20 µg/mL by transrectal insufflation every other day; group YC, treated with oral YC at 0.2 g/d; and group O₃ + YC, treated with oral YC at 0.2 g/d plus 8.1 mL/kg medical ozone at 20 µg/mL by transrectal insufflation every other day, for a total of 30 consecutive days. After preconditioning treatment, acute hepatic injury was induced in all dogs with a single intraperitoneal injection of CCl₄ mixture at a dose of 0.9 mL/kg body weight.

Measurements

The general condition of the dogs was observed before and after treatment, in terms of motor activity, mental state, eating behavior, urination and defecation. The survival time of each dog was measured accurately in hours.

For biochemical and hematological measurements, intravenous blood was sampled pre- and post-treatment at 24 h, 2, 3, 4, 7 and 14 d. All the blood samples were analyzed immediately by the clinical laboratory of Nanfang Hospital for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), prothrombin time (PT), serum ammonia (AMMO), and blood urea nitrogen (BUN).

For histopathology, multiple liver tissues were obtained through ultrasound-guided percutaneous needle biopsy from each dog before and after the experiment. The formalin-fixed, paraffin-embedded liver sections (5 µm)

Table 1 Histological assessment of drug induced acute hepatitis in dogs

Score	Centrilobular necrosis	Inflammation in centrilobular areas
0	None	None
1	Isolated necrotic hepatocytes or single row of hepatocyte drop-out in perivenular areas	Mild: inflammatory infiltrate affecting < 50% centrilobular areas
2	> 1 and up to 3 rows of perivenular necrotic hepatocytes	Moderate: inflammatory infiltrate affecting > 50% and < 75% centrilobular areas
3	> 3 rows of perivenular necrotic hepatocytes with confluent and/or bridging necrosis	Severe: dense inflammatory infiltrate affecting > 75% centrilobular areas

were stained with hematoxylin and eosin (HE) and Gomori silver. The degree of necrosis and inflammatory cell infiltrate was evaluated on a four-point scale (Table 1), using 20 random fields at 100 × and 400 × magnification per slide, by a blinded pathologist (MIF)^[16].

Statistical analysis

Overall survival was evaluated by actuarial analysis using Kaplan-Meier estimates. Independent samples test for comparison of biochemical and hematological measurements, and one-way ANOVA and LSD test for histological assessment were performed by SPSS 13.0. P -values < 0.05 were considered statistically significant.

RESULTS

General condition of the dogs

Pre-treatment, all 30 dogs were in good condition. Eating behavior, feces and urine were normal. Post CCl₄ treatment, all dogs began to vomit and lost balance immediately. Except for the O₃ + YC group, all the dogs in the other four groups appeared to prefer to stay still alone or were pacing up and down restlessly, while some of them showed poor mental health and appetites, and yellow urine. In group O₃ + YC, there were no differences before and after treatment, except for two dogs with yellow urine.

Survival analysis

Log Rank, Breslow and Tarone-Ware tests were used for comparison of survival rate in the five groups of dogs. All three tests showed that the survival rate of group O₃ + YC was significantly higher than that of the control group ($P < 0.05$), while there were no significant differences between any other two groups ($P > 0.05$) (Figure 1).

Biochemical and hematological measurements

Pre-treatment, there were no significant differences ($P > 0.05$) between the control and the other four groups for any of the measurements (AST/ALT was not being analyzed at the time). After CCl₄ injection, all the measurement index in the five groups except for AST/ALT showed a tendency to increase during the first three days and then gradually fall. There were significant differences ($P < 0.05$) between groups OAT and O₃, OAT and YC, OAT and O₃ + YC for PT, and between groups

Figure 1 Survival functions of the five groups. The record of each dog's survival time starts at the injection time, and ends in post-360 h (15 d). Plots express the survival rates in different time.

