The Effect of Coenzyme Q_{10} and Curcumin on Chronic Methanol Intoxication Induced Retinopathy in Rats

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Background: *The retinal pathophysiology of methanol intoxication is that formate inhibits retinal mitochondrial function and increases oxidative stress.*
Objective: *To investigate the effect of coenzyme Q_{10} and curcumin on chronic methanol intoxication causing retinopathy in rats.*
Material and Method: *The authors designed an experimental study of chronic methanol intoxication in rats depleted of folate with methotrexate. The studied group received methanol (2 mg/kg body weight in saline by intraperitoneal injection) and methotrexate (0.1 mg/kg body weight in saline by subcutaneous injection) every other day for ten weeks to induce chronic methanol intoxication, while another group received saline as vehicle and served as control group. The studied rats were confirmed to develop significant retinopathy after 10 weeks and then assigned to three treatment arms: either corn oil (as control) or coenzyme Q_{10} (20 mg/kg/day) or Curcuma longa extract (2.5 mg/kg/day) for four weeks. Eyes were enucleated and the retinal tissue was prepared for histological examination. The sections were evaluated by an experienced pathologist and blinded to the experimental conditions.*
Results: *Histological analysis revealed that animals treated with both methanol and methotrexate showed vacuolation of photoreceptor inner segment and disaggregation of cells in the inner and outer nuclear layers of the retina compared to a normal histological appearance in control animals. The retinal histology in the experimental animals with administration of Coenzyme Q_{10} or Curcuma longa extract appeared essentially normal and this was not found in the experimental animals which received corn oil.*
Conclusion: *Coenzyme Q_{10} and curcumin administration improves retinal histology by reversing the pathological changes due to chronic methanol and establish a morphologically normal retina.*

Keywords: *Methanol, Coenzyme Q_{10}, Curcumin, Retinopathy, Optic neuropathy*

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Methanol (MeOH) is present as a component of many products such as antifreeze, paint removers, cleaners and gasoline. The similarity of methanol to ethanol in appearance and odor leads to accidental or intentional exposure through intoxication. Methanol is metabolized in the liver to formaldehyde and to formic acid. Formic acid inhibit the cytochrome oxidase complex of the respiratory chain process in the mitochondria which interrupts both oxidative phosphorylation and electron transport and energy production[1,2]. There are clear signs of methanol-induced visual system toxicity. Ophthalmologic manifestations of methanol induced ocular abnormalities in humans have been clearly documented. After methanol intoxication, the most notable histopathological changes of the retina were swelling of photoreceptor inner segments and mitochondrial disruption. The retinal pathophysiology of methanol intoxication is that formate inhibits retinal mitochondrial function and increases oxidative stress[1-3].

Coenzyme Q_{10} (Co Q_{10}) is a potent antioxidant and has membrane stabilizing properties[4-7]. It is also an electron carrier in the mitochondrial synthesis of ATP[4]. Curcumin is the major active constituent of curcuma longa extract. It has strong antioxidant and

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free radical scavenging properties[8,9]. It also has been shown to possess anti-inflammatory activity[8,9].

The aim of this study was to examine the effect of Co Q_{10} and curcumin on the chronic methanol-compromised retina in rats depleted of folate with methotrexate (MTX). MTX is a dehydrofolate analog which selectively inhibits formate oxidation by binding dehydrofolate reductase which accordingly depletes the rats' folate stores thus allowing formate to accumulate into toxic concentrations following methanol administration[10]. After inducing chromic methanol intoxication, the animals received Co Q_{10} and curcumin in order to determine whether such antioxidant treatment restored normal retinal morphology and function.

Material and Method

Thirty-five male Spraque-Dawley rats weighing between 300-360 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya Campus, Nakornpathom Province, Thailand. Animals were individually housed, with free access to food and water in the controlled room with 12-hour light-dark schedule in a temperature-and humidity-controlled environment and acclimatized the laboratory facility for 2 weeks prior to the experiment. The experimental animal protocol was conducted in accordance with the Declaration of Helsinki and the institutional guidelines and was approved by the Siriraj Animal Care and Use Committee (SI-ACUC), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

MeOH (HPLC grade; Sigma) was diluted in sterile saline and administered intraperitoneally (ip) as a 20% weight/volume solution. MTX was also diluted in sterile saline and administered subcutaneously (sc).

