Effects of Gallic Acid and Its Derivatives on Inflammatory Regulation of Endometriotic Primary Cultures: Study on NF-κB mRNA Expression and IL-6 Secretion

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Endometriosis is a gynecologic disease in women that can cause infertility and chronic pelvic pain with a relatively high recurrence rate. This research was to prove the effects of gallic acid and its derivatives on inflammatory regulation of endometriosis primary cultures in terms of NF-κB mRNA expression and IL-6 secretions. Endometriosis cells are derived from endometriosis tissue of patients undergoing laparoscopy, isolated enzymatically and cultured primarily. The culture cells were treated with gallic acid, heptyl and octyl gallate at doses (25.6 μg/ml, 51.2 μg/ml and 102.4 μg/ml) for 48 h, then induced with 500 ng/ml LPS for 24 h. Inflammatory regulation was assessed from NF-κB mRNA expression with qRT-PCR and IL-6 secretion levels with ELISA. Gallic acid and its derivatives showed a decrease in the relative expression of NF-κB, significantly at dose 102.4 μg/ml. IL-6 although not statistically significant. The result indicated that gallic acid and its derivatives have a potential as anti-inflammatory effect. Gallic acid and its derivative compounds have an effect on decreased relative expression of mRNA NF-κB and IL-6.

Keywords: Endometriosis, inflammation, NF-κB mRNA, IL-6.
Interleukin 6 (IL-6) is a cytokine that present in various inflammatory processes in endometriosis. IL-6 is known to increase the production of other cytokines in endometriosis tissue. IL-6 plays a role in tumor growth and persistence of ectopic endometriosis tissue and inflammation in endometriosis. IL-6 found high levels in endometriosis. The up regulation of IL-6 cytokines happened through the activation of NF-κB pathway or MAPK in stromal cells of endometriosis.

The involvement of NF-κB and IL-6 cytokines on the inflammatory mechanisms in endometriosis, provides an opportunity to find a new agent in the treatment of endometriosis through NF-κB pathway inhibition, one of them is gallic acid.

Gallic acid is a class trihydroxybenzoic acid of compounds 3,4,5-hydroxybenzoic acids and its derivate including heptyl and octyl gallate have the potential inhibit the spread of cancer cells, antioxidant, anti-mutagenic, and anti-inflammatory in some cell lines.

Adding carbon atoms of gallic acid through esterification of the carboxyl groups produced alkyl ester derivative of gallates including heptyl and octyl gallate. The alkyl compounds are more hydrophobic than gallic acid, making them easier to penetrate through the cell wall.

Gallic acid as an anti-inflammatory has been demonstrated (Murase, 1999) by inhibiting cytokine TNF-α that play a role in the induction of translocation of NF-κB and decreased the expression endotelial-leukocyte adhesion molecule in cultured HUVEC (Human Umbilical Vein Endothelial Cell). This intracellular response clearly demonstrates that suppression of NF-κB activation by gallic acid can decrease the expression of genes that play a role in both inflammation and tumorogenesis but so far have not found the publication of the effects of heptyl gallate and octyl gallate as anti-inflammatory of endometriosis.

Analysis of NF-κB mRNA expression with qRT-PCR

A number of 5x10³ cells planted in well 12, then given gallic acid, heptyl gallate, and octyl gallate with concentration (25.6 µg/ml; 51.2 µg/ml; 102.4 µg/ml) for 48 h. Then induced 500 ng/ml LPS for 24 h. For positive control cells were induced by LPS only and cells without treatment for negative control. Pellets of endometriosis cells were carried out by RNA isolation of RNA mini kit Gene Science (Geneaid). Analysis of NF-κB mRNA expression used qRT-PCR with SBYR Fast Universal One Step qRT-PCR Kit.
(Kapabyosystem). β-actin as housekeeping gene and the expression level of NF-kB was assessed by the Livak method.

**IL6 Cytokine Secretions Assay**

Supernatant cell cultures prepared for assay of IL-6 cytokines were derived from the culture medium which has been given the gallic acid, heptyl gallate, and octyl gallate with various concentrations. IL-6 secretions were assessed by ELISA kit (QuantiKine, R&D, USA) according to protocol.

**RESULTS**

**Primary Culture of Endometriosis Cells**

Endometriosis cell were isolated and cultured from three samples patients. Endometriosis cells growth show in fig.1.

**NF-kB mRNA Expression**

Endometriosis cell cultures were treated with gallic acid, heptyl and octyl gallate for 48 hour later induced LPS for 24 h. NF-kB mRNA expression show in fig.2.

