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Gamijiyu-tang Decreases the Dimethylnitrosamine-Induced Hepatic Fibrosis in the Rats

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Abstract – Gamijiyu-tang (GJT) described originally in the Dong Eui Bo Gam, a traditional reference for oriental medicine in the Korea, has been clinically used for treatment of chronic liver disease. In order to evaluate scientifically a hepatoprotective effect of GJT in the liver fibrotic disease, the present study investigated how GJT improves a hepatic function in the dimethylnitrosamine (DMN)-treated rat. DMN treatment caused a significant increase of relative liver weight to the body at 28 days after DMN induction. Administration of GJT with a clinical dose decreased significantly the sAST level (158.8±7.76 IU/L) elevated by DMN injection ($p<0.01$). A similar phenomenon was also observed at change of both sALP and sALT level in the GJT and/or DMN-treated animal ($p<0.01$, $p<0.05$, respectively). A remarkable increase of hydroxyproline was observed by treatment of DMN with comparing to the normal rat (361.9±7.35 *vs.* 1278.1±52.9 μg/g tissue, $p<0.01$). This was significantly reduced by a simultaneous treatment of GJT with DMN for 21 days ($p<0.05$), but not recovered completely to its normal value. In addition, GJT administration ameliorated conspicuously the DMN-induced histopathological changes of liver such as hemorrhage, cell necrosis and fibrosis. Taken together, results described here demonstrated scientifically in first the medicinal efficacy of GJT by using in vivo animal model, indicating that GJT improves the DMN-induced hepatic injury through reducing an excessive accumulation of collagen and histopathological changes. The decreased collagen content may be a pivotal process for GJT to improve hepatic function in the DMN-induced liver fibrosis. The present study suggests that GJT may be useful for and applicable to the treatment of hepatic fibrosis in chronic liver disease.

Key words – Gamijiyu-tang; liver; fibrosis; dimethylnitrosamine; collagens; rat.

A marked accumulaton of collagen in the liver, hepatic fibrosis, is the most common lesion of clinically relevant chronic liver disease which leads to functional failure, and may progress to cirrhosis. This typical hepatic disease is related with a high rate of morbidity and mortality in the human. Although an excess accumulation of collagen is a pivotal pathological feature in the hepatic fibrosis, it is a complex process which involves additionally a remarkable deposition of extracellular matrix components caused by activation of cells capable of producing matrix materials. The extracell-

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ular matrix component are interstitial collagens (types I and III), basement membrane collagen (type IV), and noncollagenous glycoprotein such as laminin and fibronectin.[1] In order to ensure the information of functional change of extracellular matrix in the pathological condition, it is necessary to know the production of hepatic collagen *in vivo*.

Dimethylnitrosamine (DMN) is the most widely distributed carcinogen in food, pesticide formulations, tobacco smoke and industrial environments, and an agent accepted generally as causing necrosis or toxic cell death. Even many different chemicals such as alcohol and CCl_4 are well known compounds to induce liver damage in *in vivo*, DMN is one of them that are frequently used for induction hepatic fibrosis in *in vivo*. In the DMN-induced animal model for liver disease, inflammatory infiltrate is associated with activation of perisinusoidal stellate cells, progressive deposition of collagen and extracellular matrix.[2-4] This *in vivo* model has been shown to reproduce partially some clinical and morphological features of human chronic liver disease.[5]

