

# Retinal Ganglion Cell Density after Coenzyme Q10 Treatment in a Rat Model of Methanol Toxic Optic Neuropathy

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## Abstract

**Purpose:** To compare the retinal ganglion cell (RGC) density in the control and methanol-treated rats of methanol toxic optic neuropathy (MTON).  
**Methods:** The experimental study was conducted in 12 MTON rats divided into two groups: the control group (I) and the coenzyme Q10 group (II). Both groups were given a high dose of methanol (10 g/kg body weight). After 4 hours, the rats were given a high dose of coenzyme Q10 (10 g/kg body weight). After 24 hours, the eyes were examined by fundus photography. The RGC density was measured under a microscope using a retinal image analysis software.

**Results:** The RGC density was higher in the control group (I) and lower in the methanol-treated group (II). A significant difference was identified between the two groups.

**Conclusion:** Methanol toxic optic neuropathy, caused by MTON, reduced retinal ganglion cell density.

**Keywords:** methanol toxic optic neuropathy, coenzyme Q10, retinal ganglion cell density

**Introduction:** Methanol is a colorless, odorless, and tasteless liquid that is highly toxic to humans and animals. It is commonly found in alcoholic beverages, antifreeze, and industrial solvents. Methanol poisoning can lead to severe health complications, including blindness and death.

**Background:** Methanol poisoning is a medical emergency that requires immediate treatment. The primary goal of treatment is to prevent the absorption of methanol into the bloodstream. This is typically achieved by administering activated charcoal and gastric lavage.

**Objective:** The purpose of this study was to investigate the effect of coenzyme Q10 on RGC density in a rat model of MTON.

**Methods:** The study was conducted in 12 rats divided into two groups: the control group (I) and the coenzyme Q10 group (II). Both groups were given a high dose of methanol (10 g/kg body weight). After 4 hours, the rats were given a high dose of coenzyme Q10 (10 g/kg body weight). After 24 hours, the eyes were examined by fundus photography. The RGC density was measured under a microscope using a retinal image analysis software.

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「神経眼科」 第34巻 第2号 別冊

Neuro-ophthalmology Japan Vol.34. No.2.

平成29年6月25日発行

日本神経眼科学会





## Retinal Ganglion Cell Density after Coenzyme Q10 Treatment in a Rat Model of Methanol Toxic Optic Neuropathy

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### Abstract

**Purpose:** To compare the retinal ganglion cell (RGC) density in the control and coenzyme Q10-treated rat models of methanol toxic optic neuropathy (MTON).

**Methods:** This experimental animal study was conducted in 14 MTON model rats divided into two groups, the control group (I) and the coenzyme Q10 group (II). Both groups were exposed to N<sub>2</sub>O: O<sub>2</sub> and orally administered methanol (3 g/kg body weight). After 4 h, the rats in the group II were orally administered coenzyme Q10. After 8 h, the eyes were enucleated and histopathological specimens were prepared. The specimens were imaged under a microscope at 200× magnification and the RGC densities were counted.

**Results:** The average RGC densities for the group I and group II were 36.57 (SD 5.84) and 57.67 (SD 7.63), respectively. A significant difference was identified between the two groups ( $p < 0.001$ ).

**Conclusion:** RGC density was higher in the MTON model rats that were treated with coenzyme Q10 compared to the control group. (*Neuro-ophthalmol Jpn 34: 239~243, 2017*)

**Key Words:** methanol toxic optic neuropathy, coenzyme Q10, retinal ganglion cell density

### Introduction

Acute methanol intoxication cause central nervous system depression followed by acidosis, coma, and death. Methanol intoxication also affects visual function, as indicated by the development of blurred vision, which leads to blindness at 18–48 h after ingestion. This is attributed to optic nerve damaged and is called methanol toxic optic neuropathy (MTON)<sup>1,2)</sup>.

Methanol is a commonly used industrial chemical, which can enter the body through gastrointestinal ingestion or inhalation. The enzyme alcohol dehydrogenase is responsible for the metabolism of methanol to formaldehyde, which is subsequently metabolized to formate by formaldehyde dehydrogenase. Formate causes acidosis and damages

cytochrome oxidase, an essential component of mitochondrial ATP synthesis<sup>3-8)</sup>. The reduction in ATP synthesis generates a disturbance in the Na-K pump that later disrupts axoplasmic flow. This causes intra-axonal cell to swell, which compresses cells and leads to retinal cell and optic nerve damage<sup>2)</sup>. Methanol was also reported to increase reactive oxidative species (ROS), tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-4<sup>9,10)</sup>.

