

Effect of Resveratrol in Expression of Caspase-7 and Retinal Ganglion Cells in a Rat Model With Traumatic Optic Neuropathy

Yunita^{1,2} Budu³, Andi Muhammad Ichsan⁴, Habibah S Muhiddin⁴,

¹Research in Postgraduate Program, Medical Faculty, Hasanuddin University, Indonesia, ²Ophthalmologist At Hasanuddin University Hospital Indonesia, ³Professor In Medical Faculty University Hasanuddin, Indonesia, ⁴Asisten Professor Ophthalmology Departement University Hasanuddin, Indonesia

Abstract

Objective: To analyze the effect of resveratrol to the expression of caspase-7 and density of retinal ganglion cells in a rat model with Traumatic Optic Neuropathy (TON) compared to control.

Methods: This is an experimental study using Wistar rats. Samples were divided into four groups. One normal control group, 1 TON control group and 2 TON resveratrol groups. TON induced by clamping the optic nerve retrobulbar with Hartmann Mosquito 2,5 inch. Resveratrol was given 10 mg/Kg and 20 mg/Kg in two resveratrol groups. Enucleation was performed 1 day after to evaluate the expression of Caspase-7 by immunohistochemistry and density of retinal ganglion cell by hematoxylin eosin staining.

Results: Caspase-7 expression was lowest in the resveratrol 20 mg/kg treatment group (4.00 ± 2.00) compared to resveratrol 10 mg/kg treatment group (6.00 ± 2.23), TON control group (6.00 ± 6.78) and normal control group (7.20 ± 2.58). Retinal ganglion cell density was highest in the resveratrol 10 mg/kg treatment group (27.23 ± 2.75) compared to resveratrol 20 mg/kg treatment group (24.89 ± 5.83), normal control group (24.19 ± 2.39) and the lowest in TON control group (22.96 ± 4.62). Based on the Pearson correlation test, it was found that there was no correlation between Caspase-7 expression and retinal ganglion cell density in a rat model with TON ($p: 0.178$; $r = 0.314$)

Conclusions: Resveratrol administration shows effect in lowering apoptosis marker caspase-7 and preserving retinal ganglion cell in traumatic optic neuropathy.

Keywords: Caspase-7, Traumatic optic neuropathy, Resveratrol, Retinal ganglion cell

Introduction

Retinal ganglion cell (RGC) death can occur due to apoptosis and necrosis mechanisms. Apoptosis is a cellular mechanism for programmed cell death but can be triggered by several factors including ischemia, toxic substances, and radiation¹. Traumatic optic neuropathy (TON) is a disorder of the optic nerve caused by an acute injury with a clinical picture of a sudden decrease

in visual acuity²

The pathophysiology of TON involves several factors that are associated with the concept of primary injury and secondary injury that initiates retinal ganglion cell death². Primary injury generally results from direct injury to the optic nerve, either compression or damage of the tissue, whereas secondary injury is a mechanism of optic nerve damage caused by inflammation or vascular disorders¹.

Coressponding author:

Yunita

Email: yunique_us@yahoo.com

Apoptosis can be lead by actvation of caspase. Caspase is a very interesting area of research on developmental disorders, cancer, infections, and

degenerative diseases. To date, two types of caspases have been defined, namely the caspase initiator and the effector / caspase executioner. As mentioned earlier, caspase-8 and -9 are initiator caspases while caspase 3 is effector caspases. Furthermore, other caspases such as caspase-2, -10, and -11 are included in caspase initiators while caspase-6 and -7 are included in the effector category^{6,2}. Still very little is known about caspase-7 in apoptosis of RGC. Caspase-7 was generally believed not exist in central nervous system. It was speculated that caspase-7 was acting as a redundant version of caspas-3 in apoptosis cascade. However, it was found that caspase-7 cleaves substrate different from caspase-3. Several non ocular studies suggest that caspase-7 play critical role in apoptosis and normal development. Decreased in caspase-7 protect the optic nerve from RGC death.^(3,4)