In order to know the biochemical activity of DMN which is related to tissue injury *in vivo*, its bioactivation mechanism could be understood at a molecular and cellular level. In general consideration, two enzyme species are biochemically responsible for the *N*-demethylation of DMN through an oxidative N-demethylation reaction, namely DMN-N-demethylase I and II.[6,7] Kamendulis and Corcoran[8] suggested that DMN produces DNA damage arising in part through Ca^{2+}-dependent endonuclease activation in a cultured hepatocyte, and this injury is an early event in the DMN-induced toxic cell death. Moreover, this chemical can lead to cause an excessive deposition of extracellular matrix protein, specially collagen in the rat liver.[9,10] In the previous reports,[3,11,12] DMN-induced hepatic cirrhosis model has an inflammatory infiltrate which is associated with activation of perisinusoidal stellate cells and the progressive deposition of collagen and extracellular matrix components. This process is mainly mediated by fibrogenic and/or proliferation of cytokines. And then, therapeutic strategies for hepatic fibrosis is mainly targeted to reduce the process of fibrogenesis regulated by perisinusoidal stellate cells that may be a final common pathway for liver fibrosis.[13] Although a several different types of therapeutics for treatment of hepatic fibrosis and chronic liver disease are currently used, medicines which have a high efficacy for them are not known yet.

Gamijiyu-tang (GJT) composed with several different herbs is a noted prescription based on the traditional medicine in Korea. Clinically, it has been mainly used in the chronic liver disease including cirrhosis.[14] Although it has being used clinically for treatment of human disease, no scientific evidence is available, demonstrating that GJT improves physiologically the liver function which was reduced by a certain pathological condition, specifically hepatic fibrosis and chronic liver disease, in the animal model. And then, the present study was done to evaluate whether GJT has a hepatoprotective activity in the DMN-induced liver fibrosis model *in vivo*. Results provided here demonstrated in first that daily administration of GJT reduced the increased serum aminotransferase activity, collagen content, and ameliorated the histopathological changes which are associated

with the development of DMN-induced liver fibrosis *in vivo*.

MATERIALS AND METHODS

Animals – Male Sprague-Dawley rats (210~250 g) purchased from Korea Research Institute of Chemical Technology, Daejun were used. They were allowed free access to a commercial diet (Samyang) and tap water, and maintained under 12-hour light-dark cycles. Liver damage (hepatic fibrosis) was induced by injecting DMN intraperitoneally at doses of 1 μl (diluted to 1: 100 with 0.15 M NaCl) per 100 g body weight. The injections were given weekly on the first three consecutive days over a period of 21 days. Control animals also received an equal volume of 0.15 M NaCl without DMN. Animals used in this study were sacrificed at 28 days after beginning of experiment by anesthetizing with diethyl ether. After blood for biochemical measurement was obtained from abdominal vein, the liver was rapidly removed, and rinsed in cold physiological saline. Liver weight on the each animal was measured after blotting with filter paper, and stored at -70℃ until analyzed.

Preparation and treatment of Gamijiyu-tang – As shown in Table I, GJT is a prescription mixed with a several different herbs. All herbs used were purchased from the Korea Medicine Herbs Association, Seoul, and authenticated by pharmacognosist, Korea Institute of Oriental Medicine. In order to prepare GJT extract, mixed herbs were boiled with 1 *l* of distilled water by using herbal medicine decocter (traditional type) for 1.5 hrs. The extract was filtered and the residue was boiled for 1 hour and filtered again. The filtrates were mixed together. Final volume was adjusted volumetrically to around 300 ml to prepare an appropriate volume for administration (10 ml/Kg body weight). The extract was administered orally at doses of clinical use for a consecutive 21 days after 1 week DMN induction. In order to study the effect of GJT on the preinduced liver injury, GJT administration started at 1 week after the first DMN injection.

Table I. Composition of Gamijiyu-tang (GJT)

Constituent	Weight (g)
Sanguisorbae Radix	20
Angelicae gigantis Radix	20
Cnidii Rhizoma	20
Asini Relatinum	8
Sophorae Flos	4
Zingiberis Rhizoma	4
Schizonepetae Herba	4
Cervi pantotrichum Cornu	4
Glycyrrhizae Radix	2

Assessment of hepatic injury – At 28 days after starting experiment, all animals were fasted for overnight, and anesthetized with diethyl ether to sacrifice. After blood sample was obtained from abdominal vein, it allowed to clot at room temperature for 3-5 min, and centrifuged at 900×g for 10 min at 4℃ (Avanti 30, Beckman) to collect serum. Serum levels of alkaline phosphatase (sALP), aspartate aminotransferase (sAST) and alanine aminotransferase (sALT) were assayed by standard spectrophotometric methods by using commercial test reagents (Trace scientific) and automated system (Airone 200, Crony).

