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Effects of Nanogold on the Alleviation of Carbon Tetrachloride-Induced Hepatic Injury in Rats

Yi-Peng Chen[1], Zong-Her Dai[1], Pan-Chen Liu[2], Jiunn-Jye Chuu[1], Kuo-Yang Lee[1], Shun-Lai Lee[1], and Yun-Ju Chen[3]

[1]*Department of Biotechnology, College of Engineering, Southern Taiwan University, Tainan 71005*
[2]*Department of Veterinary Medicine, College of Veterinary Medicine, National Chung Hsing University, Taichung 40227*
and
[3]*Department of Child Care, College of Humanities and Social Sciences, Southern Taiwan University, Tainan 71005, Taiwan, Republic of China*

Abstract

Gold particles have been used in complementary medicine for decades, and many beneficial effects have been reported. Our present study sought to evaluate the therapeutic effects of nanogold in carbon tetrachloride (CCl_4)-injured liver of rats. Male SD rats were subjected to liver injury induction by CCl_4, then the rats were fed with zero to high dose (0, 1, 5 or 10 ppm) of nanogold water every day for 4 weeks. Biochemical analyses on liver functions were then performed to evaluate the therapeutic effects of nanogold. Our results revealed that gold nanoparticles lowered serum aspartate aminotransaminase (AST) and alanine aminotransferase and exerted serum total protein-recovering effects, which might be partially associated with the elevation of anti-inflammatory cytokine IL-10 level. In addition, serum triglyceride level fell after continuous ingestion of nanogold. Finally, the experimental animals recovered body weight after 4 weeks of nanogold ingestion. This is the first report indicating inflammation-alleviating effects of nanogold on hepatic injury.

Key Words: alanine aminotransferase, aspartate aminotransferase, hepatic injury, IL-10, nanogold

Introduction

Pure colloidal gold has been known for decades to exhibit beneficial effects on rejuvenating weak organs, especially the brain and digestive system, and on improving blood circulation (†). Colloidal gold has also been successfully used in treating rheumatoid arthritis, alcoholism and addiction and tuberculosis, and in healing burns, scrapes and open sores (†, §). Colloidal gold is tasteless and non-toxic relative to gold salts which accumulate in the body and cause toxic side effects (2). World Health Organization also approved gold as a food additive in 1983. In the past 10 years, nanogold has been reported to be therapeutically effective in treating experimental arthritis by inhibiting the activity of vascular endothelial growth factor (VEGF) in the synovial fluid (17), and in reducing cancer mass (10) as well as plaques of Alzheimer's disease (¶) by enhancing the efficacy of photothermal therapy. This is the first study demonstrating the inflammation-alleviating effect of nanogold upon hepatic injury.

Corresponding author: Dr. Yun-Ju Chen, Department of Child Care, College of Humanities and Social Sciences, Southern Taiwan University, No. 1, Nan-Tai Street, Yongkang Dist., Tainan City 71005, Taiwan, ROC. Tel: +886-6-2533131 ext. 8193, Fax: +886-6-2368201, E-mail: yunjuchen@mail.stut.edu.tw

†Colloidal Gold. Available at: http://www.colloidalworld.com/colloidal-gold.html. Accessed February 20, 2011.
§Colloidal Gold Benefits. Available at: http://www.buzzle.com/articles/colloidal-gold-benefits.html. Accessed April 12, 2011.
¶Gold is newest weapon in battle against Alzheimer's. *Health News*; **12**:10, 2006.

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Table 1. Grouping of experimental animals

Groups	Intraperitoneal injection	Per os [tube-fed]
Group A (standard control group): normal diet	olive oil	normal saline
Group B (sham control group): carbon tetrachloride treatment	40% CCl_4/olive oil	normal saline
Group C (experimental group): low dose of nanogold water	40% CCl_4/olive oil	nanogold water (1 ppm)
Group D (experimental group): medium dose of nanogold water	40% CCl_4/olive oil	nanogold water (5 ppm)
Group E (experimental group): high dose of nanogold water	40% CCl_4/olive oil	nanogold water (10 ppm)

Materials and Methods

Preparation of Gold Nanoparticles

The 2-5 nm nanogold particles were manufactured by Gold NanoTech, Inc., Taipei, Taiwan, R.O.C. using the innovative technology of physical metal miniaturization to guarantee that the nanogold was 100% gold.