OAT and O₃ + YC, O₃ and O₃ + YC for BUN. In order to compare the measurements post CCl₄ injection, all the index values for each dog were adjusted to the same level. Post CCl₄ injection, the measurement values of ALT, TBIL, PT and AMMO were significantly higher ($P < 0.05$) in the control group than in the other four groups, but there were no significant differences for BUN in all groups ($P > 0.05$). ALT was significantly lower ($P < 0.05$) in group OAT than in group YC, while ALT, TBIL, PT and BUN were significantly higher ($P < 0.05$) in group OAT than in group O₃ + YC. ALT, TBIL, PT and BUN were significantly higher ($P < 0.05$) in group O₃ and YC than in group O₃ + YC. AST/ALT in the control and YC groups showed a little below 1.0 at 24 h after CCl₄ was administrated, then increased to > 1.5 in the control group, and fell to < 0.5 on d 3-4. On d 5-14, AST/ALT in the control group continued to fall (0.1 on d 14). However, in group YC AST/ALT started to increase after 7 d (1.0 on d 14). AST/ALT in the OAT and O₃ groups was approximately 1.0 on first day, fell to approximately 0.4-0.5, and began to increase from d 4 (about 1.0 on d 14) (Table 2).

Liver histopathology

Liver sections from the five groups of dogs, after death or survival for 15 d, were examined and graded for the degree of necrosis and accompanying inflammation, as shown in Table 1. Control group livers demonstrated higher scores for necrosis ($P < 0.05$) and inflammation ($P < 0.05$) compared with the OAT, O₃, YC and O₃ + YC groups (Figure 2).

DISCUSSION

Drug-induced hepatitis is becoming a big problem in clinical practice, and proper management of this problem has not been found so far. For searching new strategy to overcome drug-induced hepatitis, medical ozone and traditional Chinese medicine were first tried in animal model. The effects of medical ozone combined with traditional Chinese medicine on CCl₄-induced acute hepatic injury in dogs were evaluated in this study. The novel method decreased the incidence of jaundice and hepatic encephalopathy and prolonged survival time. Medical ozone combined with YC showed a better effect than OAT, O₃ and YC alone in decreasing transaminases,

relieving jaundice and promoting recovery of blood clotting activity.

A number of hepatotoxins have been used to induce fulminant hepatic failure (FHF) in animal models: d-galactosamine, N-acetyl-P-aminophenol and CCl₄^[17,18]. CCl₄ causes serious hepatotoxicity, and has been used extensively and successfully to induce liver damage, including fibrosis^[19,20]. CCl₄ is metabolized by cytochrome P450 2E1 in the liver to produce a toxic metabolite. It has been demonstrated to induce liver necrosis, as well as apoptosis^[21]. Peroxidation of membrane lipids secondary to the formation of trichloromethyl radicals is believed to be the basis for the toxic effects of CCl₄.

Medical ozone is characterized by its safety and multiple effects in low dose. The main mechanisms of action of ozone pre-conditioning on hepatic injury are as follows. It enhances erythrocyte metabolism^[22-26], which can promote the oxygen-carrying capacity of hemoglobin and promote liver oxygenation. Ozone can induce intracellular anti-oxidant enzymes of the liver and scavenging free radicals^[14,27-29]. When hepatocytes are equipped with strength against peroxidative radicals by the preconditioning of medical ozone, they can become prone to survival under the attack of toxic CCl₄ radicals. The range of ozone concentrations within which it can exert a therapeutic effect without toxicity is wide from 10 mg/mL to 80 mg/mL^[25,30]. YCs have been prescribed clinically for several years. *Astragalus mongholicus*, *Hedyotis diffusa* and *Radix polygoni* prepared as a prescription can promote production of lymphoblasts, regulate the ratio of T-lymphocyte subsets, strengthen immune function, increase activity of lymph active cells and natural killer cells, and induce interferon. Giant knotweed rhizome, *Hedyotis diffusa*, *Rhizome* and *Phyllanthus amarusniruri* have also shown antiviral effects. Honeycomb of paper wasps, red peony root and *Salvia miltiorrhiza* can promote blood flow, mitigate liver injury, and improve hepatocyte oxygenation and promote their recovery. Therefore, the combined effects of O₃ and YC were to up-regulate immune function and promote liver oxygenation. This study may spur the use of a new strategy for the clinical management of some drug-related hepatitis. Clinical trials are needed to confirm the effects of this novel regime.