Colorimetric determination of collagen – Hydroxyproline content, as an indicator for the amount of collagen in the liver,[1,2,9-11] was determined by using a slightly modified method of previous one.[15] Briefly, liver was excised, blotted with filter paper, weighed, cut into 0.2 g for each sample with du-

plicate. Each tissue was homogenized with 4 ml of 6 N HCl, and hydrolyzed in Teflon-sealed glass vial at 110℃ for 18 hrs. The hydrolysate was aliquoted to 50 μl with duplicate in a separated vial, and allowed to evaporate at 65℃ for 4 hrs. Isopropanol (1.2 ml) was added into each vial to dissolve a sediment, then 0.2 ml aliquots of 0.56%-buffered chloramine-T solution was added. After taking 10 min of intermediated time, 1.0 ml of Ehrlich's solution was added to each sample. All sample were mixed completely, and incubated to develop color at 50℃ for 90 min. The absorbance of each sample was measured spectrophotometrically at 558 nm by using water as reference, and corrected for the reagent blank.

Liver histopathology - After blood collection, the median lobe of liver was immediately excised to continue histopathological observation. Briefly, liver slice was fixed in 10% neutral-buffered formalin. The hepatic tissue was dehydrated, and embedded in paraffin. Tissue was sliced with 5 μm thickness, and stained with hematoxylin/eosin for histological examination and trichrome to identify specifically the hepatic collagen.

Statistical analysis - Experimental results were statistically analyzed by one-way analysis of variance (ANOVA). The statistical significance between control and drug-treated group was evaluated by using Student's *t*-test. $P<0.05$ was considered as a significant difference in all comparison.

RESULTS

Changes of liver and body weight - When animals were treated with DMN, their body weight were dramatically decreased, and a few animal did not survived more than 2 weeks. Decrease of body weight by DMN administration did not restored by GJT treatment through a period of the present study (data not shown). By the way, as shown in Fig. 1, a relative weight of liver to body in DMN-treated rats was significantly elevated compared to it in the normal rat ($p<0.01$). Although coadministration of GJT reduced the DMN-induced increase of relative liver weight to body, statistical significance was not observed between DMN- and DMN plus GJT-treated animals. This may indicate a partial recovery of liver injury by GJT in the DMN-received animals.

Improvement of the hepatic function by GJT treatment - sALP, sAST and sALT activities were determined as indicators of parenchymal liver cell damage and hepatic function.[16] The values of sALP, sAST and sALT activities in the normal control animals were 157.8±11.51, 108.8±9.01 and 41.3 ±1.03 IU/l, respectively (mean±SE, n=7). At 28 days after beginning experiment, their activities were significantly elevated by treatment of DMN, representing that individual

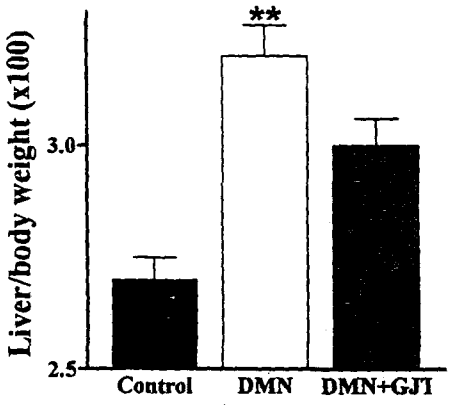

Fig. 1. Effect of GJT on liver weight in the DMN-treated rat. Liver weight was measured at 28 days after beginning experiment in the rats treated with DMN alone and DMN plus GJT. GJT was adminstered orally for 3 weeks after 1 week of DMN treatment. Results are expressed as mean±S.E. **$p<0.01$ vs. normal control rat.

