Research Article

Evaluating the Role of Curcumin and CoQ10 in CCl_4-Induced Liver Fibrosis

Dalia H.M.A. Abu-Baih*, Asmaa M.A. Bayoumi P.H.D., Ahmed M.Mahmoud Okasha*****

* Department of Biochemistry, Faculty of Pharmacy, Deraya University.
** Department of Biochemistry, Faculty of Pharmacy, Minia University.
***Department of Biochemistry, Faculty of Medicine, Minia University

Abstract

Background: Coenzyme Q10 and curcumin were evidenced by several studies as potent antioxidant agents and work as ROS scavengers. It has been documented that they have potential role in preventing liver fibrosis. **Objective**: The objective of this study is to evaluate the role of curcumin and/or Co-Q_{10} in carbon tetrachloride (CCl_4)-induced liver fibrosis in rats. **Materials and methods:** 50 rats divided into 5 groups: group I was normal control, group II received I.P injection of CCl_4, group III received oral CoQ_{10} and I.P CCl_4, group IV received curcumin and I.P CCl_4, group V received oral curcumina nd coQ10 along with CCl_4. Hepatic level of MDA and GSH was determined as well as catalase activity. **Results:** CCl_4-induced liver fibrosis was demonstrated by an increase in the hepatic levels of MDA and a decrease in the GSH and catalase in the liver. Treatment with Co-Q_{10} and/or curcumin decreased the levels of MDA. The level of catalase and GSH increased significantly and liver fibrosis and inflammation were attenuated on treatment. **Conclusion:** Our data suggested that CoQ_{10} and/or curcumin protect the liver from chronic CCl_4-induced liver fibrosis in rats. Therefore, Co-Q_{10} and curcumin are potential therapeutic antioxidant agents against chronic liver injury.

Keywords: Liver fibrosis, CCl_4, oxidative stress, Curcumin, CoQ_{10}.

Introduction:

Liver fibrosis is the wound healing response to a variety of acute and chronic stimuli, including, viral infection, ethanol, drugs, toxins, cholestasis, and metabolic disease. Hepatic fibrosis develops due to an increase in fibrillar collagen synthesis and deposition along with insufficient remodeling (Wynn, 2007).

Liver fibrosis is the characterized by the accumulation of extracellular matrix proteins. Synthesis and degradation of ECM is regulated by extracellular matrix - modifying enzymes, matrix metalloproteinase (MMPs) and the tissue inhibitors (TIMPs) (Aghemo and Colombo, 2009).

Oxidative stress plays an important role in the establishment of fibrosis Therefore, the use of molecules with antioxidant properties has been proposed as a treatment for fibrosis and cirrhosis caused by oxidative stress. Oxidative stress contributes to fibrogenesis by increasing harmful cytokines such as tumor necrosis factor-α (TNF-α).

CCl_4 is widely used to induce liver damage because it is metabolized in hepatocytes by cytochrome P450, generating a highly reactive carbon centered trichloromethyl radical, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis (Fang, et al., 2008).

Curcumin was shown to have beneficial effects on a variety of inflammatory conditions**.** The other salient feature of curcumin is that exhibits strong antioxidant activity, comparable to vitamin C and E, and it was shown to be a potent scavenger of a variety of reactive oxygen species (Joe et al., 2004).

Co-enzyme Q_{10}, an endogenous lipid-soluble benzoquinone- acts as a powerful

antioxidant that scavenges free radicals and prevents the initiation and propagation of lipid peroxidation in cellular membranes. In addition, Co-enzyme Q_{10} has anti-inflammatory properties, decreasing the production of proinflammatory cytokines such as tumor necrosis factor-α (Fouad and Jresat, 2012).

Materials and methods

Experimental design:

50 male western albino rats were equally divided into five groups. Group 1 serves as a control healthy group. Liver fibrosis was induced in group 2 by intraperitoneal (IP) injection of CCl_4: mineral oil 1:1 (0.8 ml/kg) twice weekly for six weeks (Roderfeld, M., et al., 2006). Group 3 was administered Co- Q_{10} once daily for 6 weeks at 10 mg/ Kg along with CCl_4 from day one of experiment (Fouad et al., 2010). Group 4 was administered curcumin at 100 mg/kg/day along with CCl_4 from day one of experiment for six weeks (Gangarapu et al., 2013). Group 5 was administered both Co-Q_{10} and curcumin once daily at doses 10 mg/Kg and 100 mg/kg respectively along with CCl_4 from day one of experiment for six weeks.