Experimental Design

Male Sprague Dawley (SD) rats weighing 400-420 g were used in this study. Twenty-five male SD rats were randomly divided into five groups: A, B, C, D and E (Table 1). nimals were subjected to induction of liver injury twice a week (Monday and Thursday) for 4 weeks. Group A (standard control group) rats were given intraperitoneal injection of olive oil (0.1 ml/100 g BW), and rats in groups B to D were i.p. injected with 40% CCl_4 in olive oil (0.1 ml/100 g BW). For the nanogold treatment program post hepatic injury induction, groups A and B rats were tube-fed with normal saline (1 ml/100 g BW) every day. Groups C, D and E rats were given low, medium or high dose (1, 5, 10 ppm; 1 ml/100 g BW) of nanogold water every day, respectively, for 4 weeks. Rats were sacrificed with diethyl ether at the end of the nanogold treatment period.

Blood Collection

Each animal was anaesthetized with diethyl ether, and 2 ml of blood was collected once a week from the tail vein 2 h after nanogold ingestion. All rat blood samples were kept at room temperature for 1 h to allow clotting. The samples were then centrifuged with 12,000 rpm at 4°C for 5 min to separate the serum (12). Serum was separated and preserved in a cuvette at -20°C until analysis.

Biochemical Analysis of Serum Samples

Serum samples collected from different groups of rats were analyzed for serum ALT, AST, TP and TG by commercially available standard assay kits as previously described in the study of Sancheti *et al*. (Asan Pharmaceutical, Seoul, Gyeonggi-do, South Korea) (16). The absorption was recorded using an automated serum analyzer.

Cell Culture

The mouse macrophage cell line RAW264.7 was maintained at 37°C with 5% CO_2 in supplemented RPMI 1640 (10% fetal bovine serum, 0.2 mM L-glutamine, 100 U of penicillin/ml and 100 µg of streptomycin/ml). 5×10^3 RAW264.7 cells were co-incubated with 0, 1, or 10 ppm of nanogold for 24 h in 96-well plates. Cell supernatants were collected, spun down at 12,000 ×g for 5 seconds before being subjected to enzyme-linked immunosorbent assay (ELISA) for IL-10 secretion from the macrophages.

IL-10 ELISA

For quantification of IL-10, a commercially available ELISA (Biosource International, Camarillo, CA, USA) was used according to the manufacturer's instructions. In short, 100 µl samples, including standards of known mouse IL-10 content, were pipetted into the wells coated with an antibody specific for mouse IL-10, followed by incubation for 1 h at 37°C. Then 100 µl of a biotinylated second antibody was added. The plate was incubated for another hour at room temperature. After removal of excessive second antibody, 100 µl of streptavidin-peroxidase was added to each well. After incubating the plate for 30 min at room temperature, the wells were washed to remove all unbound enzyme. Chromogen solution (100 µl) was added, followed by incubation for 30 min at room

temperature in the dark to produce color. Stop solution (100 µl) was added to each well, and the absorbance of each well was read at 450 nm. The intensity of the colored product was directly proportional to the concentration of mouse IL-10 present in the sample.

Statistical Analysis

All of the data are expressed as means ± SD. The significance of difference was evaluated by one-way ANOVA followed by Duncan's pairwise multiple comparison tests. Differences were considered significant if $P < 0.05$.

Results

Therapeutic Effects of Nanogold Ingestion in Repairing Injured Liver Induced by CCl_4

To analyze the effects of nanogold on CCl_4-damaged liver, markers of hepatocyte injury aspartate aminotransferase (AST, formerly called serum GOT) and alanine aminotransferase (ALT, formerly serum GPT) were examined (Fig. 1). Liver was the main site of synthesis of plasma proteins including globulin, albumin, fibrinogen, lipoproteins and other coagulation factors. Therefore, serum total protein (TP) was also measured as an indication of liver function. AST, ALT and TP values were examined once every week for 4 weeks. Intermediate to high dose (5, 10 ppm) of nanogold showed a significant lowering effect on AST and ALT values in the later course of CCl_4 induction, especially in the 4th week (Fig. 1, A and B). A significant recovery of serum TP value was observed in nanogold-treated rats in the later course of nanogold treatment (Fig. 1C). Our results indicate that nanogold is therapeutically effective in treating hepatic injury.