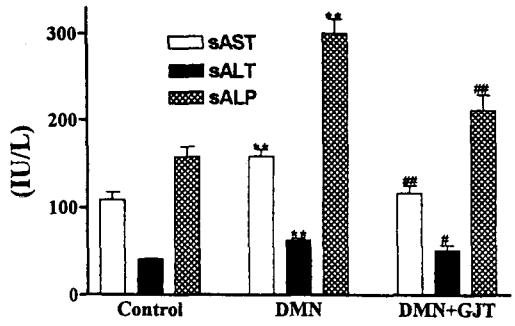

Fig. 2. Effect of GJT on the serum AST, ALT and ALP in the DMN-treated rats. Oral GJT administration started on the 7th day after DMN induction, and continued for 21 days. Results are expressed as mean±S.E. **p<0.01 vs. control. #p<0.05 and ##p<0.01 vs. DMN alone.

values of them were 301.5±16.85, 158.8±7.76 and 63.1±2.21 IU/l, respectively (mean± SE, n=7). This increment was lowered remarkably by administration of GJT (p<0.01, Fig. 2). Even though the elevated enzyme activity was not completely recovered to its normal level, reduction of enzyme activity by GJT could indicate that there was a tendency towards a reduced parenchymal liver cell damage and an improvement of hepatic function.

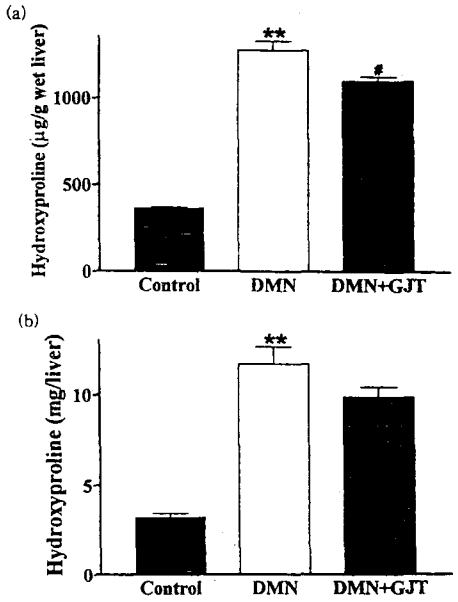

Fig. 3. Effect of GJT on hepatic hydroxyproline content in DMN-treated rats. The hydroxyproline content was measured in the rats treated with DMN alone and DMN plus GJT. Oral treatment of GJT began at 7 days after DMN induction, and treated concurrently with DMN for 3 weeks as described in Materials and Methods. (a) Hydroxyproline content normalized as wet tissue weight. (b) Total hydroxyproline amount represented as whole liver. Results expressed as mean±S.E. **p<0.01 vs. control value. #p<0.05 vs. the level of DMN-treated rats.

Decrease of collagen content by GJT administration–As described previously, one of typical features in hepatic damage by DMN is a proliferation of extracellular matrix. In regarding this hypothesis, the present study measured a hydroxyproline content to estimate a change of hepatic collagen content by DMN treatment and to evaluate the effect of GJT on the DMN-induced hepatic cirrhosis. The level of hydroxyproline content in the normal rats was 361.9±7.35 μg/g tissue (3.19±0.23 mg/liver). As shown in Fig. 3a and 3b, when DMN administered to the rats, both a hydroxyproline content normalized as liver weight (1,278.1 μg/g wet liver weight) and a total hydroxyproline amount in whole liver (11.7 mg/liver) were significantly increased by about 3.5 folds as compared to those in the normal control rats, respectively (p<0.01). Interestingly, the hydroxyproline content increased by treatment of DMN alone was significantly reduced by coadministration of DMN plus GJT (Fig. 3a, p<0.05). This result was consistent with the serum biochemical study, indicating that administration of GJT reduced a parenchymal liver cell damage and improved a hepatic function in the DMN-treated rats.