At the end of experiment, animals were anesthetized with ether and sacrificed. The liver tissue specimens were excised and weighed and imbedded immediately in liquefied nitrogen and stored at -80°C for further investigations.

Assessment of lipid peroxides levels in the liver: Liver lipid peroxidation measured by spectrophotometric kit *(Biodiagnostic, catalog no CA 2529).* Liver lipid peroxidation was determined as thiobarbituric acid reacting substance substance and is expressed as equivalents of malondialdehyde using 1,1,3,3 tetrathoxy-propane as a standard. Results were xpressed as nmol/ g liver tissue (Buege and Aust, 1978).

Assessment of reduced glutathion levels in the liver: GSH spectrophotometric kit *(Biodiagnostic, catalog no GR 2511)* was used. Briefly, the method is based on that the sulfhydryl group of GSH reacts with 5,5-dithio-bis-2-thio-2-nitobenzoic acid which was measured calorimetrically at 450 nm using Bechman DU-64 UV/VIS spectrometer. Results were expressed as µmol/g of liver tissue (Beutler et al., 1963).

Assessment of catalase activity in the liver: Catalase activity measured by spectrophotometric kit *(Biodiagnostic, catalog no CA 2517).*Catalase activity was determined from the rate of decomposition of H_2O_2 at 520 nm after the addition of tissue homogenate as described by colorimetric kit. The results were expressed as unit/ g liver tissue (Aebi, 1984).

Results

CCl_4-induced liver fibrosis was demonstrated by the significant increase in the hepatic levels of MDA. Also GSH level and catalase activity were significantly decreased in the liver. Treatment with Co-Q_{10} decreased the levels of MDA and cause significant increase in the catalase activity and GSH level. Interestingly administration of curcumin along with CCl4 prevented oxidative stress as revealed by the significant decrease in the level of MDA and the significant increase in the level of GSH and catalase activity. Moreover co-administration of Co-Q10 and curcumin significantly decreased the levels of MDA and significantly increased catalase activity and GSH level when compared with curcumin or Co-Q10 groups (Table 1).

Table 1: Assessment of liver MDA, Catalase and GSH:

	C-ve	CCl_4	Co-Q_{10}	Curcumin	Co-Q_{10} +Cur	P-value
CAT	25.9-30	13.1-16.9	16.4-19.9	19.8-21.9	23.2-26	<0.001
Mean ±SE	27.7±0.423	15.1±0.380	18.1±0.380	20.6±0.252	24.9±0.314	
GSH	1.2-3	0.3-0.6	0.6-0.9	0.7-1.1	0.8-1.5	<0.001
Mean ±SE	1.77±0.157	0.47±0.036	0.78±0.032	0.84±0.042	1.18±0.069	
MDA	54-60	69-72	66-69	63-65.5	60-62	<0.001
Mean ±SE	56.6±0.561	70.6±0.371	67.2±0.326	63.9±0.266	61.1±0.276	

Discussion

The principal mechanism by which CCl4 causes hepatic damage are lipid peroxidation, decreased activities of antioxidant enzymes and generation of free radicals (Kuzu et al., 2007). ROS are known to cause hepatocyte necrosis and further stimulate the progression of hepatic fibrosis.

In agreement with preceding studies, the oxidative stress in the fibrosis group is manifested by a significant decline in the GSH and CAT accompanied by increase in the MDA level which reflects the oxidative stress induced damage (Abd-Allah et al., 2016).

Treatment with curcumin improved the oxidative status induced by CCl4. In this context, a decrease in ROS production, reflected by a significant reduction in the mean MDA concentration level, was accompanied by an increase in the antioxidant capacity level as seen by a significant increase in GSH concentration and CAT enzymatic activity levels as compared to that in CCl4 treated group. This findings suggest that antioxidant activity of curcumin and it is ability to scavenge free radicals may be involved in the protective mechanism against CCl4-induced liver toxicity. These results is in accordance with prior studies (lee et al., 2016).

On the other hand, CoQ10 was found to significantly attenuate the increase in oxidative stress markers. As shown by the decreased MDA level and increased GSH content and catalase activity. Co-Q10 is known to have antioxidant properties and free radical scavenging properties (Tsuneki et al., 2007). GSH non-enzymatic anti-oxidant and CAT enzymatic antioxidant act as a defense against released free radicals. But when level of free radicals released increase, depletion of these antioxidants occurs. Co Q10 prevents this depletion of GSH and CAT as well as reducing MDA level. In harmony, preceding reports supported this theory (Sohet et al., 2009).