All the animals were weighed weekly. The animals were randomly divided into four groups. The first three groups were set as control and the last group was treated with MeOH and MTX. On the third week, group 1 (5 untreated rats) and 2 (5 rats treated with MeOH) received saline sc, while group 3 (5 rats treated with MTX) and 4 (20 rats treated with MTX-Me OH) received MTX sc at a dose of 0.2 mg/kg body weight every other day for two weeks (0.2 mg/kg/EOD). After the fourth week, MTX was reduced to 0.1 mg/kg in group 3 and 4 while groups 1 and 2 received an equivalent volume of saline sc. Twenty percentage of MeOH (2g/kg/EOD) was administered into group 2 (MeOH) and 4 (MTx-MeOH) ip, but the methanol was replaced by saline in group 1 (untreated) and 3 (MTX). All animals were treated for 10 weeks (Table 1).

After ten weeks of chronic methanol exposure, all the rats in groups 1, 2, 3 and five rats in group 4 were euthanized by an overdose of pentobarbital. Eyes were enucleated and placed in 10% formalin. Within 24 hours, the globes were bisected and processed by routine histology. The samples were cut one micron thick for toluidine blue staining for light microscopic examination.

To evaluate the effect of Co Q_{10} and curcumin, the remaining rats of group 4 were divided randomly into three groups. Groups 4A, 4B and 4C were treated with corn oil, Co Q_{10} and curcumin solution, respectively.

Curcumin used was extracted from *Curcuma longa*. It was kindly provided by Prof. Dr. Pawinee Piyachaturawat, Faculty of Science, Mahidol University and Prof. Dr. Apichart Suksamrarn, Faculty of Science, Ramkhamhaeng University. For treatment, Curcumin was mixed with corn oil and was given orally to rats in the experimental group (4C) with an 18-gauge gavage needle at a dose of 2.5 mg/kg body weight every day for four weeks. In experiments with Co Q_{10}, rats in group 4B received oral Co Q_{10} mixed with corn oil at a dose of 20 mg/kg per day. The animals in the corresponding control group (4A) were given the same amount of corn oil only. The dietary regimen of the three groups is summarized in Table 2.

The animals were scarified by an overdose of pentobarbital. Eyes were enucleated and the retinal

Table 1. Experimental design of chronic methanol intoxication

Group	2 wks	10 wks
Group 1 (untreated) (n = 5)	Saline sc	- Saline sc- Saline ip
Group 2 (MeOH) (n = 5)	Saline sc	- Saline sc- 20% MeOH (2 g/kg/EOD) ip
Group 3 (MTX) (n = 5)	MTX0.2 mg/kg/EOD sc	- MTX (0.1 mg/kg/EOD) sc- Saline ip
Group 4 (MTX-MeOH) (n = 20)	MTX0.2 mg/kg/EOD sc	- MTX (0.1 mg/kg/EOD) sc- 20% Methanol (2 g/kg/EOD) ip

sc = subcutaneous, ip = intraperitoneal, MeOH = methanol, MTX = methotrexate, EOD = every other day

tissue was prepared for histological analysis. The retina was carefully removed under a dissecting microscope. All sections were evaluated by an experienced pathologist blinded to the experimental conditions.

Statistical analysis

All values are expressed as means ± Standard error of mean (SEM). Kruskal Wallis test was used to determine whether any significant difference existed among the groups for weight. In all cases, the minimum level of significance was taken as $p < 0.05$.

Results

Rats in group 1 (176 ± 11.40 g) gained slightly more weight than rats in group 2 (166 ± 31.30) group 3 (154 ± 19.49 g) and group 4 (158 ± 38.63 g). However, there was no statistically significant difference on gaining weight among different experimental groups ($p = 0.381$).

Rats in group 4A, 4B and 4C gained the same weight. There was also no statistically significant difference in these groups.