Release of Anti-Inflammatory Cytokine IL-10 following Nanogold Treatment

The AST and ALT-lowering effects suggest induction of anti-inflammatory action by the nanogold. Therefore, we sought to understand one step further the anti-inflammatory mechanism of nanogold. After 24 h of nanogold incubation, there was a dose-dependent increase in anti-inflammatory cytokine IL-10 secretion from macrophages, indicating the involvement of IL-10 in the anti-inflammatory action of nanogold (Fig. 2).

Effect of Nanogold Ingestion in Lowering Serum Triglyceride Level

Since high serum triglyceride (TG) level is as-

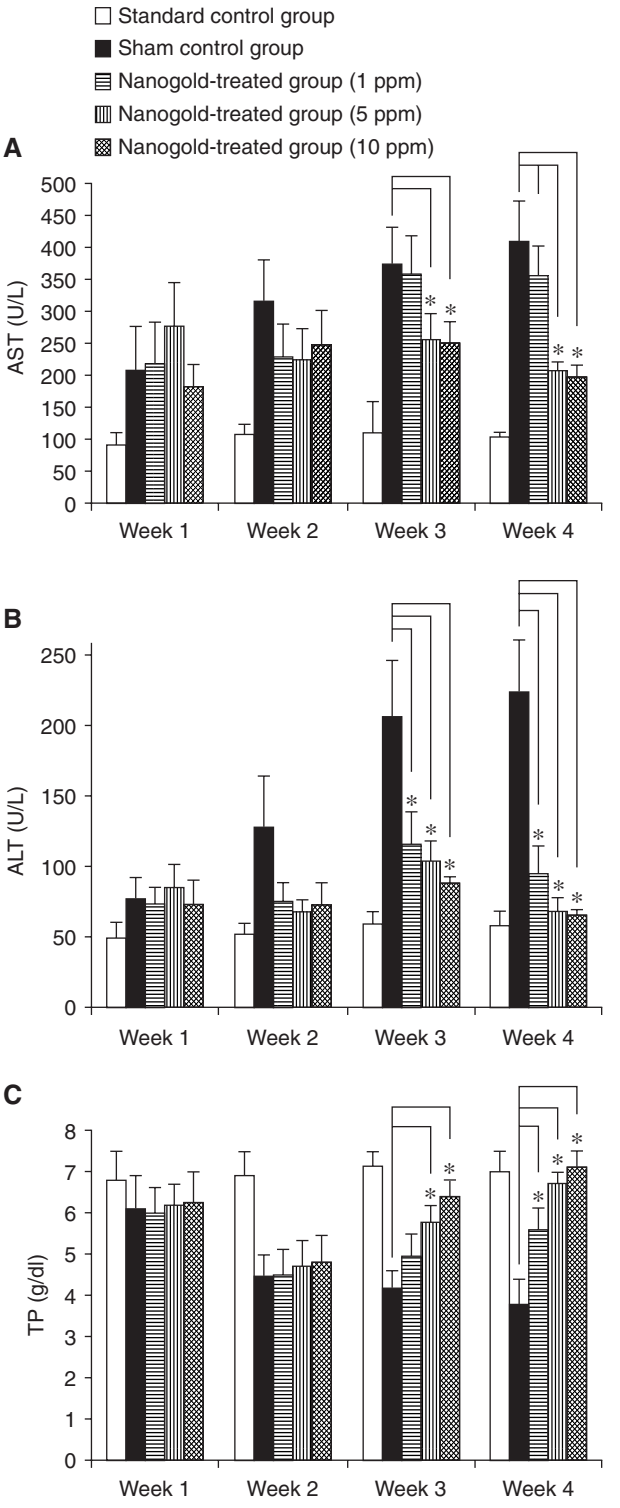

Fig. 1. Effects of nanogold on liver function markers *in vivo*. Blood was withdrawn at the end of each week from the tail vein of SD rats (n = 5) 2 h after nanogold ingestion, and the collected sera were subjected to biochemical analyses. The results were plotted as (A) AST, (B) ALT and (C) TP over the 4 weeks. The data are presented as means ± SD, and the experiments were repeated twice with similar results. *$P < 0.05$, compared with the sham control group induced with CCl_4.