Histopathological changes–In the histopathological observation on day 28 of the experiment, hepatocytes were enlarged with

hemorrhage and congestion in the DMN-treated animal. Centrilobular damages consisting of inflammatory cell infiltration and fibrosis were observed with disintegration of hepatocyte (Fig. 4b and 4e). A special staining method, Masson Trichrome, was employed to identify the hepatic collagen and proliferation of extracellular matrix. A trichrome-positive cells in DMN-treated liver was much more prominent than that in normal hepatocytes (Fig. 4d and 4e). In animals receiving GJT plus DMN, liver structure identified by using both H&E and Trichrome staining strategies was more regular compared to it of animal receiving DMN alone, but not recovered completely (Fig. 4c and 4f). This result also demonstrated that GJT could ameliorate the DMN-induced hepatic damage. Histopathological finding obtained by here indicated that DMN leads to hepatic fibrosis, and consistent with the biochemical results evaluated by measurement of hydroxylated amino acid, hydroxyproline.

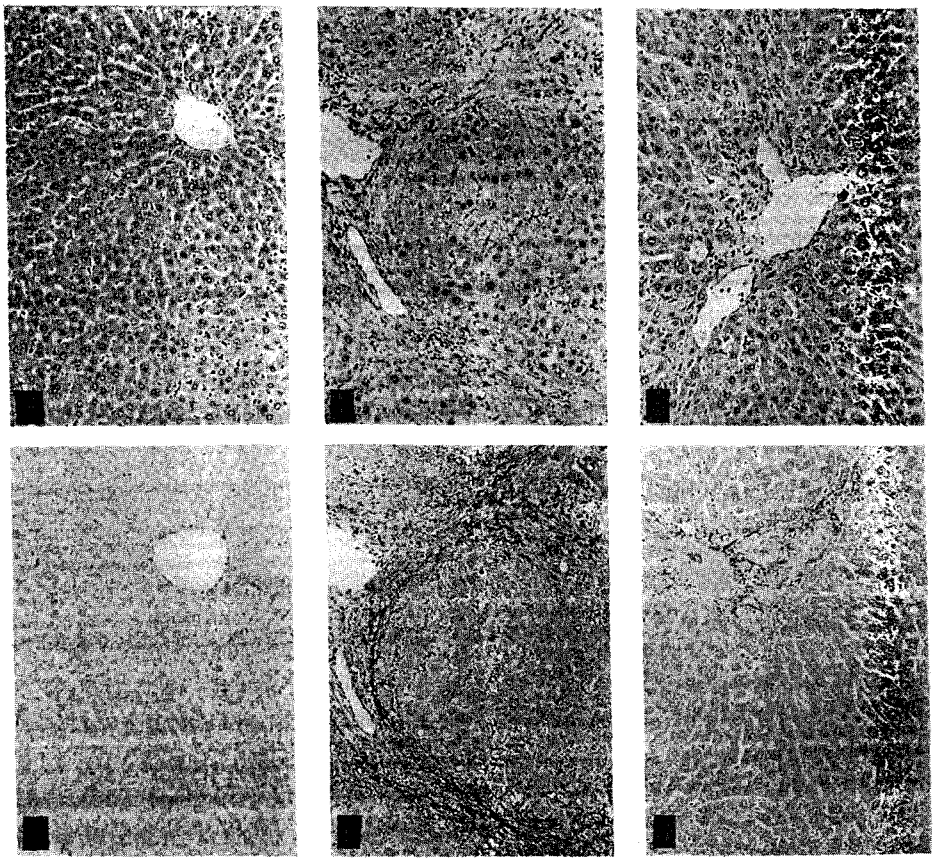

Fig. 4. Liver histopathology in the DMN- and DMN plus GJT-treated rats. Liver fibrosis was induced by administrating DMN for 3 weeks in the male Sprague-Dawley rats. GJT was treated concurrently with DMN for 3 weeks after 1 week of DMN induction. Liver tissue was fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin-eosin (a, b and c) or by Masson trichrome (d, e, and f). Photomicrographs represent the normal liver (a and d), DMN-treated one (b and e) and DMN plus GJT-administered one (c and f). DMN treatment resulted in the centrilobular damages consisting of hemorrhage, congestion, inflammatory cell infiltration and fibrosis.