COMMENTS

Background

Medical ozone has been widely used, especially in Europe, in surgery and medicine for 50 years. The technique of ozonotherapy, such as major autohemotherapy, rectal insufflation and topical ozonotherapy, were gradually developed in recent decades and served for more than 10 million patients who suffered from skin ulcerative lesion or trauma, diabetic foot, arthritis, arterial obstruction, ulcerative colitis and spinal disk herniation, etc. The property of ozone and its therapeutic effects were partially illuminated. Ozone has opposite faces toward human body. It is harmful gas in high concentration or administration by respiratory tract but therapeutic in low concentration. The milestone fact sheets were that: (1) the ozone reacts with red blood cells (RBC) and reactivates RBC metabolism by increasing 2, 3-DPG and ATP concentration; (2) ozone reacts with immunocompetent cells and induces cytokines release, including IL-2, 4, 10, IFN- β , γ , TGF- β 1; (3) ozone activates tissue cells' antioxidative enzymes and enhances capacity of radical scavenger. Each discovery mentioned above will mould scientific base for the novel application scope of ozone. For example, the finding of effect of ozone on immune system is the scientific foundation of using

Table 2 Changes of biochemical and hematological indexes of the five groups (Data represents median $\pm$ quartile)

Group	Pre-injection	Post-injection CCl ₄					
		Post-24 h	Post-2 d	Post-3 d	Post-4 d	Post-7 d	Post-14 d
ALT/(U/L)							
Control	26.00 (16.50-33.75)	1156.00 (245.00-1641.75)	43.50 (13.25-4399.75)	1018.00 (66.00-1970.00)	1790.50 (1763.00-1818.00)	1349.50 (1307.00-1392.00)	297.00 (244.00-350.00)
OAT	24.50 (17.75-26.25)	113.00 (26.50-439.00)	95.00 (43.50-427.50)	173.00 (61.25-918.50)	135.00 (52.50-1390.50)	465.00 ^a (109.75-875.00)	77.50 ^a (16.00-155.50)
O ₃	25.50 (12.00-37.25)	564.00 (75.00-1054.50)	474.00 (132.00-1025.50)	209.00 (126.50-1136.00)	259.00 (119.00-1377.00)	139.00 ^a (57.50-538.00)	32.00 ^a (25.00-70.00)
YC	18.00 (11.75-52.50)	1310.00 ^c (919.75-1740.25)	662.00 ^c (355.00-2034.50)	1610.50 (158.75-1682.50)	1064.50 (235.25-2394.00)	465.00 ^a (109.75-875.00)	77.50 ^a (16.00-155.50)
O ₃ + YC	26.50 (20.00-32.75)	28.50 ^{b,f,h} (23.50-41.75)	34.00 ^{c,f,h} (27.25-40.25)	33.50 ^{a,d,f,h} (27.75-37.75)	30.50 ^{a,d,f,h} (26.50-38.75)	23.50 ^{a,d,f,g} (20.00-36.00)	12.00 ^{a,d,f,g} (3.75-12.50)
AST/ALT							
Control		0.78 (0.45-1.38)	1.36 (0.28-6.94)	5.71 (0.13-11.29)	0.06 (0.03-0.09)	0.05 (0.03-0.07)	0.10 (0.09-0.10)
OAT		0.80 (0.50-2.97)	0.49 (0.17-0.85)	0.41 (0.18-0.70)	0.43 (0.10-0.77)	0.57 (0.15-0.79)	0.99 ^a (0.29-1.70)
O ₃		1.02 (0.77-1.35)	0.81 (0.38-1.36)	0.45 (0.15-0.65)	0.62 (0.22-0.94)	0.71 ^a (0.33-1.05)	1.52 ^a (0.75-2.24)
YC		0.73 (0.48-1.11)	1.09 (0.62-7.86)	0.43 (0.26-0.78)	0.21 (0.07-0.45)	0.24 (0.07-0.87)	1.00 ^a (0.20-2.02)