With apoptosis in research, the profound therapeutic potential of apoptosis has enabled researchers to develop promising therapeutic solutions that focus on the voluntary death of aberrant cells. A spectrum of drugs and therapies that exploit apoptosis has been shown to be effective against disease³. Resveratrol (3,5,4-trihydroxystilbene) is a natural polyphenol found in grapes, red wine, berries and other plants. has been identified as an inhibitor of carcinogenesis, cardiovascular diseases, neurodegenerative and aging. Many studies showed resveratrol effect on eye and related disorders. Similar effect in cancer, cardiovascular and neurodegenerative diseases, also seen in eye disorder including anti-oxidative, anti-apoptosis, anti-tumorigenic, anti-inflammation and anti-angiogenic.⁵

Resveratrol can protect retina by inhibiting inflammation biomarkers interleukin-6 (IL-6) and interleukin-8 (IL-8), by transforming growth factor- β 1 (TGF- β 1), cyclooxy- genase-2 (COX-2), and through VEGF accumulation. Resveratrol is known to activate sirtuins. The SIRT1 activating compounds promote longevity in different species and provide protective effects against acute or chronic neurodegenerative diseases, including retinal injury. Resveratrol prevented neuronal loss and delayed the visual decline.⁶

Thus our study was aim to analyze the effect of resveratrol to the expression of caspase-7 and density of retinal ganglion cells in a rat model with Traumatic Optic Neuropathy.

Material and Method

This is an experimental study using rats. The research was carried out in the animal testing laboratory and the Anatomical Pathology Laboratory of the RSPTN UNHAS Makassar. The sample size was 24 rats.

Work procedures

a) Twenty-four rats were divided into 4 groups (6 per group), 1 normal control group without treatment, 1 TON control group without therapy and 2 groups of TON treated with resveratrol (10mg / kgBW and 20mg / kgBW).

b) TON induction is performed by clamping the optic nerve retrobulbar with Hartmann Mosquito 2,5 inch for 15 seconds until afferent pupillary response was negative.

c) Resveratrol was given post-induction orally at a dose of 10 and 20mg / kgBW using oral gavage to ensure drug distribution according to the expected dose.

d) The examination is carried out after going through the therapy interval. The induced eyeball then enucleated and prepared in a paraffin block with a sagittal cut and focuses on the inferior area of the optic nerve.

Histopathological examination;

Enucleated eyes were fixed with formaldehyde, embedded in paraffin and cut in 5 μ m thick section. For routine histological analysis. Section were stained with hematoxylin and eosin (H&E) and examined with light microscope. The number of retinal ganglion cell nuclei was counted in 40x magnification. The amount obtained is calculated per field of view and the average is calculated.

Immunohistochemistry examination;

a) Deparaffinized the dried preparations with xylene 2 times (5 minutes each) and rehydrated with alcohol 96%, alcohol 80% and alcohol 70% (5 minutes each). Then washed for 5 minutes

b) Put the preparation in TRS solution, and heated in microwave for 20 minutes. After cooling down, washed with PBS 2 times (5 minutes each)

c) Tissue margins were marked. Peroxide block was done for 15 minutes. Washed with PBS and put in protein block for 5 minutes and washed again in PBS twice (5 minutes each)

d) Antibody caspase-7 (Invitrogen) monoclonal antibody were diluted 1/100 and incubated in -20°C.

e) The preparation then washed with PBS twice (5 minutes each) and put in Ultratek anti-polyvalent (ScyTek) for 10 minutes.

f) The preparation then washed with PBS twice (5 minutes each) and put in Ultratek HRP (ScyTek) for 10 minutes. Then washed again twice (5 minutes each)

g) Preparation was incubated with *chromogen Diaminobenzidine* (DAB) and washed with PBS twice (5 minutes each, and soaked in hematoxylin solution for 5 minutes.

h) Preparation washed in running water. And dehydrated with alcohol 70%, alcohol 80%, alcohol 96% and clearing with xylol I and II 5 minutes each.

i) Slide then dried and prepared on the object glass.

j) The preparations were read under a light

microscope. The number of caspase-7 expression based on brownish staining in sitoplasm and was counted in 40x magnification. The amount obtained is calculated per field of view and the average is calculated.

