IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 8 Ser. 9 (August. 2019), PP 72-76
www.iosrjournals.org

The Effect of Curcuma Longa on IL1-αand Cox2 in Endotoxin Induced Uveitis in Rabbit

Khalid.M.R.Abdulla[*], Winarto[**], LisyaniSuromo[***]
Corresponding Author: Khaled.M.R.Abdalla
[*]*Department of Blood Bank, Al Jala Hospital, Benghazi,Libya*
[**]*Department of Microbiologyand Opthalmology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia*
[***]*Department of Clinical Pathology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia*

Background:*Endotoxin InduceUveitis (EIU) is an experimental inflammation mimics the real acute uveitis, involving iris, ciliary body and choroid, in which many cytokines had been involved. Curcuma longa (CL) has anti-inflammatory and pro-inflammatory effects in several tissues including the eye.*
Objective: *to determine the anti-inflammatory effects of curcuma longa extract on endotoxin induced uveitis.*
Methods: *EIU was generated by intravitreally injection of lipopolysaccharide LPS of E.coli (LPS; 100 µg).The study group consist of control group and treatment group which was given curcuma longa extract (80 mg/kg body weight) intra gastric for 3 days. Atday 7 the rabbits were killed, eyes were enucleated, a stained by immunohistochemistry using monoclonal antibody of IL-1α and COX-2. The expression of IL-1α and COX-2 were determined by Allerd score.*
Results*: Clinical sign and symptom of acute anterior uveitis were prominent at day 3. The control group showed a ocular inflammation with high degree than treatment group. The expression of IL-1α and COX-2 in control group significantly higher than treatment group (p=0.001). This results showed that curcuma longa extract 80mg/kg body weight/day for 3 days had anti IL-1α and anti COX-2 in EIU.*
Conclusions: *Curcuma longa extract 80mg/kg has anti-inflammatory effect to uveitis induced by endotoxin of E.coli.*
Keywords:*curcuma longa, endotoxin, uveitis, IL1-α, COX-2, rabbits.*

Date of Submission: 06-08-2019 Date of Acceptance: 22-08-2019

I. Introduction

Uveitis is the term used to describe many forms of intraocular inflammation involving the uveal tract, iris, ciliary body and choroid, and adjacent ocular structures (retina, vitreous and optic nerve). Inflammation of the anterior uvea called anterior uveitis or iridocyclitis, inflammation of iris called "iritis", inflammation of the ciliary body called "cyclitis," and inflammation of choroid called "choroiditis." In clinical usage, uveitis has been used to describe most forms of intraocular inflammations,[1] and requires an urgent referral and thorough examination by an ophthalmologist due toneed of urgent treatment to control the inflammation.[2]Uveitis estimated to be responsible for approximately 10% of the blindness in the United States.

Immunopathogenesis of uveitis are not well understood, therefore it need a model of uveitis to study such process in vitro. Animal model is extensively used to understanding the mechanisms and the potency of medicine. Rabbit has been used for many studies on endotoxin-induced uveitis, because a rabbit has a large eye in which makes it easy for clinical or histopathology examination. Systemic or intraocular administration of endotoxininanimal experiments; the lipopolysaccharide (LPS), a component of outermembranesof gram-negative bacteria, could induced an acute anterior uveitis, known as endotoxin-induced uveitis (EIU). It has been hypothesized that EIU may serve as a model for human uveitis associated with gram-negative bacterial infection, because LPS induces the release of several inflammatory cytokines, such as IL-1,tumor necrosis factor (TNF), and interleukin-6.In vitro and in vivo, it is likely that these mediators also involved in the pathogenesis of EIU and, possibly, of human uveitis. This is supported by the data that intraocular injection of IL-1 or TNF in rabbits results in acute uveitis and in human,anddetection of up to 50,000-fold raised of IL-6 levels in the aqueous humor of patients with uveitis.[2, 3]

The pro-inflammatory cytokines such as IL-1α, IL-1β, IL-6, interferon-γ and tumor necrosis factor-α have all been detected within the ocular fluids or tissues in the inflamed eye. IL-1 is expressed by many cells and has multiple functions including local inflammation. Cells known to express IL-1α include astrocytes, fibroblasts, hepatocytes, keratinocytes, brown fat adipocytes, dendritic cells, macrophages. IL-1α and IL-1β are produced by macrophages, monocytes, fibroblasts, and dendritic cells. They form an important part of the

inflammatory response of the body against infection. These cytokines increase the expression of adhesion factors on endothelial cells to enable transmigration of leukocytes to sites of infection and re-set the hypothalamus thermoregulatory center, leading to an increased body temperature.