On histological examination, there was disorganization with vacuoles in the photoreceptors in rats treated with MTX-MeOH and cells were disaggregated in the inner and outer nuclear layers (Fig. 1). Electron microscopic studies demonstrated mitochondrial swelling in photoreceptors of rats treated with MTX-MeOH (Fig. 2). Rats treated with Me OH also showed similar histological changes but none were seen in the control and MTX treated groups (Fig. 1).

Co Q_{10} and Curcumin administrations groups showed a much improved photoreceptor histology with no evidence of edema and minimal degree of vacuolation, and similar to the ultrastructural findings of the corn oil (control) group which showed no change (Fig. 3).

Discussion

The folate-depleted and methanol-treated animals (MTx-MeOH) gained less weight than the three other groups of rats, and their retinas showed histological disorganization, disaggregation and vacuolization with areas of edema, while electron microscopy showed mitochondrial impairment. This is consistent with retinal damage in experimental animal models with chronic methanol exposure, as reported in

Table 2. Experimental design of the effect of Co Q_{10} and curcumin in chronic methanol intoxication

Group	2 weeks
4A (N=5) Corn oil	Oral corn oil
4B (N=5) Co Q_{10}	Oral Co Q_{10} 20 mg/kg/day
4C (N=5) Curcuma longa	Oral curcuma longa extract 2.5mg/kg/day

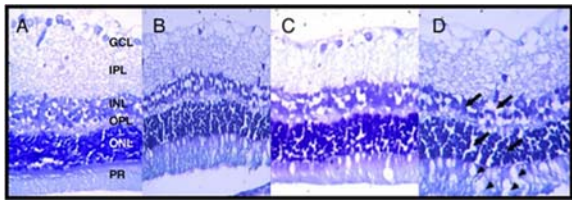

Fig. 1 Effect of chronic methanol intoxication on the retina histology. Light micrographs of retinal tissue prepared from untreated rat (A), MTX treated rat (B), MeOH treated rat (C), and MTX – MeOH treated rat (D). Sections were taken from the posterior pole of the retina within two disc diameters of the optic disc in any direction. (A) GC, ganglion cell; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PR, photoreceptor. (A)No remarkable changes were seen at the light microscopic level in untreated-control group. (D) Arrows: Disaggregation of cells in the inner and outer nuclear layer; Arrowheads: disorganization and vacuole in the photoreceptors in rat treated with MTX-MeOH. (C) There is some degree of histological changes in MeOH group. (Toluidine blue, magnification x 100)

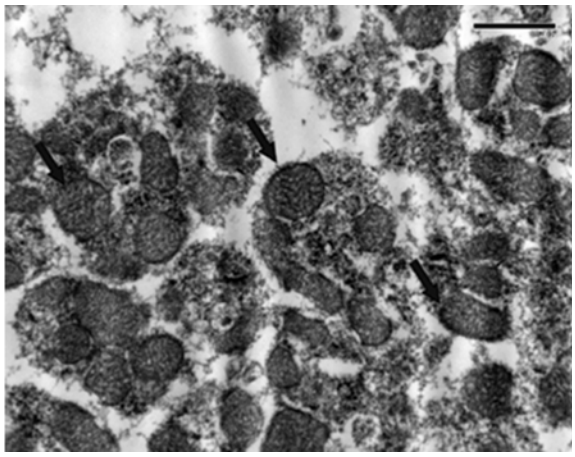

Fig. 2 Effect of chronic methanol intoxication on ultrastructural histology. Electron micrograph of photoreceptor of retinal tissue prepared from MTX-MeOH treated rat. The arrows indicate swollen mitochondria. (Magnification x 22000)

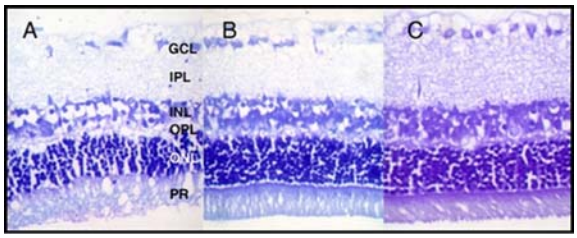

Fig. 3 Effect of CoQ10 or curcumin in methanol intoxication on retinal histology. Light micrographs of retinal tissue from corn oil control rat (4A), CoQ10 treated rat (4B), and curcumin treated rat (4C). Histopathologic changes were improved to nearly normal retina with minimal degree of vacuolation in photoreceptor in rats treated with Co Q10 (4 B) or curcumin (4C), while no histopathologic changes were presented in corn oil treated control rat (4A). (Toluidine blue, magnification x 100)

previous studies[1-3,11].