Data Analysis

All data obtained were processed using the SPSS program and analyzed with a significance level of ≤ 0.05 Independent t-test was used to compare two independent groups. Pearson's correlation test was used to see the relationship between two different groups

Result

Expression of caspase-7

Caspase-7 expression was lowest in the resveratrol 20 mg/kg treatment group (4.00 ± 2.00) compared to resveratrol 10 mg/kg treatment group (6.00 ± 2.23), TON control group (6.00 ± 6.78) and normal control group (7.20 ± 2.58) (table 1). Figure 1 showed immunohistochemical staining of the rat retinal ganglion cell with caspase-7 antibody. The effect of resveratrol to the expression of caspase-7 in rat with TON showed that treatment with resveratrol decreased the expression of caspase-7 compared to TON control and normal control, treatment with higher dose (20 mg/kg) showed lower expression compared to 10 mg/kg dose. Although there was not significantly different (Table 2).

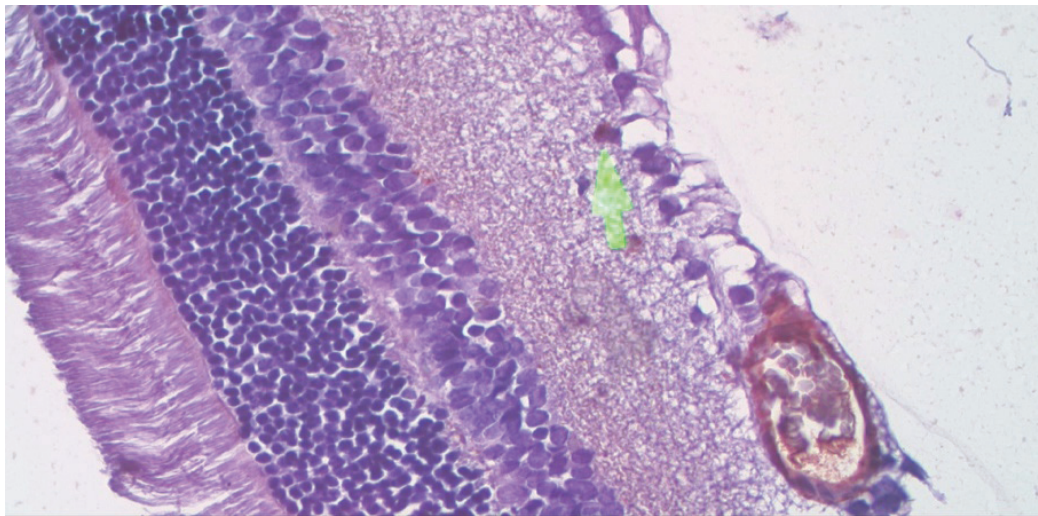


Figure 1

Microscopic description of caspase-7 expression in retinal ganglion cell (arrow) with immunohistochemistry.

Retinal ganglion cell density

Retinal ganglion cell density was highest in the resveratrol 10 mg/kg treatment group (27.23 ± 2.75) compared to resveratrol 20 mg/kg treatment group (24.89 ± 5.83), normal control group (24.19 ± 2.39) and the lowest in TON control group (22.96 ± 4.62) (table 1). Figure 2 showed retinal ganglion cell with hematoxylin eosin staining.