Cyclooxygenase-2 (COX-2) is unexpressed under normal conditions in most cells, but elevated levels are found during inflammation. COX-2 is generally expressed only in cells where prostaglandins are up regulated (e.g., during inflammation). COX-2 associated with an inflammatory reaction during the early phase of an inflammatory response.[5, 6]

Curcuma longa L., which belongs to the *Zingiberaceae family*, is a perennial herb, distributed throughout tropical and subtropical regions of the world, being widely cultivated in Asian countries, mainly in India and China. In Malaysia, Indonesia, India and China has been used as traditional medicine and well studied due to its economic importance.[4]Research shows curcumin is a highly pleiotropic molecule capable of interacting with numerous molecular targets involved in inflammation. Curcumin modulates the inflammatory response by down-regulating the activity of COX-2, lipoxygenase, and inducible nitric oxide synthase (iNOS) enzymes; inhibits the production of the inflammatory cytokines tumor necrosis factor-alpha (TNF-a), IL-1, IL-2, IL-6, IL-8, and IL-12, monocyte chemo-attractant protein (MCP), and migration inhibitory protein; and down-regulates mitogen-activated and Janus kinases.[4, 5]

The understanding of cytokine and anti inflammatory mediators are importance since its manipulation are usefull strategies for the tratment of ocular inflammation. In this study we investigated the anti inflammatory effects of *curcuma longa* 80 mg/kg body weight/day for 3 days towards anterior uveitis in rabbit, in relation to IL-1α and COX-2 expressions.

II. Methods

Preparation of *Curcuma longa* extract

The coarse powder of *Curcuma longa* (25 g) was extracted with ethanol (300ml) using a soxhlet apparatus for 48 h at 60°C. The ethanolic extract thus obtained was dried under reduced pressure at a room temperature not exceeding $40C^0$.

Rabbit

White rabbit's inbred strain was obtained from and reared at Animal Research Laboratory, Faculty of Veterinary Medicine, Gajah Mada University Yogyakarta. The animals were feed and care according to the rule of Helsinki animals rights.

Experimental design

Using proparacain 0.5% as topical anesthesia, the rabbits was injected intravitreally by LPS 20 µL (100 ng) using 30-gauge needle. The rabbits was grouped as experimental and control with seven rabbits in each group. Experimental group was given *curcuma longa* extract 80 mg/kg body weight/day for 3 days by intragastric tube. Clinical assesment of EIU was based on redness and discharge of the eye, cloudy of anterior chamber, and lack of pupillary reactivity to the light. At day 6, all rabbit were killed, the eyes were enucleated and stored in buffered formalin for immunohistochemistry staining.

Immunohistochemistry

The eyes were immersed in 4% glutaraldehyde for 30 minutes, transferred to and fixed in 10% buffer formaline for at least 24 hours, dehydrated in series of alcohol solutions, and embedded in parafin, then make 4 µm vertical section cut through pupillary optic nerve axis. Staining were applied after reacted to monoclonal antibody of IL-1α and monoclonal antibody of COX-2 to. A pathologist assess microsocopically using a light microscope with objective of 20 and 40 magnification.

Statistical Analysis

Values were expressed as mean ± SD, and unpaired student t test were used to assess statistical siginifiance of difference, in which $p < 0.01$ regarded as signifaicant.

III. Results

Clinical sign of uveitis

All rabbits were still a live up to the final of study. Before injection, the rabbit eyes were looks normal without any sign and symptom of inflammation. After 3 days of LPS injection, EIU were developed and showed an overt clinical signs of anterior uveitis (fig. 1).