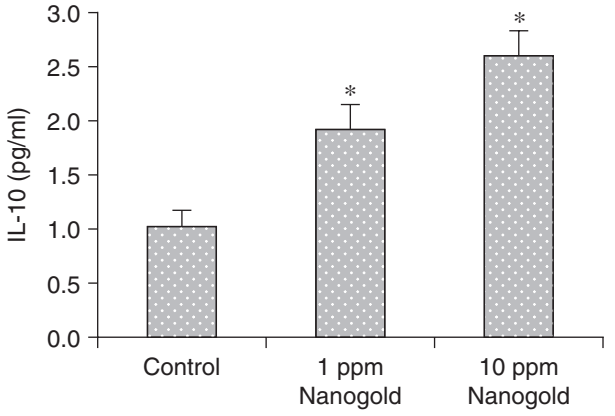

Fig. 2. Effects of nanogold on IL-10 secretion *in vitro*. RAW 264.7 macrophages were treated with 0, 1, or 10 ppm of nanogold for 24 h and secretion of IL-10 into the medium was measured by ELISA. Values represent means ± SD of three independent experiments. $*P < 0.05$, compared with the control group without nanogold treatment.

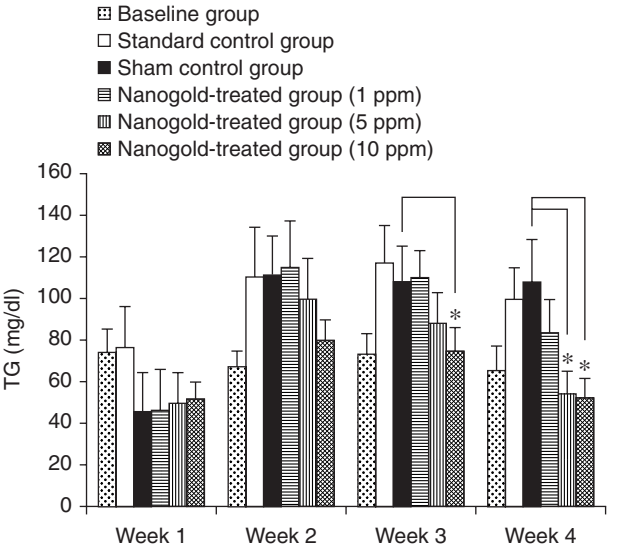

Fig. 3. Effects of nanogold on serum TG level *in vivo*. The collected sera were subjected to serum TG level determination (n = 5). The results were plotted and presented as means ± SD, and the experiments were repeated twice with similar results. $*P < 0.05$, compared with the sham control group induced with CCl_4.

sociated with fatty liver formation which leads to prolonged increased AST and ALT values, serum TG level was analyzed to evaluate the effects of nanogold. It seemed that olive oil tended to raise serum TG value throughout the experiment, and intermediate to high concentrations (5 and 10 ppm) of nanogold ingestion resulted in a significant reduction in serum TG levels in the 4th week of CCl_4 induction when compared with the group without nanogold treatment. Serum TG values for an additional baseline group without any treatment were measured to demonstrate the normal level of serum TG unaffected by olive oil. Our results indicate that nanogold lowers blood lipids, thereby inhibiting fatty liver formation and subsequent elevation of AST and ALT values.

Recovery of Body Weight as a Result of Nanogold Ingestion

To evaluate the effects of nanogold treatment on the body weight of hepatic-injured rats, body weight was recorded every week and plotted. CCl_4 caused a significant decrease in body weight after 4 weeks of induction (Fig. 4, A and B). On the other hand, rats treated with intermediate to high dose (5 or 10 ppm) of nanogold showed a gradual increase in body weight after 4 weeks of CCl_4 induction. Therefore, rats treated with intermediate to high dose (5 or 10 ppm) of nanogold showed a prominent body-weight difference after 4 weeks compared with rats induced only with CCl_4 (Fig. 4, A and B). Here we found a positive correlation between recovery of liver functions and reestablishment of body weight.