DISCUSSION

The present study used biochemical endpoints to investigate how GJT could affect hepatocellular pathogenesis after DMN intoxication used previously,[4,9,10,12,16,17] and demonstrated scientifically in first that GJT has a hepatoprotective activity in the DMN-induced hepatic fibrosis. Moreover, the experimental results reported here provide a scientific information on the medicinal efficacy of GJT regarding in the clinical utility, which is originally referred in the traditional oriental medicine, Korea.[14]

Hepatic fibrosis is a common response to chronic liver injury caused by various origins such as viruses, metabolites, and toxicants. Even a lot of etiological factors are considered for chronic liver disease, hepatic fibrosis is characterized by a conspicuous accumulation of extracellular matrix components that form the hepatic scars and consist of fibril-forming collagens, matrix glycoconjugates and hyaluronic acid.[18,19] In addition to a general pathological feature of chronic hepatic disease, the pathological and biochemical characteristic of DMN-induced hepatic damage was suggested by several different laboratories. Ala-KoKko et al.[20] demonstrated that hepatic damage by DMN treatment was characterized histopathologically by a hemorrhage, cellular necrosis and prominent fibrosis after 28 days of DMN induction. In immunohistochemical study, hepatic stellate cells, namely fat-storing or Ito cell, which is identified as the primary source of excess extracellular matrix proliferation were activated by DMN treatment in the rats.[21] This means that DMN can induce an excessive accumulation of extracellular matrix components such as collagens, laminin, fibronectin. Although many researchers are using DMN for preparation of hepatic disease in *in vivo* animal model, in which represents a certain special feature of chronic liver disease in the human, the CCl_4 is also applicable to the animal for investigating a human hepatic disease. In the CCl_4-induced liver fibrosis, an increase of desmin-positive cells is a prominent evidence in the liver fibrotic area, and these cells also express α-sm-actin.[22] In DMN-induced hepatic fibrosis, the number of demin-positive cells also increases in fibrotic area, and most of them are interestingly α-sm-actin-positive, indicating that DMN- and CCl_4-induced liver fibrosis is associated with an acceleration of the proliferation and activation of lipocytes.[17,23] From these studies, it is generally accepted that fibrosis-related genes such as collagen, desmin, and α-sm-actin are produced primarily by nonparenchymal cells, indicating that nonparechymal cells could be regarded as a playing a pivotal role in the DMN- and CCl_4-induced hepatic fibrosis. In the present study, hepatic damage by administration of DMN was also demonstrated histopathologically by hemorrhage, cellular necrosis and excess fibrosis after 28 days of DMN induction, and this is consistent well with the previous report.[20] Microscopically, liver fibrosis demonstrated by Masson trichrome-staining technique was conspicuously observed in the rat treated with DMN (Fig. 4e). This result suggests that *in vivo* model used in this study may be good enough to investigate the hepatoprotective activity of GJT, and that GJT ameliorates the severity of liver fibrosis induced by DMN as reflected by low sALP, sAST and sALT level (Fig. 2).