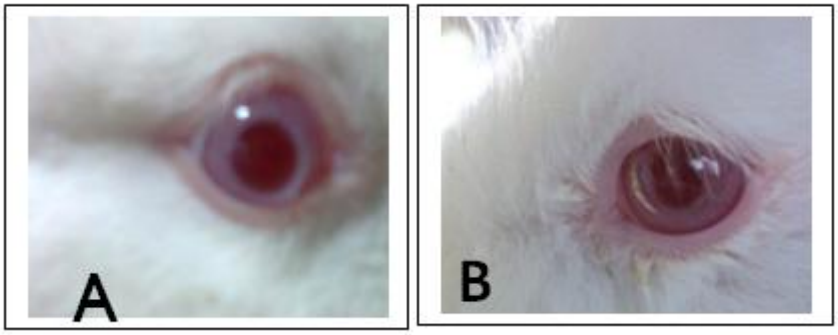

Figure 1. Clinical sign of anterior uveitis before LPS injection (A) and at day 3 (B).

Rabbit eyes in the control group showed a higher grade of ocular inflammation than experimental group at day 6 of experiment (fig.2).

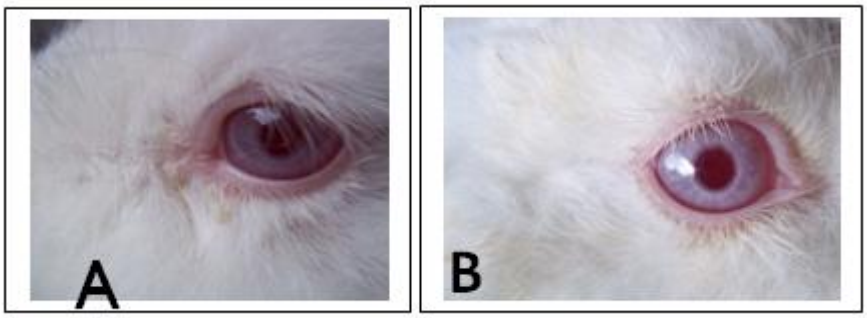

Figure 2. Clinical sign of anterior uveitis in control (A) and experimental group (B) at day 6.

Immunohistochemistry

Eye tissue IL-lα and COX-2 expression was measured by immunohistochemistry and quantified according to Allred score. IL-1αexpression of eye tissues showed in treatment group were significantly less than in non-treatment groupasseen in the figure 3.

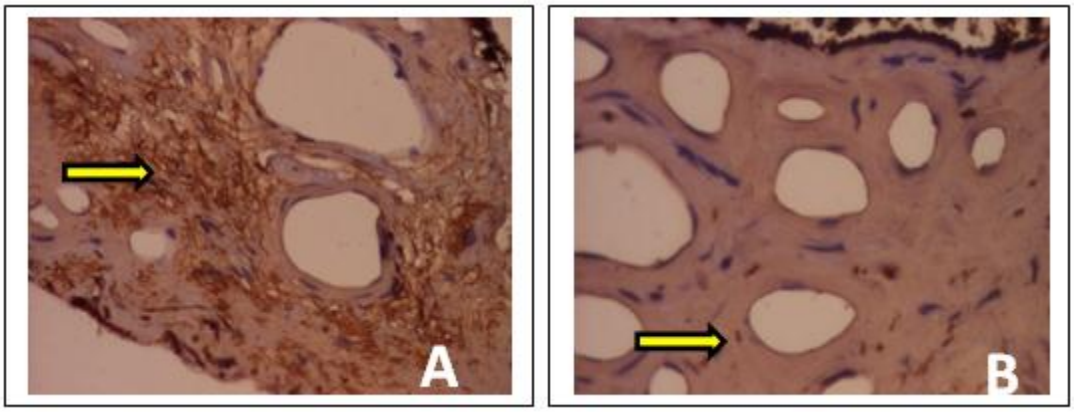

Figure 3. IL-1α expressions of anterior uveitis in control group (A) and experimental group (B) at day 6.

COX-2 expression of eye tissues showed in treatment group were significantly less than in non-treatment groupasseen in the figure 4.