Discussion

Pure gold has been used in complementary medicine for many decades. Besides its many beneficial effects, some toxic side effects have been reported to be associated with the use of gold compounds, but not pure gold (2, 13). It has been proposed that most of the toxicities associated with the use of gold aurothiolates were probably due to the gold trichloride formed *in vivo* by disproportionation (2). On the other hand, no evidence indicates that pure colloidal gold causes toxicity at the clinical, histological, cellular and molecular levels (3, 6-9, 11). Metallic gold is excreted mainly *via* the kidneys according to Perrelli and Piolatto (15). A case study in one male subject from Abraham in 2008 demonstrated that whole-blood levels peaked at 30 min and became undetectable by 8 h post-ingestion of 30 mg colloidal gold in a liquid suspension, suggesting a rapid clearance of colloidal gold from the peripheral circulation (1). Gold compounds like gold aurothiolates, however, have a very long half life with over 90% of gold bound to serum albumin in the blood (4). The fraction of gold thiolate associated with red blood cells is not internalized in the cells but is bound to the erythrocyte membrane (5). Mukherjee *et al*. also performed renal and hepatic toxicity assays of nanogold in which nanogold was given to normal mice for 7 consecutive days, then the mice were sacrificed and the serum was collected. They found no significant differences be-

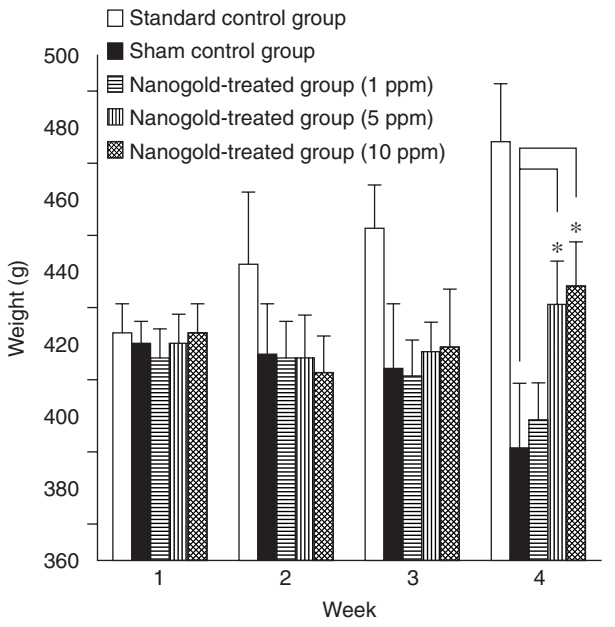

Fig. 4. Effects of nanogold on body-weight recovery *in vivo*. The body weight was measured every 7 days since the onset of terpinen-4-ol treatment and plotted as means ± SD (n = 5). The experiments were repeated twice with similar results. $*P < 0.05$, compared with the sham control group induced with CCl_4.

tween serum levels of creatinine, blood urea nitrogen, bilirubin alkaline phosphatase, AST and ALT between the nanogold treated and untreated control animals, indicating again the safety of using pure colloidal gold (14).

Furthermore, our nanogold was manufactured with physical vapor deposition (PVD) process which maintains 99.99% of gold nanoparticles, and the unique technology was applied to allow our gold nanoparticles to be evenly dispersed in pharmaceutical graded water. Therefore, unlike nanogold made with chemical reduction which requires the addition of dispersing agent to avoid the aggregation of nanoparticles, the gold nanoparticles used in this study are evenly suspended in water without the addition of dispersing agent. This further increases the purity of nanogold. In addition, over 95% of the 2-5 nm nanogold used in this study can be efficiently excreted, and the nanogold does not accumulate inside the body (James Tang — Chairman & CEO of Gold NanoTech, Inc., Taipei, Taiwan, R.O.C., personal communication).

Nanogold has been known to have anti-inflammatory and enhanced collagen formation effects and is used in traditional medicine worldwide. However, the underlying mechanism is unclear. A previous study by Tsai *et al*. demonstrated that nanogold could bind to and thereby inhibit the activity of VEGF in the synovial fluid of rheumatoid arthritis, resulting in the amelioration of rheumatoid arthritis (17). Our present results indicated that nanogold could elevate anti-inflammatory cytokine IL-10 level in supernatants of macrophages co-incubated with nanogold, which might partially explain the anti-inflammatory effects of nanogold on liver as macrophage infiltration is a common phenomenon for hepatic inflammation.