O ₃ + YC		2.39 ^{b,f,h} (1.56-2.59)	2.08 ^{d,f} (1.43-2.17)	1.09 ^{d,f,h} (1.00-1.70)	1.39 ^{a,d,f,h} (1.26-1.79)	1.43 ^{a,d,f,g} (1.04-1.92)	2.08 ^{a,c} (1.77-3.79)
TBIL/(μmol/L)							
Control	2.10 (0.18-4.73)	7.15 (2.73-31.83)	9.80 (6.15-33.85)	6.65 (6.60-6.70)	6.10 (5.30-6.90)	4.95 (4.70-5.20)	1.80 (0.60-3.00)
OAT	3.80 (0.85-4.70)	5.20 (2.50-6.40)	5.30 (1.90-14.30)	6.55 (3.35-21.15)	3.50 ^a (2.05-4.20)	5.45 (1.88-10.90)	3.30 (1.20-6.08)
O ₃	1.55 (0.20-4.85)	4.30 (2.45-5.65)	4.30 (3.05-7.05)	3.30 (1.95-6.45)	1.90 ^a (1.10-4.35)	2.50 ^a (1.85-4.20)	3.00 (0.80-4.95)
YC	2.65 (0.35-5.18)	3.40 (1.60-26.15)	7.00 (2.95-33.65)	3.40 ^a (1.15-3.55)	4.45 (1.55-5.78)	1.95 ^a (0.80-3.93)	2.70 (0.98-10.73)
O ₃ + YC	4.20 (3.83-4.33)	1.95 ^{a,c,e} (0.10-2.40)	1.00 ^{b,c,e,h} (0.30-2.38)	1.45 ^{a,c} (0.80-5.10)	1.30 ^{a,d,g} (0.70-1.55)	0.85 ^{a,c,e} (0.50-2.13)	3.50 (0.50-4.28)
PT/s							
Control	6.80 (6.50-13.05)	13.20 (8.93-38.93)	18.90 (12.40-60.70)	12.25 (11.70-12.80)	8.70 (8.40-9.00)	7.80 ^a (7.60-8.00)	8.55 ^a (8.20-8.90)
OAT	6.50 (6.50-7.28)	7.20 ^a (6.50-8.35)	7.85 ^a (7.60-9.83)	7.95 (6.50-9.40)	6.50 (6.50-8.70)	6.50 (6.50-6.50)	6.50 (6.95-7.93)
O ₃	7.30 (6.95-8.03)	8.60 (7.30-11.35)	9.50 (8.65-16.35)	7.80 ^a (7.50-10.00)	7.30 (6.90-8.45)	7.50 (7.00-7.70)	7.10 ^a (6.80-7.30)
YC	7.70 (7.18-8.20)	12.55 (8.23-24.75)	9.90 (8.70-36.40)	7.40 ^a (7.20-10.60)	7.20 (6.68-8.70)	7.55 (6.68-8.20)	6.90 (6.50-8.13)
O ₃ + YC	7.65 (7.35-8.15)	7.25 ^{a,c,g} (6.50-8.13)	7.40 ^{b,c,f,h} (7.20-8.65)	7.30 ^a (7.18-8.28)	7.30 ^a (7.28-7.40)	6.85 ^a (6.50-7.50)	7.45 ^a (7.03-7.70)
AMMO/(μmol/L)							
Control	69.55 (35.47-106.35)	96.50 (53.20-161.33)	114.80 (80.90-133.90)	132.80 (82.30-183.30)	143.40 (67.40-219.40)	53.00 (32.50-73.50)	91.40 (40.60-142.20)
OAT	41.20 (35.45-54.50)	54.70 (43.70-79.70)	44.35 ^a (25.93-83.10)	49.85 ^a (38.48-62.28)	55.10 ^a (31.35-64.53)	49.65 (38.55-75.45)	33.30 (13.40-52.50)
O ₃	49.75 (39.33-62.03)	54.70 (52.40-76.65)	51.50 ^a (43.00-73.50)	65.70 ^a (52.20-72.40)	48.30 (44.85-74.05)	65.40 (44.50-94.80)	47.10 (32.63-63.75)
YC	47.50 (34.25-73.23)	53.80 (49.45-173.23)	54.70 ^a (43.70-79.70)	55.70 (45.83-86.95)	77.35 (57.63-168.25)	45.40 (39.18-97.68)	45.50 (23.35-110.85)
O ₃ + YC	63.50 (15.60-74.78)	77.35 (56.73-158.20)	55.30 ^a (44.90-69.58)	59.65 ^a (41.70-63.45)	58.40 (45.95-69.25)	67.10 (51.20-93.95)	41.90 (37.08-61.48)
BUN/(mmol/L)							
Control	3.30 (2.45-4.45)	5.50 (3.85-7.15)	3.80 (2.70-6.20)	2.40 (1.60-3.20)	3.60 (3.10-4.10)	3.65 (3.20-4.10)	5.15 (1.20-9.10)
OAT	3.85 (3.53-4.38)	6.20 (5.20-8.30)	4.50 (4.10-6.25)	3.70 (3.25-4.60)	4.65 (3.10-7.33)	3.20 (2.38-6.65)	2.75 (2.10-4.00)
O ₃	3.75 (3.18-5.55)	5.70 (4.65-8.70)	5.80 (4.75-7.05)	4.00 (3.20-5.80)	3.90 (2.40-6.45)	2.60 (1.90-6.35)	2.60 (2.20-3.30)
YC	3.50 (2.88-5.73)	7.55 (3.90-13.58)	4.30 (3.55-13.00)	4.10 (1.98-7.13)	3.00 (2.55-6.68)	2.00 (1.68-6.75)	3.20 (2.83-3.20)
O ₃ + YC	2.85 (2.28-3.65)	3.10 ^{d,e,g} (1.50-3.98)	3.50 ^e (2.70-4.15)	3.75 (2.30-4.68)	3.45 (3.30-3.95)	2.20 (1.80-3.28)	1.80 ^g (1.70-2.40)