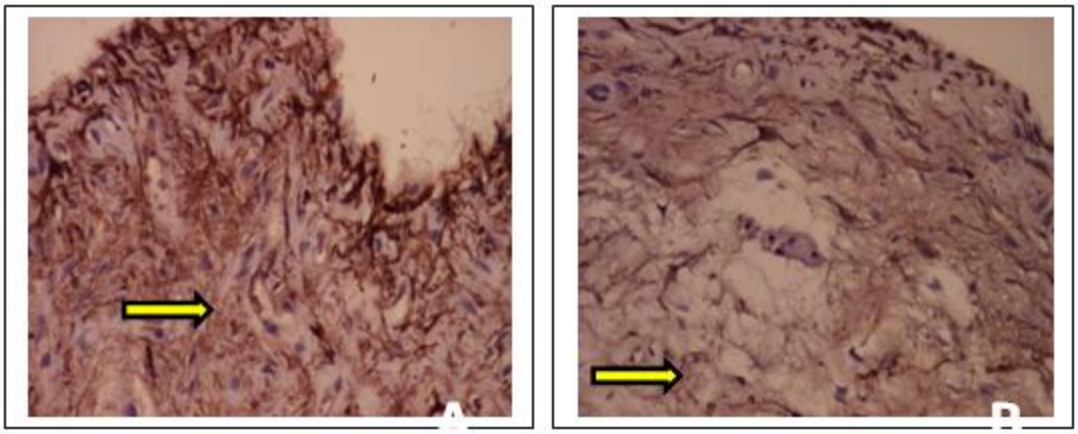

Figure 4. Cox-2 expressions of anterior uveitis in control group (A) and experimental group (B) at day 6.

IV. Discussion

In this study, anterior uveitis or EIU was succesfully develop by intravitreal injection of LPS. There were some eye discharge present in both groups and redness of the eye, watery, cloudy of anterior chamber and no pupillary light reflex, as seen in figure 1. Other reseacher was succes develop EIU by injection out site of the eye, e.g. intraperitoneal or footpad injection. By intravitreal injection, endotoxin directly exposed to uvea tissue and causes a local inflammation, eventhough there were an immune priviledge presennt within the eye.

In the control group they were significantly higher the expression of IL-1α and COX-2. It mean that both of them has a major role in the inflammation process of the uvea. The aim of this study was to elucidate the effect of *Curcuma longa* on IL-1α and COX-2 expressions in EIU by making comparison between control group and experimental group.The results showed the effect of treatment by curcuma longa extract for three days, either clinically or IL-1α and COX-2 expressionswere significantly decreased, as seen in figure 2, 3 and 4.

Curcumin is the active component of C. longa, comprising about 2-8% of the spice, and has been found to have potent antioxidant, anti-inflammatory, and anti-carcinogenic properties.

The therapeutic response to topical application of aqueous extracts of C. longa has demonstrated for the first time the possible utility of this drug as a topical application for the treatment of anterior uveitis. The reduced severity of inflammatory changes observed in histopathologic examination and clinical manifestations in the inflamed eye was the result of significant inhibition of vascular and cellular inflammatory responses. The release of chemical mediators of inflammation and inflammatory enzyme is also suppressed secondary to inhibition of the cellular response. The suppression of cellular inflammatory responses by herbal extracts was evidenced by significantly low levels of inflammatory cells, proteins, and IL1-α level and COX2 in aqueous humor of treated animals.. Direct binding to LPS of some of the extract constituents may be another mechanism of suppression of endotoxin-triggered uveitis. Inhibition of cyclooxygenase (COX)-2 and IL1-α may also contribute to the anti-inflammatory effect in anterior uveitis, as studies have shown selective COX 2 inhibitory activity of curcumin. A further study of the isolated active principle components of this herbal extracts is warranted, to explore fully the mechanisms of anti-inflammatory activity in endotoxin-induced uveitis. [(8)]

V. Conclusion

The conclusion of this study has showed that the curcuma longa extract has potent anti-inflammatory effect toward, endotoxin induced uvitis in rabbit is based on**:**

1. IL1-α expression of endotoxin-induced uveitis in rabbit that treated with *Curcuma longa* extract was significantly lower than without *Curcuma longa* extract.
2. COX-2 expression of endotoxin-induced uveitis in rabbit treated with *Curcuma longa* extract was significantly lower than without *Curcuma longa* extract.

SUGGESTION

IL-1α and COX-2 may be can use as the parameter to measure the effect of*curcumalonga*.*Curcuma longa* can be used as anti-inflammatory and alternative due to uvitis induced by LPs.

Acknowledgement

I would like to send my full gratitude to The Pathological Anatomy Department of and Animal Laboratory at Gajah Mada University facilities for conductingthis study. I also full thank to the research team of Biomedical Science Department of DiponegoroUniversity Semarang.