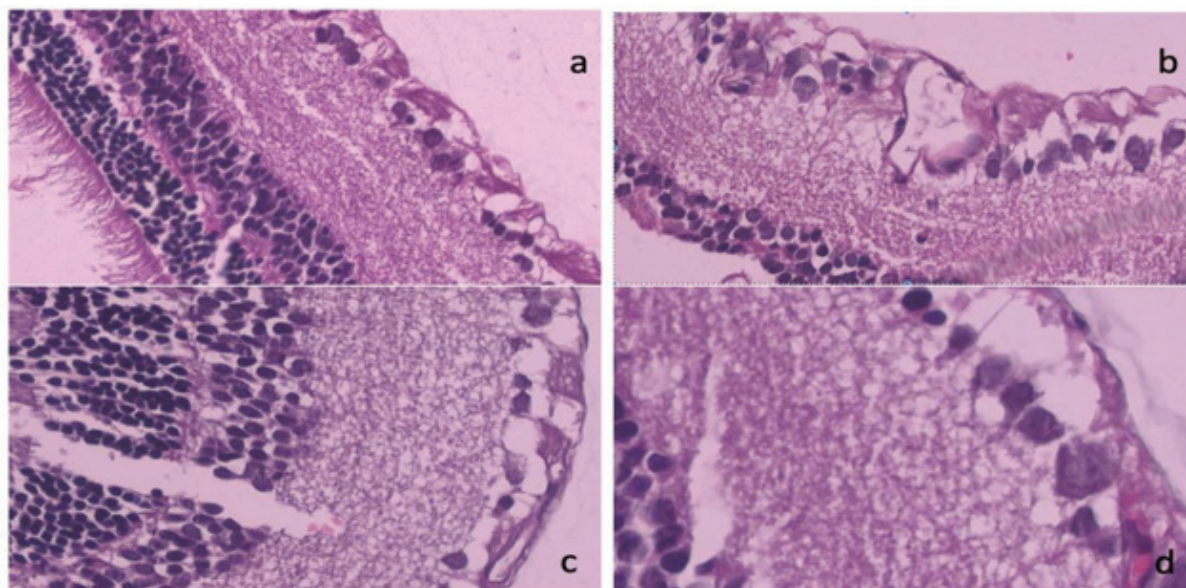


Figure 2: microscopic retinal ganglion cells with H&E staining on (a) normal control, (b) TON control, (c) resveratrol TON mouse 10mg / kgBW, (d) resveratrol TON mouse 20mg / kgBb)

Table 1: Expression of caspase-7 and retinal ganglion cell density

Groups	n	Mean \pm SD Expression of caspase-7	Mean \pm SD retinal ganglion cell density
Normal Control	5	7.20 \pm 2.58	24.19 \pm 2.39
TON control	5	6.00 \pm 6.78	22.96 \pm 4.62
Resveratrol dosis 10 mg/kg	5	6.00 \pm 2.23	27.23 \pm 2.75
Resveratrol dosis 20 mg/kg	5	4.00 \pm 2.00	24.89 \pm 5.83

Table 2: Differences in Expression of Caspase-7 and retinal ganglion cell density in Rat Models with TON between groups

Group	n	Caspase-7	Difference (P value)	Value	Difference (P value)
TON control	5	6.00±6.78	1.20 ±3.24 (0.726)	22.96±4.62	1.22 ±2.32 (0,611)
Normal Control	5	7.20±2.58		24.19±2.39	
TON control	5	6.00±6.78	0.00±3.19 (1.000)	22.96±4.62	4.27± 2.40 (0,114)
Resveratrol 10 mg/kg	5	6.00±2.23		27.23±2.75	
TON control	5	6.00±6.78	2.00± 3.16 (0.758)	22.96±4.62	1.93± 3.32 (0.578)
Resveratrol 20 mg/kg	5	4.00±2.00		24.89±5.83	
Normal Control	5	7.20±2.58	1.20 ± 1.53 (0,100)	24.19±2.39	3.04 ± 1.63 (0,100)
Resveratrol 10 mg/kg	5	6.00±2.23		27.23±2.75	
Normal Control	5	7.20±2.58	3.20 ±2.46 (0,180)	24.19±2.39	0.70 ±2. 81 (0,810)
Resveratrol 20 mg/kg	5	4.00±2.00		24.89±5.83	
Resveratrol 10 mg/kg	5	6.00±2.23.	2.00 ± 1.34 (0.141)	27.23±2.75	2.32 ± 2.88 (0.441)
Resveratrol 20 mg/kg	5	4.00±2.00		24.89±5.83	

Independent T test

Based on the results of the Pearson correlation test, it was found that there was no correlation between Caspase-7 expression and retinal ganglion cell density in a rat model with TON (p: 0.178; T= 0.314)

Table 3: Correlation Of Caspase-7 Expression And Retinal Ganglion Cell Density On Rat Model With TON

Variable	Pearson Correlation	
	T	P
Caspase-7 Levels Vs Retinal Ganglion Cells	0.314	0.178

Discussion

Effectivity of resveratrol on expression of caspase-7 in rat with TON

The results of this study showed that there was no difference in the expression of Caspase-7 in rats with TON between the TON control group and the normal control group, resveratrol at a dose of 10mg / kg, and resveratrol at a dose of 20mg / kg ($p > 0.05$). However, based on the average number, the levels of caspase-7 were at the lowest in the 20mg / kgBW resveratrol group compared to the other groups. This implied that resveratrol can decrease the expression or caspase-7 in TON rats compared to controls.