The present study shows that improvements in retinal histology were observed in methanol-exposed rats after administration of Co Q_{10} or curcumin and this was not a finding in control untreated rats.

Impaired energy metabolism of mitochondrial transport chain and increased oxidative stress from reactive oxygen species secondary to electron transport chain blockage are considered to be the factors in the pathogenesis of methanol intoxication[1,2,12]. Sadun also suggested that the papillomacular bundle, the small caliper and long axon in the optic nerve, is restricted in transporting mitochondria and unable to meet transport demands[1].

Application of Co Q_{10} (ubiguinone) has been shown to provide clinical benefit in treating cardiomyopathy and neuro-muscular disorders with mitochondrial dysfunction in both animal experiments and clinical studies[4,6,7,13]. Co Q_{10} is an electron carrier in the mitochondrial synthesis of ATP[4,13]. Relatively high concentrations of Co Q_{10} are found in the mitochondria where it has a critical role in energy production[6]. The papillomacular bundle, a tissue with high energy demands, may, therefore be affected when the availability of Co Q_{10} becomes limiting. It was also found to improve impaired mitochondrial function[14]. This is consistent with the hypothesis that mitochondrial dysfunction play a role in the pathogenesis of methanol intoxication and that Co Q_{10} could ameliorate impaired methanol intoxicated retinas by improving mitochondrial function. Furthermore, CoQ_{10} has a potent antioxidant effect[4-7], membrane stabilizing properties[4-7] and inhibition of cell death properties[6], all of which may play a role in protecting the mitochondria from free radical damage and helping restored their integrity and function. Based on this, it may be expected that Co Q_{10} improves both antioxidant ability and impaired mitochondrial function in methanol-compromised retinas. Co Q_{10} may enhance recovery from retinal injury and other ocular diseases in which mitochondrial dysfunction are postulated to play a role. The present results support this hypothesis and suggest a clinical interest that requires further investigation.

Curcumin is the major active constituent of *Curcuma longa* extract and it has well known properties as an antioxidant, anti-inflammatory and free radical scavenging activities[8,9]. The antioxidant and anti-inflammatory properties of curcumin may provide protection against the development of diabetic retinopathy, age-related macular degeneration (AMD), and cataract[8,9,15]. It has also been shown to protect against the necrotic and apoptotic cell death induced by N-Methyl-D-aspartate excitotoxicity in rat retinas[16]. Since methanol intoxication produces an elevation of intracellular [Ca^{2+}][16,17], it is possible that curcumin administration may act to encounter this effect. Another possible effect of curcumin in alleviating methanol intoxication could be due to a decrease in oxidative stress. The authors have demonstrated that curcumin and its dietary source, turmeric, improve retina integrity in methanol intoxication. Further studies are required to determine whether the antioxidant properties of curcumin provide relief in the clinical treatment of methanol optic neuropathy.

In an earlier study, it was shown that ranitidine, an antioxidant and inhibitor of alcohol dehydrogenase, may inhibit the metabolism of methanol and improve histological changes of methanol intoxicated retina[18]. Furthermore, photobiomodulation with red to near-IR light protected the methanol-exposed retina by improving mitochondrial respiratory chain function and promoting cellular survival[19].

The clinical characteristics of methanol intoxication are extraordinarily similar to those of Leber's hereditary optic neuropathy (LHON), Cuban epidemic of optic neuropathy (CEON) and nutritional or toxic optic neuropathy[1]. These neuropathies show selective degeneration of papillo-macular bundle of the optic nerves and present primarily with central visual loss[1].