References

[1]. Lowder CY, Char DH: Uveitis-A review [Medical Progress]. West J Med 1984; 140:421-432.
[2]. UIG. Uveitis. [2012 April 9; cited 21012 April 13].availablefrom:http://en.wikipedia.org/wiki/Uveitis.
[3]. Hoekzema R, Verhagen C, van Hagen M, Kijlstra A: Endotoxin-induced uveitis in the rat: The significance of intraocular interleukin-6. Invest Ophthalmol Vis Sci 1992; 33; 532-9.
[4]. Araujo, C.A.C. and L.L. Leon. Biological activities of Curcuma longa L. Mem. Inst. Oswaldo Cruz.2001; 96(5):723-8.
[5]. Surenka JS. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: A review of preclinical and clinical research. Altern. Med. Rev. 2009;14:141-53
[6]. Caspi, R. R. Animal models of autoimmune and immune-mediated uveitis. Drug Discovery Today: Disease Models 2006; 3(1): 1-9.
[7]. Ghazali, Mohammed. 2012. The Anti-Inflammatory Effects Of*Curcumalonga* On The Uveitis Induced By Endotoxin In Mater Thesis, Diponegoro University, 2012.
[8]. The anti-inflammatory Effects of Curcuma longa and Berberisaristata in Endotoxin-InducedUveitis in Rabbits.3 Gupta SK, et al. Invest Ophthalmol Vis Sci. 2008; 49(9):4036
[9]. Kalariya NM, Shoeb M, Reddy AB, Zhang M, van Kuijk FJ, Ramana KV. Prevention of endotoxin-induced uveitis in rats by plant sterolguggulsterone. Invest Ophthalmol Vis Sci . 2010; 51(10):5105-13.
[10]. Induced Uveitis in Rabbits. Oztürk F, et al. Jpn J Ophthalmol. 1999;43(4):285-9
[11]. Moorfield eye hospital.uvitis.[update 2005 april;cited 2012 mar 25].available from: http://ebookbrowse.com/meh-leaflet-macularoedema-pdf-d147693852
[12]. UIG. Anterior uvitis. [2009 October: cited 2012 April 17].available from: http://www.uveitis.net/patient/pdf/UIG-anterior.pdf
[13]. American Uvitis Society. Acute Anterior Uvitis. [Update jan2003; cited 2012apri10].available from: http://www.uveitissociety.org/pages/diseases/aau.pdf.
[14]. PN SR. Anatomy Of Bacteria.[update 2009 mar;cited2012 mar 27].available from: http://www.microrao.com/micronotes/anatomy.pdf.
[15]. Leonardo MR, Silva LA, Assed S, Nelson-Filho P. Importance of bacterial endotoxin (LPS) in Endodontics. J Appl Oral Sci. 2004; 12:93-8.
[16]. Capsi,R,R .Immune Mechanisim In Uvitis .1999 springer seminimmunopathal. 21,113-4.
[17]. Priya CG, Rathinam SR, Muthukkaruppan V. Evidence for endotoxin as a causative factor for leptospiral uveitis in humans. InvestOphthalmol Vis Sci. 2008;49(12):5419-24.
[18]. Huhtinen, M. Acute anterior uveitis and HLA-B27: infectious background, systemic inflammation, and prognosis of the patients thesis eity:Medical Faculty of the University of Helsinki. 2002.
[19]. Kijlstra A. Cytokines: their role in uveal disease. Eye. 1997. 11, 200-5
[20]. Abbas, A, K. et.al, Cellular and Molecular Immunology .6th ed., 2007, Chapter 12.
[21]. Mukhopadhyay A, Basu N, Ghatak N, Gujral PK. "Anti-inflammatory and irritant activities of curcumin analogues in rats." Agents & Actions. 1982, 12(4): 508-15.
[22]. Tuaillon N, Shen de F, Berger RB, Lu B, Rollins BJ, Chan CC. MCP-1expression in endotoxin induced uveitis. Invest Ophthalmol Vis Sci.2002; 43:1493-1498.

*Khaled.M.R.Abdalla. " The Effect of Curcuma Longa on Il1-A And Cox2 In Endotoxin Induced Uveitis In Rabbit". IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 8, 2019, pp 72-76.