It is now well established that hepatic fi-

brosis is accompained by deterioration of liver functions and accumulation of collagen in the liver.[10,24] The amount of collagen deposited depend on the balance between the rates of biosynthesis and degradation. During biosynthesis of collagen, collagen is modified by a series of post translational modifications which are necessary to carry the nascent polypeptide chains to the extracellular matrix and to ensure proper fibril formation.[25] The enhanced collagen production has been also shown to be accompanied by many changes in the biosynthesis pathway such as increases in the pool of free proline, the concentration of functional prolyl tRNA.[20] In addition to the morphological finding in this study, chemical assessment of hepatic collagen content by measurement of hydroxyproline content confirmed the histological observation of an excess accumulation of collagen in DMN-treated rats (Fig. 3a, 3b, 4b and 4e), and evaluated that post treatment of GJT improved the liver function reduced by the preinduction of hepatic injury/fibrosis (Fig. 2, 3a and 3b). Results shown in Figs. 4a and 4b may indicate that DMN treatment resulted in the enlargement of liver, but it was not significantly affected by GJT administration, because hydroxyproline content normalized as tissue weight was significantly different between DMN- and DMN plus GJT-treated rats (Fig. 4a), but not in the comparison of total hydroxyproline amount to the whole liver (Fig. 4b). This also supported by the result shown in Fig. 1. Although study described in here could not clarify a detailed biochemical mechanism of hepatoprotective activity of GJT to DMN-induced liver damage, it might be arisen from a decreased content of liver collagen as shown in Fig. 3 and 4. The further study is needed to evaluate the involvement of an enhanced biosynthesis and/or a reduced degradation of collagen by GJT in the DMN-treated rat.

In conclusion, GJT was shown to improve a reduced hepatic function in the DMN-treated rat model, and this may be occurred through a decreased content of liver collagen. In regarding pharmacological mechanism of GJT, we could not completely rule out the direct effect of GJT on the enzyme activity which are involved in the biotransformation pathway of DMN in *in vivo*. The further study is needed to evaluate the pharmacological activity of GJT and the biochemical mechanism of hepatic collagen synthesis and/or degradation by GJT in the DMN-treated liver damage, specially the involvement of hepatic stellate cells which is the primary source of excess extracellular matrix proliferation in the liver.

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LITERATURE CITED

1. Maher, J. J. and McGuire, R. F. (1990) Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis *in vivo*. *J. Clin. Invest.* 86: 1641-1648.
2. Jenkins, S. A., Grandison, A., Baxter, J. N., Day, D. W., Taylor, I. and Shields, R. (1985) A dimethylnitrosamine-induced model of cirrhosis and portal hypertension in the rat. *J. Hepatol.* 1: 489-499.
3. Mancini, R., Paolucci, F., Svegliati, B. G., Jeze-