In conclusion, our results suggest that nanogold may be used as a complementary medicinal agent in patients with hepatic diseases.

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References

1. Abraham, G.E. Clinical applications of gold and silver nanocolloids. *Original Internist* 15: 132-157, 2008.
2. Abraham, G.E. and Himmel, P.B. Management of rheumatoid arthritis: Rationale for the use of colloidal metallic gold. *J. Nutr. Environ. Med*. 7: 295-305, 1997.
3. Ackerman, G.A.M. and Wolken, K.W. Histochemical evidence for the differential surface labeling. Uptake, and intracellular transport of a colloidal gold-labeled insulin complex by normal human blood cells. *J. Histochem. Cytochem*. 29: 1137-1149, 1981.
4. Blocka, K.L., Paulus, H.E. and Furst, D.E. Clinical pharmacokinetics of oral and injectable gold compounds. *Clin. Pharmacokinet*. 11: 133-143, 1986.
5. Campbell, J.M., Reglinski, J., Smith, W.E., Porter, D. and Sturrock, R.D. Action of sodium aurothiomalate on erythrocyte membrane. *Ann. Rheum. Dis*. 51: 969-971, 1992.
6. Danien, B.J., Sims, P.A. and Kruse-Elliott, K.T. Use of colloidal gold and neutronactivation in correlative microscopic labeling and label quantitation. *Scanning Microsc*. 9: 773-780, 1995.
7. De Roe, C., Courtoy, P.J., Quintart, J. and Baudhuin, P. Molecular aspects of the interactions between protein, colloidal gold and cultured cells: Applications to galactosylated serum albumin and rat hepatocytes. *Arch. Intern. Physiol. Biochem*. 90: 186, 1982.
8. Feldherr, C. and Akin, D. The permeability of nuclear envelope in dividing and nondividing cell culture. *J. Cell Biol*. 111: 1-8, 1990.
9. Feldherr, C. and Akin, D. Signal-mediated nuclear transport in proliferating and growth-arrested BALA/c 3T3 cells. *J. Cell Biol*. 115: 933-999, 1991.
10. Huang, X., Qian, W., El-Sayed, I.H. and El-Sayed, M.A. The potential use of the enhanced nonlinear properties of gold nanospheres in photothermal cancer therapy. *Laser Surg. Med*. 39: 747-753, 2007.
11. Juurlink, B.H. and Devon, R.M. Colloidal gold as a permanent marker of cells. *Experientia* 47: 75-77, 1991.
12. Lin, S.C., Lin, Y.H., Chen, C.F., Chung, C.Y. and Hsu, S.H. The hepatoprotective and therapeutic effects of propolis ethanol extract on chronic alcohol-induced liver injuries. *Am. J. Chin. Med*. 25: 325-332, 1997.
13. Merchant, B. Gold, the noble metal and the paradoxes of its toxicology. *Biologicals* 26: 49-59, 1998.
14. Mukherjee, P., Bhattacharya, R., Wang, P., Wang, L., Basu, S.,

Nagy, J.A., Atala, A., Mukhopadhyay, D. and Soker, S. Antiangiogenic properties of gold nanoparticles. *Clin. Cancer Res*. 11: 3530-3534, 2005.

15. Perrelli, G. and Piolatto, G. Tentative reference values for gold, silver, and platinum: Literature data analysis. *Sci. Total Environ*. 120: 93-96, 1992.
16. Sancheti, S., Sancheti, S., Bafna, M. and Seo, S.Y. Antihyperglycemic, antihyperlipidemic, and antioxidant effects of *Chaenomeles sinensis* fruit extract in streptozotocin-induced diabetic rats. *Eur. Food Res. Technol*. 231: 415-421, 2010.
17. Tsai, C.Y., Shiau, A.L., Chen, S.Y., Chen, Y.H., Cheng, P.C., Chang, M.Y., Chen, D.H., Chou, C.H., Wang, C.R. and Wu, C.L. Amelioration of collagen-induced arthritis in rats by nanogold. *Arthritis Rheum.* 56: 544-554, 2007.