The relative mRNA expression of NF-kB in endometriosis cells with positive controls decreased compared to the relative expression of NF-kB with negative control assessed by q-RTPCR. There was no difference observed from NF-kB relative expression in gallic acid, heptyl, and octyl gallate at doses of 25.6 and 51.2 μg/ml. Significant statistic result of gallic acid, heptyl, and octyl gallate show mRNA NFkB suppression in a dose of 102.4 mg/mL (p value 0.049). The highest suppression was in the octyl group with a dose of 102.4 μg/mL. Suppression of mRNA NF-kB expression in octyl gallate greater than heptyl gallate and gallic acid.

**IL-6 Secretions**

After rationalization with negative control, levels of IL-6 decreased in cells were given gallic acid and octyl gallate compared to positive control, especially in octyl gallate dose 51.2 dose and 102.4 μg / mL. The effect of gallic acid and its derivatives to IL-6 secretions show in fig.3.

**DISCUSSION**

Gallic acid proved effective in several cell line as an anti-tumor by suppressing tumor cell viability, inhibiting proliferation, invasion and angiogenesis of tumors by inducing apoptosis also suppressing NFKB activation [21-24].
The results of our study of gallic acid, heptyl gallate and octyl gallate indicate the ability of suppression mRNA NF-κB, in line with earlier studies that gallic acid and synthetic derivatives of alkyl ester gallates effectively influence viability and cell proliferation in any type of cell line [16, 25-27].

Gallic acid is a candidate for antitumor and cancer agents with selective ability, the cytotoxic acid is cancerous but safe for normal cells. Weisburg (2014) found the gallic acid selective cytotoxic activity against oral cancer cells HSC-2 but not on gingival fibroblast cells. Gallic acid induces apoptosis by increasing caspase 3 and cleavage of poly-ADP ribose polymerase causing morphological changes in cancer cells. The antioxidant properties of gallic acid are highly significant in biological activity thus increasing normal and cytotoxic cell protection against cancer cells [28]. The gallic acid contained in the Solanum Surattense had antioxidant property also shows activity as free radical scavenging which is indispensable in the development of anti-oxidant therapy [29]. This encourages the gallic acid as a potential anti-cancer agent that is safe and effective. Molecular docking results also show that gallic acid and its derivatives meet criteria of pharmacokinetic parameters to be developed as new drugs [30].

Gallic acid has 1 H group on the carbon side chains with ClogP value 0.89, while heptyl gallate with the chemical formula -(CH2)6-CH3 with ClogP value 2.32, and octyl gallate (CH2)7-CH3 with ClogP value 3.72 [17]. Heptyl and Octyl gallate is a synthetic derivative of gallic acid by adding an -OH group on the carbon side chains. The addition of OH groups to the group of gallic acid derivatives increases the solubility and hydrophobicity of the substance so as to facilitate the penetration and increase of the biological activity of the natural substances within the cell. Lipophilic chain length on the side chain alkyl ester form affect the affinity and cell membrane permeability to these substances [31-32]. This supports the findings that heptyl and octyl gallate as an alkyl ester derivatives more potent as an agent blocking of NF-κB through suppression mRNA expression of endometriosis cell rather than pure gallic acid.

Suppression the relative expression of mRNA NF-κB with positive control was shown in groups conditioned by inflammation with LPS and the treatment resulted in relatively lower levels of expression than the ratio of untreated groups. The degree of relative suppression in the octyl group is higher than heptyl gallate and gallic acid. This proves the work of octyl gallate with more OH groups on carbon side chain is more potent in inhibiting NF-κB activity than heptyl gallate and gallic acid. The greater number of OH groups on the acidic carbon side chain increases the penetration ability into the cell, thus affecting the cell’s biological activity. These findings were parallel with previous studies which underlined that gallic acid was able to suppress the expression of genes involved in inflammation and tumorogenesis [19].

In this study, IL-6 level decreased when cells were given by octyl gallate doses 51.2 and 102.4 compared to positive controls (p value = 0.004). The results show that octyl gallate may potentially suppress IL-6 levels compared to gallic acid and heptyl gallate. In the previous research by Soo-jin Jeong, et al (2016) the gallic acid contained in natural materials Radix Sanguisorbae give effect to decrease the levels of IL-6 on the cell line RAW 254.7. There was a reduction of activity Myeloperoxidase and p-STAT3 activity that cause suppress mRNA IL-6, TNF-a, iNOS, and COX-2 expressions [33].

CONCLUSIONS

The results of this study demonstrated that treatment of octyl gallate into endometriosis cells is capable of suppressing NFκB mRNA expression and IL-6 secretion. Octyl gallate has a more potential effects as an anti-inflammatory than gallic acid and heptyl gallate.

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REFERENCES