Histopathologically, swollen myelin and axons of the optic nerve have been found, which leads to retrolaminar demyelination. Damage occurs in the photoreceptor cells, including retinal ganglion cells (RGCs), which leads to a decrease in RGC density<sup>6,7)</sup>.

As MTON impairs mitochondrial function, studies have been conducted on the neuroprotective effect of



antioxidant compounds such, as  $\alpha$ -lipoic acid, caffeic acid phenethyl ester (CAPE), and docosahexaenoic acid (DHA)<sup>11-14</sup>.

Another agent designed to target mitochondria is coenzyme Q10, also known as ubiquinone; the compound is lipophilic and water insoluble, and performs a key function in electron transport and ATP synthesis in mitochondria. Coenzyme Q10 has been widely studied in mitochondria-related diseases such as congestive heart failure, mitochondrial encephalopathy, Parkinson's disease, Huntington's disease, infertility, degenerative diseases, and macular diseases<sup>15-17</sup>. This substance produces an effective response in these diseases. Coenzyme Q10 functions through two mechanisms in neurological diseases that result from alcohol toxicity: electron transport in mitochondria to maintain ATP synthesis, and antioxidant properties that reduce free radicals<sup>18,19</sup>.

In MTON, the effect of coenzyme Q10 as antioxidant and promote of mitochondrial metabolism in RGC density has not been previously studied.

The objective of this study was to compare RGC density in the control and coenzyme Q10-treated MTON rat models.

### Material and Methods

A completely randomized study using model animals was conducted. Fourteen male wistar rats of weight 300-350 g were used as model MTON animals. The rats were divided into two groups: control (I), and coenzyme Q10 treated (II). Both groups were administered  $N_2O:O_2$ , which produces equivalent effects in humans. Four hours after this procedure, the animals were orally administered methanol at a concentration of 3 g/kg body weight. The group I was untreated control and received no additional treatment; the group II was orally administered coenzyme Q10 of 100 mg/kg body weight. After 8 h, the eyes were enucleated and histopathological specimens were prepared. The RGC density was counted under a microscope at 200x magnification.

All rats procedures performed in accordance with the Association for Research in Vision and Ophthal-

mology statement for the use of Animals in Ophthalmic and Vision Research.

Data normality was assessed by Shaphiro Wilk analysis, in which  $p > 0.05$  indicated normally distributed data that could be analyzed by parametric tests. The average difference in RGC density among treatment groups was compared by analysis of variance in which significant results were further analyzed with post hoc test. Value of  $p < 0.05$  were considered statistically significant.

### Results

The data in the present study were normally distributed ( $p > 0.05$ ) in each group. (Table 1)

The present study showed average of RGC density 36.57 (SD 5.84) in the group I and 57.67 (SD 7.63) in the group II. (Figure 1) with a significant difference between the groups ( $p < 0.001$ ) (Table 2).

Table 1. Normality Test

Normality test of RGC density		
Groups	p-value	Data distribution
I	0.214	Normal
II	0.205	Normal

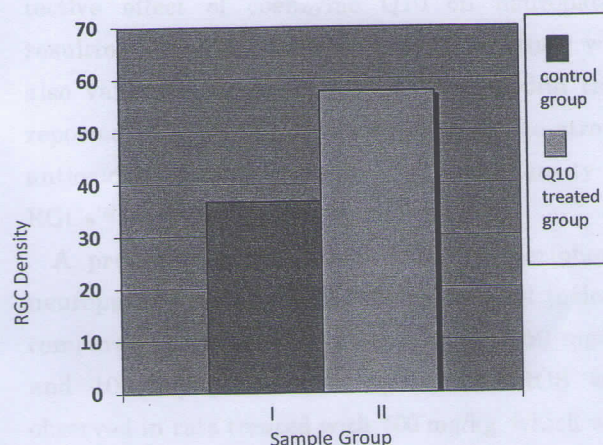
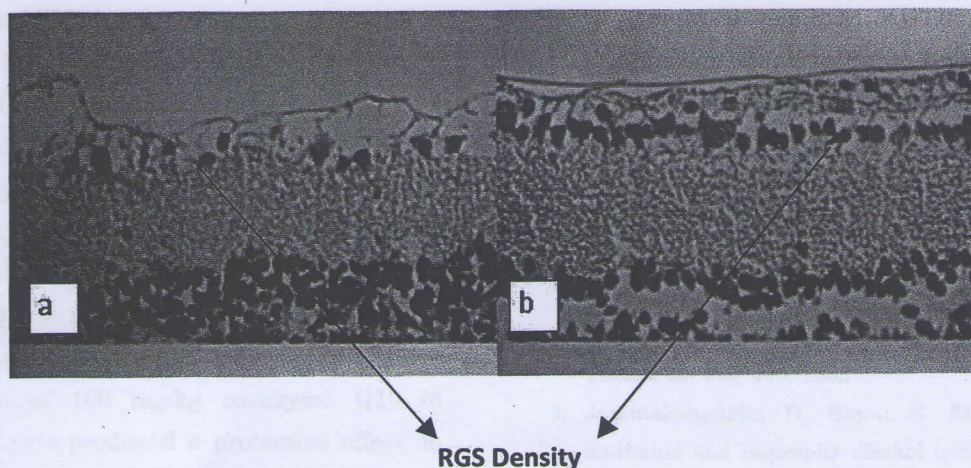