The ultrastructural features of rat tissue with methanol intoxication and tissue from patients with Leber's are surprisingly similar, with changes in

abnormal shapes and cristae of the mitochondria[1].These histopathological features matched closely with those revealed in experimental rats exposed to high levels of formate. There is evidence to suggest that a common pathophysiological mechanism exists which involves mitochondrial phosphorylation and inhibition of ATP production causing disruption of neuronal function. These might reflect the same pathophysiological cause and thus impaired mitochondrial function may be the common feature of both retinal and optic nerve dysfunction in these diseases[1-3].

Patients with CEON often revealed high levels of formate and low level of folic acid in their blood and, interestingly, a higher concentration of MeOH than normally was reported in non-commercial rum in the epidemic area[1]. It has also been reported that serum methanol levels in the acute stage of LHON were higher with normo-tension glaucoma compared with patients without optic nerve disease[20]. These findings suggested that the small intake of MeOH in alcoholic beverages together with a reduction of folate might be the cause of optic neuropathy in some patients. In the present study, the authors used lower methanol doses and relatively older rats to achieve slow progression of methanol optic neuropathy similar to that of chronic exposure in humans.

The authors conclude that Co Q_{10} and curcumin administration produced direct effects on chronic methanol intoxication retinas. These effects may be due to their antioxidant and free radical scavenging actions and the effect of Co Q_{10} on mitochondrial dysfunction. These findings suggest that Co Q_{10} and curcumin may be of therapeutic value in the prevention and/or treatment of patients with chronic methanol optic neuropathy and optic neuropathy due to mitochondrial dysfunction.

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Potential conflicts of inferest

None.

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ผลของ coenzyme Q_{10} และ curcumin ต่อจอตาอักเสบจากการได้รับสาร methanol เรื้อรังในหนู

นิพนธ์ จิรภาไพศาล มงคล อุยประเสริฐกุล อาภรณ์ จันท์จารุณี

ภูมิหลัง*: จอตาอักเสบจากสาร methanol เกิดจากการที่ formate ยับยั้งการทำงานของ mitochondria และเพิ่ม oxidative stress การได้รับยาหรือสารบางชนิด อาจลดภาวะ oxidative stress และเพิ่มการทำงานของ mitochondria*

วัตถุประสงค์*: เพื่อศึกษาผลของ coenzyme Q_{10} และ curcumin ต่อการเกิดจอตาอักเสบจากสาร methanol ในหนู*

วัสดุและวิธีการ*: การทดลองเริ่มด้วยให้สาร methotaxate (0.1 มก./กก./วัน) แก่หนู เพื่อให้ขาด folate แล้วทำให้หนูเหล่านี้เกิดจอตาอักเสบ โดยใช้ methanol (2 มก./กก./วัน) ฉีดเข้าทางช่องท้อง เป็นเวลา 10 สัปดาห์ โดยหนูกลุ่มควบคุมจะให้เป็นสารน้ำแทน จากนั้นตรวจดูชิ้นเนื้อจอตาว่ามีพยาธิสภาพจริง แล้วแบ่งกลุ่มหนูที่มีจอตาอักเสบจาก methanol เป็น 3 กลุ่ม กลุ่มละ 5 ตัว แล้วให้กินน้ำมันข้าวโพด (กลุ่มควบคุม) หรือ coenzyme Q_{10} (20 มก./กก./วัน) หรือสารสกัด curcuma longa (2.5 มก./กก./วัน) เป็นเวลา 4 สัปดาห์ จากนั้นตรวจดูชิ้นเนื้อจอตาโดยพยาธิแพทย์โดยวิธีปิดบังมิให้ทราบว่าหนูกลุ่มใดได้รับสารอะไร*

ผลการศึกษา*: ผลทางพยาธิวิทยาพบว่าจอตาของหนูที่ได้รับสาร methanol และ methotrexate จะมี vacuolation ใน photoreceptor inner segment และเซลล์ในชั้น inner และ outer nuclear จะมีการแยกตัวออกจากกัน แต่ถ้าหนูเหล่านี้ได้รับสาร coenzyme Q_{10} หรือ สารสกัด curcuma longa จะไม่พบพยาธิสภาพต่างๆ ดังกล่าว*

สรุป*: สาร coenzyme Q_{10} และ curcumin จะช่วยทำให้พยาธิสภาพในจอตาอักเสบจากสาร methanol กลับมาดีขึ้น*