quel, A. M. and Orlandi, F. (1991) Phenotypic analysis of inflammatory infiltrate in rats with dimethylnitrosamine induced cirrhosis. *Int. J. Exp. Pathol.* 72: 119-128.
4. Mancini, R., Jezequel, A. M., Benedetti, A., Paolucci, F., Trozzi, L. and Orlandi, F. (1992) Quantitative analysis of proliferating sinusoidal cells in dimethylnitrosamine-induced cirrhosis. *J. Hepatol.* 15: 361-366.
5. Jezequel, A. M., Mancini, R., Rinaldesi, M. L., Macarri, G., Venturini, C. and Orlandi, F. (1987) A morphological study of the early stages of hepatic fibrosis induces by low doses of dimethylnitrosamine in the rat. *J. Hepatol.* 5: 174-181.
6. Mostafa, M. H. and Weisburger, E. K. (1980) Effect of chloropromazine hydrochloride on carcinogen metabolizing enzymes: Liver microsomal dimethylnitrosamine demethylase, 4-aminoazobenzene reductase and arylhydrocarbon hydroxylase. *J. Natl. Cancer Inst.* 64: 924-929.
7. Sheweita, S. A. and Mostafa, M. H. (1996) N-nitroso compounds induce changes in carcinogen-metabolizing enzyme. *Cancer Lett.* 106: 243-249.
8. Kamendulis, L. M. and Corcoran, G. B. (1995) Dimethylnitrosamine-induced DNA damage and toxic cell death in cultured mouse hepatocyte. *J. Toxicol. Environ. Health* 46: 31-46.
9. Ala-Kokko, L., Pihlajaniemi, T., Myers, J. C., Kivirikio, K. I. and Savolainen, E. R. (1987) Gene expression of type I, III and IV collagens in hepatic fibrosis induced by dimethylnitrosamine in the rat. *Biochem. J.* 244: 75-79.
10. George, J. and Chandrakasan, G. (1996) Molecular characteristics of dimethylnitrosamine induced fibrotic liver collagen. *Biochim. Biophys. Acta* 1292: 215-222.
11. Mancini, R., Jezequel, A. M., Benedetti, A., Paolucci, F., Trozzi, L. and Orlandi, F. (1992) Quantitative analysis of proliferating sinusoidal cells in dimethylnitrosamine-induced cirrhosis. *J. Hepatol.* 15: 361-366.
12. Mancini, R., Benedetti, A. and Jezequel, A. M. (1994) An interleukin-1 receptor antagonist decreases fibrosis induced by dimethylnitrosamine in rat liver. *Virchows Arch.* 424: 25-31.
13. Friedman, S. L. (1993) The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. *N. Eng. J. Med.* 328: 1828-1835.
14. Huh, J. (1994) Dong Eui Bo Gam. Haisungsa, Seoul.
15. Jamall, I. S., Finelli, V. N. and Que Hee, S. S. (1981) A simple method to determine nanogram levels of 4-hydroxyproline in biological tissues. *Anal. Biochem.* 112: 70-75.
16. Seifert, W. F., Bosma, A., Hendriks, H. F. J., Van Leeuwen, R. E. W., Van Thiel-de Ruiter, G. C. F., Seifert-Bock, I., Knook, D. L. and Brouwer, A. (1995) Beta-carotene (provitamin A) decreases the severity of CCl_4-induced hepatic inflammation and fibrosis in rats. *Liver* 15: 1-8.
17. Jezequel, A. M., Ballardini, G., Mancini, R., Paolucci, F., Bianchi, F. B. and Orlandi, F. (1990) Modulation of extracellular matrix components during dimethylnitrosamine-induced cirrhosis. *J. Hepatol.* 11: 206-214.
18. Brenner, D. A., Westwick, J. and Breindl, M. (1993) Type I collagen gene regulation and the molecular pathogenesis of cirrhosis. *Am. J. Physiol.* 264: G589-G595.
19. Gressner, A. M. and Bachem, M. G. (1990) Cellular sources of noncollagenous matrix proteins: role of fat-storing cells in fibrogenesis. Semin. *Liver Dis.* 10: 30-46.
20. Ala-Kokko, L., Stenback, F. and Ryhanen, L. (1989) Preventive effect of malotilate on dimethylnitrosamine-induced liver fibrosis in the rat. *J. Lab. Clin. Med.* 113: 177-183.
21. Baroni, G. S., Ambrosio, L. D., Curto, P., Casini, A., Mancini, R., Jezequel, A. M. and Benedetti, A. (1996) Interferon gamma decreases hepatic stellate cell activation and extracellular matrix deposition in rat liver fibrosis. *Hepatol.* 23: 1189-1199.
22. Tanaka, Y., Nouchi, T., Yamane, M., Irie, T., Miyakawa, H., Sato, C. and Marumo, F. (1991) Phenotypic modulation in lipocytes in experimental liver fibrosis. *J. Pathol.* 164: 273-278.
23. Yasuda, H., Imai, E., Shiota, A., Fujise, N., Morinaga, T. and Higashio, K. (1996) Antifibrogenic effect of a deletion variant of hepatocyte growth factor on liver fibrosis in rats. *Hepatol.* 24: 636-642.
24. Sherlock, S. (1989) Disease of the liver and biliary system. 8th ed., 410-448. Blackwell, Oxford.

25. Prockop, D. J., Berg, R. A., Kivirikko, K. I. and Uitto, J. (1976) Intracellular steps in the biosysthesis of collagen. *In* Ramachandran, C. N. and Reddi, A. H. eds., Biochemistry of collagen, 163-273. Plenum Press, New York.

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