Figure 1. RGC density





**Figure 2. Histopathology of RGC (magnification 200x)**

(a) Control Group (b) Coenzyme Q10 Group

It showed that RGC density is higher in Coenzyme Q10 group.

**Table 2. Comparison of density of retinal ganglion in MTON between control and coenzyme Q10**

Gropus	Density of retinal ganglion cell				<i>P-value</i>
	$\chi$	SD	Median	Minimum - Maximum	
I	36.57	5.84	38	25.50-43.50	] <0.001*)
II	57.67	7.63	58	50.00-69.00	

\*)Post Hoc

## Discussion

The toxic effects of methanol on RGCs occur through its metabolite, formate<sup>2,8,20,21</sup>. Data in MTON model rats showed that methanol caused structural damaged in RGCs, reduced RGC density, and increased retinal oxidative stress<sup>10,20-22</sup>.

In this study, RGC density in MTON rats treated with coenzyme Q10 was significantly higher than the control ( $p < 0.001$ ). Coenzyme Q10 is required for the maintenance of electron transport that facilitates ATP synthesis in mitochondria and act as a strong antioxidant to prevent oxidative stress<sup>15,18,19</sup>. Sadli N et al. reported the potency of coenzyme Q10 as a preventive therapy for neurodegenerative diseases<sup>14</sup>. In addition, a study conducted by Khandare demonstrated the neuropro-

TECTIVE effect of coenzyme Q10 on neuropathy resulting from alcohol toxicity<sup>18</sup>. This study was also validated by previous work from Sahin that reported MTON model rats exposed to the strong antioxidant CAPE exhibited a higher density of RGCs<sup>13</sup>.

A previous study in rats model of peripheral neuropathy resulting from chronic alcohol toxicity compared the neuroprotective effects of 50 mg/kg and 100 mg/kg coenzyme Q10 lower ROS was observed in rats treated with 100 mg/kg, which was administered as the standard dosage in our study. However, ethanol was used in the previous study, whilst our study used methanol. Moreover, the exposure times were different: the administration period used by Khandare was 10 weeks, whereas our study investigated acute methanol exposure over



12-h period. In MTON, ROS elevate rapidly, which requires high level of antioxidants to counteract<sup>22)</sup>. Coenzyme Q10 has a high molecular weight which hinders its ability to cross the blood-brain barrier. Higher coenzyme Q10 level can be reached in cardiac tissue, muscle, the kidney, and the heart than in central neurons. Therefore, a higher dosage of coenzyme Q10 is required to achieve a high level in central neuron mitochondria<sup>23)</sup>. This single administration of 100 mg/kg coenzyme Q10 in MTON model rats produced a protective effect on RGCs. Further studies regarding the optimal dosage of coenzyme Q10 in MTON should be performed.

Many disease processes associated with coenzyme Q10 deficiency benefit from supplementation of coenzyme Q10 including mitochondrial diseases<sup>24)</sup>. Most of toxic optic neuropathies impair mitochondrial function and display clinical presentation, which involves a symmetric, progressive loss of vision associated with dyschromatopsia, centrocecal scotomas, and the loss of high spatial frequency contrast sensitivity<sup>25)</sup>.

Mitochondrial dysfunction occurs mainly through the impairment of oxidative phosphorylation and increased production of ROS. Both pathways may contribute to the opening of the mitochondrial permeability transition pore (mtPTP) which activate the apoptotic cascade and induce cellular death<sup>25)</sup>.

In many disease conditions with increased ROS generation and activity, the concentration of coenzyme Q10 in the human body is decreased and leads to respiratory chain dysfunction. Thus, the supplementation of coenzyme Q10 is reasonable remedy<sup>25)</sup>.

### Conclusion

In this study, RGC density in MTON model rats coenzyme Q10 treated group was significantly higher than control group ( $p < 0.001$ ).

The potency of neuroprotection provided by coenzyme Q10 treatment is very promising; further studies are warranted in neuro-ophthalmology cases such as optic neuropathy arising from methanol toxicity.

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