Moreover combination group showed better effect in maintaining enzymatic activity levels and prevented lipid peroxidation as revealed by the significant reduction in MDA level and significant increase in GSH and CAT when compared to other groups. We hypothesized that this may be due to synergistic effect of the combination.

Conclusion

Our data suggested that Co-Q_{10} and/or curcumin protect the liver from chronic CCl_4-induced liver fibrosis in rats by suppressing hepatic oxidative stress as revealed by levels of GSH and MDA and catalase activity. Therefore, Co-Q_{10} and curcumin are potential therapeutic antioxidant agents against chronic liver injury.

References

1. Abd-allah G. A., El- Bakry K. A., Bahnasawy M. H. and El- Kodary S. R., (2016): protective effects of curcu-min and gingier on liver cirrhosis induced by CCl4 in rats, int. J. of pharmacology, 12: 361-369.
2. Aebi H. (1984); Methods Enzymol 105: 121-6.

3. Aghemo A. and Colombo M. (2009): Cirrhosis regression in chronic heaptitis C: an old tale with a new ending. Gastroenterology. 136: 1447-49.
4. Beutler E., Duron O. and Kelly MB, (1963): J. Lab Clin. Med. 61: 881.
5. Buege J. A. and Aust S. D. (1978): "Microsomal lipid peroxidation," Methods in Enzymology. 52: 302–310.
6. Fang H.L., Lai J.T. and Lin W.C. (2008) Inhibitory effect of olive oil on fibrosis induced by carbon tetrachloride in rat liver. Clin Nutr. 27: 900-7.
7. Fouad A.A. and Jresat I. (2012): Hepatoprotective effect of CoQ10 Q10 in rats with acetaminophen toxicity. Environ Toxicol Pharmacol. 33(2): 158-67.
8. Fouad A. A., Al-Sultan A. L., Refaie S. M. and Yacoubi M. T. (2010): CoQ10 Q10 treatment ameliorates acute cisplatin nephrotoxicity in mice. Toxicology. 274 (1–3): 49–56.
9. Gangarapu V., Gujjala S., Korivi R. and Pala I., (2013): Combined effect of curcumin and vitamin E against CCl_4 induced liver injury in rats. American Journal of Life Sciences. 1(3): 117-124.
10. Joe B., Vijaykumar M. and Lokesh B. (2004): Biological properties of curcumin-cellular and molecular mechanisms of action. Critical reviews in food science and nutrition; 44(2): 97-111.
11. Kuzu N, Metin K, Dagli AF, Akdemir F, Orhan C, Yalniz M, et al. Protective role of genistein in acute liver damage induced by carbon tetrachloride. Mediators Inflamm 2007;2007:36381.
12. Lee H-Y, Kim S-Y, Lee G-H, Choi M-K, Jung H-W, Kim Y-J, Kwon H-J and Chae H-J, (2016): Turmeric extract and its active compound, curcumin, protect against chronic CCl4-induced liver damage by enhancing Antioxidation. BMC Complementary and Alternative Medicine 16:316.
13. Li A.N., Li S., Zhang Y.J., Xu X.R., Chen Y.M. and Li H.B. (2014): Resources and biological activities of natural polyphenols. Nutrients. 6: 6020–6047.
14. Roderfeld M., weiskirchen R., wagner S., Berres M.L., Henkel C., Grotzinger J., Gressner A.M, Matern S. and Roeb E., (2006): inhibition of hepatic fibrogenesis by matrix metalloprotinase-9 mutants in mice. FASEB J. 20: 444-454.
15. Sohet FM, Neyrinck AM, Pachikian BD, de Backer FC, Bindels LB, Niklowitz P, Menke T, Cani PD, Delzenne NM. Coenzyme Q10 supplementation lowers hepatic oxide-tive stress and inflammation associated with diet-induced obesity in mice. Biochem Pharmacol 2009; 78: 1391–400.
16. Tsuneki, H., Sekizaki, N., Suzuki, T., Kobayashi, S., Wada, T., Okamoto, T., Kimura, I., Sasaoka, T., (2007): Coenzyme Q10 prevents high glucose-induced oxidative stress in human umbilical vein endothelial cells. Eur. J. Pharmacol. 566, 1–10.
17. Wynn TA. (2007): Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest. 117:524–9.