^a*P* < 0.05 vs Control; ^b*P* < 0.01 vs Control; ^c*P* < 0.05 vs OAT; ^d*P* < 0.01 vs OAT; ^e*P* < 0.05 vs O₃; ^f*P* < 0.01 vs O₃; ^g*P* < 0.05 vs YC; ^h*P* < 0.01 vs YC. ALT of each dog was within the normal level (ALT < 60.0 U/L)^[5] pre-injection, and thus the values of AST/ALT made no sense at that time.

Figure 2 Histological assessment of livers following death or living for 15 d (original magnification, $\times 100$, **A**, **B**; $\times 400$, **C**, **D**, **E**). Hepatocellular bridged necrosis, mononuclear and lymphoid inflammatory infiltration, necrosis and hemorrhage (**A**) accompanied reticular fibers structures collapsing in control group (**B**). Increased mononuclear and lymphoid inflammatory infiltration, and necrosis in groups OAT, O₃, and YC (**C**, **D**) compared with group O₃ + YC (**E**). Inflammation and necrosis were graded on a 4-point scale (Table 1) by a blinded pathologist in 20 random high power fields per animal ($n = 6$ in each group), represented graphically in panels (**F**) errorbars: 95.00% CI and (**G**) errorbars: 95.00% CI. Data represent mean $\pm$ SE; ^a $P < 0.05$ vs Control.

medical ozone to treat infectious diseases, including viral hepatitis B, and to treat rheumatic diseases. Although effort has been done to elucidate the mechanism of ozonotherapy, but its practices in whole still remained in an empirical fashion and have not been accepted by sutra legitimacy medicine.

Research frontiers

Drug-induced liver injury is an increased important problem in modern medical practice. Apart from avoiding using hepatotoxic drugs, none of effective methods to prevent drug induced hepatitis are confirmed so far. There are several articles that introduced using medical ozone to prevent liver or renal ischaemia and reperfusion (I/R) injury in animal models. Free radical and reactive oxygen species during I/R process are the main cause of organ I/R injury. The mechanism of some drug-induced hepatitis is the same way as I/R injury. So, the ozone's property of inducing antioxi enzymes and enhancing capacity of radical scavenger should confer preventive effect for protecting liver from hepatotoxic drug-induced injury.

Innovation and breakthroughs

This study gives us a new knowledge of medical ozone and impels us to have following deduction, i.e. medical ozone and herb medicine rectal administration may be a new strategy to prevent drug-induced hepatitis. From design point of view, big animal as dog as animal model in this study imitates human being more likely than small canine as mouse. Rectal administration of ozone and herb medicine was proved effective in this experiment, and provided convenient way for future clinical application.

Application

The results from this animal study confirm the protective effect of ozone and prescription of traditional Chinese medicine on hepatotoxic drug-induced liver injury. It encourages us to apply medical ozone in preventing some drug-induced hepatitis in the future clinical trials.

Terminology

Medical ozone is a mixture gas of ozone and oxygen. It is made from pure oxygen by an ozone generator. This machine used in medical therapy must have capacity to adjust ozone concentration in a precision and stabilization pattern.

Peer review

This study further confirmed the protective effect of medical ozone and herb medicine on drug-induced hepatitis and was a steady step toward its clinical application. Medical ozone and traditional Chinese medicine are both ancient antiques, its worthiness was not staying in ancient history and its merits are also useful in modern medicine, just simply needs to be added modern decoration, i.e. the evidence based medicine (EBM).

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