Resveratrol has an inhibitory effect on cell continuity signals by downregulating survivin, one of the inhibitors of apoptosis proteins (IAPs). In general, apoptosis will trigger caspase activation. IAPs inhibit apoptosis by directly blocking activation of caspase-3 and -7 and caspase-9.⁷ This is in accordance with the results of our study which showed that TON rats given resveratrol had lower caspase-7 expression than normal control and TON control mice, and caspase-7 expression in TON mice given 20 mg / KgBW of resveratrol was lower than TON mice given a dose of 10 mg / kgBW. Thus showed the effect of different dosages on caspase-7 expression. Although statistically there was no significant difference.

The evaluated the role of resveratrol in mitochondrial biogenesis in inhibiting apoptosis in retinal ganglion cells. Resveratrol therapy is thought to inhibit the apoptosis process, maintain mitochondrial membrane potential, reduce caspase and reduce the release of cytochrome C thereby maintaining cell continuity⁸ In Parkinson's disease, low-dose resveratrol (5 μ M) was thought to reduce dopamine-induced cell death in neuroblastoma cells by activating the antiapoptotic

factor Bcl-2 and inhibiting caspase-3/7.⁹ Resveratrol's effect on the pancreatic cancer cell renewal system by activating caspase-3 and -7 and inhibiting the expression of Bcl-2 and XIAP in cancer stem cells¹⁰.

Apart from a role in apoptosis, caspase-7 also has a role in inflammation. For example, low mortality rates in mice low in caspase-7 were associated with significant protection against salivary cell death. The role of caspase-7 in apoptosis and inflammation indicates its function in cell death and / or inflammation that contributes to pathological conditions including neurodegenerative diseases¹¹.

On the other hand, this study showed the highest caspase-7 expression in normal control mice. This implied the role of caspase was not only in the regulation of apoptosis, but also in the phenomenon of non-apoptotic cells. And caspase-7 has a function in the progression of cell mitosis. This stated that caspase-3 and -7 are functionally present in cells in excessive amounts. This is also consistent with research^{11,12}.

Effectivity of resveratrol on retinal ganglion cell density in rat with TON

This study shows the effect of resveratrol on retinal ganglion cell density in TON rats at the highest dose of 10 mg / kgBW compared to the dose of 20mg / kgBW. This is probably due to the bioavailability of resveratrol in experimental animals. Resveratrol is very easily absorbed in humans and experimental animals but is quickly metabolized into sulfo- or gluco- or hydrogen derivatives, which will then be excreted in the urine. Resveratrol, which was taken orally at a single dose (25 mg = 110 μ mol), the absorption was around 70%, with the concentration of resveratrol and its metabolites peaking at 2 μ m after 1 hour⁶.

Price et al found that low and moderate doses of resveratrol were required by AMPK activation and increased NAD⁺. However, at high doses, this effect is independent, indicating that the mechanism is dose dependent. Resveratrol shows its effectiveness even at low concentrations. The specific dose of resveratrol required for maximum effect is unknown. With regard to the method of administration of resveratrol therapy orally, several studies in mice have indicated that after oral administration of resveratrol, there is significant bioavailability in the cardiovascular system and binding to the kidneys and liver. Another study using mice, confirmed the same with mice regarding their bioavailability. It can even be said that resveratrol and its various derivatives can be found in various organs^{13,14}

This study showed no relationship between caspase-7 and retinal ganglion cell thickness in TON mice. This againts previous studies which showed that caspase-7 has an important role in retinal ganglion cell survival in conditions of optic nerve injury, where the absence of caspase-7 protects against loss of retinal ganglion cells. However, this study also explains that caspase-7 is not the only mediator in the pathology of the injured optic nerve, caspase-3 may also plays a role.¹⁵

Conclusion

Resveratrol administration shows effect in lowering apoptosis marker caspase-7 and preserving retinal ganglion cell in traumatic optic neuropathy model in rat.

Source of Funding - Self-funding

Conflict of Interest- None of the authors has competing interests

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 317 / UN4.6.4.5.31 // PP36 / 2020), and all research subjects give written informed consent.

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