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Original research article

The Effect of Mangiferin Against Brain Damage Caused by Oxidative Stress and Inflammation Induced by Doxorubicin

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ABSTRACT

Doxorubicin (DOX) is an anthracycline antibiotic used for anticancer therapy. However, this agent can cause various systemic side effects including cognitive impairments in chronic use. Brain damage due to DOX is caused by an increase of tumor necrosis factor-alpha (TNF-α) level in the brain. Increased TNF-α can further lead to chronic inflammation which can lead to neuronal deaths or neurodegenerative diseases. Mangiferin (MAG), a compound extracted from *Mangifera indica*, has been found neuroprotective activities, but its effect on DOX-induced brain damage is unknown. This study aims to determine the effect of MAG on brain damage induced by DOX. Male Sprague-Dawley rats were induced by DOX intraperitoneally. MAG was given orally at the doses of 30 and 60 mg/kg bw for 7 consecutive weeks. The parameters measured were inflammatory and oxidative stress markers in brain tissue. Coadministration of MAG with DOX reduced inflammation which was marked by the reduction of TNF-α mRNA expression, decreased TNF-α level and reduction of oxidative stress marked by increase of superoxide dismutase level and decrease of malondialdehyde level. In conclusion, MAG was shown to have a neuroprotective effect on brain damage induced by DOX, partly due to inhibition of inflammation and oxidative stress.

1. Introduction

Doxorubicin (DOX), an anthracycline antibiotic, is widely used for anticancer agent, including breast cancer, cancer in children such as *Wilms tumor*, soft tissue sarcomas, also *Hodgkin's* and *non-Hodgkin's* lymphoma (Hortobagyi 1997). Despite having a wide anticancer effect, the utilization of DOX can cause side effects manifested as heart cell damage (Hortobagyi 1997) and cognitive disorders (Tannock *et al.* 2004). Cognitive disorders may decrease daily activity performances, such as work performances, access to health service, and interaction and awareness to family members (Janelsins *et al.* 2011). Long-term research on DOX-based chemotherapy showed that 76% of patients experienced cognitive degradation on acute phase, and 61% of patients experienced cognitive degradation on a low phase (Wefel *et al.* 2010).

Although the biochemical basis for these cognitive problems is unknown, it has been demonstrated that cancer therapeutics agents such as DOX can modulate endogenous levels of cytokines such as tumor necrosis factor (TNF) alpha (Usta *et al.* 2004). In addition, enhanced circulating TNF-α can initiate local TNF production via activation of glial cells leading to production of reactive oxygen or nitrogen species (Szelényi 2001). DOX causes an increase of peripheral TNF-α (Tangpong *et al.* 2006; Aluise *et al.* 2010; Gilliam *et al.* 2011). The increase of peripheral TNF-α is caused by raised TNF-α production by heart muscles (Mukherjee *et al.* 2003) and immune cells (Ujhazy *et al.* 2003). The increase of TNF-α in brain tissue may most likely be caused by receptor uptake at the blood-brain barrier (Osburg *et al.* 2002) and activation of glia which causes increased production of local TNF-α through signal activation of nuclear factor–kappa B (NF-κB) (Mohamed *et al.* 2011; McCoy and Tansey 2008). Further escalation of TNF-α can induce mitochondrial damage (Tangpong *et al.* 2006; Joshi *et al.* 2005) and constant glia activation, which in turn play roles in chronic inflammation that can lead to neuronal deaths or neurodegenerative diseases (Gonzales-Scarano and Baltuch 1999). Thus, it is

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possible that an increase in TNF-α level may be a link between DOX-induced oxidative stress and central nervous system injury.

Mangiferin (MAG) is a xanton glycoside, initially isolated from *Mangifera indica* L, which is found in many types of mango trees. A particular species of mango tree that grows in Indonesia, *Mangifera foetida* Lour. (*Anacardiaceae*; locally called *bacang*), has been proved to contain higher levels of MAG compared with other mango varieties. MAG is a potent antioxidant (Pal *et al.* 2013), with hepatoprotective (Das *et al.* 2012) and neuroprotective (Liu *et al.* 2013) activities. Our recently published studies showed that MAG also has cardioprotective effects, especially on DOX-induced rats, by regulating the intracellular calcium homeostasis (Arozal *et al.* 2015; Agustini *et al.* 2015). MAG has also been found to decrease inflammation and cell damage in the brain through the decrease of TNF-α and negative regulation of NF-κB (Marquez *et al.* 2012). The neuroprotective effect of MAG has also been studied on diabetic rat model by delivering the mentioned MAG dosage for 8 weeks (Liu *et al.* 2013).

To the best of our knowledge, there has not been any research on the effect of MAG toward brain damage induced by DOX. The focus of the present study was to understand the potential protective effect of MAG to prevent brain damage induced by DOX as an effort to enrich the use of evidence-based Indonesian natural herbal medicine.

2. Materials and Methods

2.1. Materials

Unless otherwise stated, all reagents were of analytical grade and purchased from Sigma-Aldrich (Singapore) or Merck Millipore (Jakarta, Indonesia). DOX hydrochloride injection was obtained from Kalbe Pharma (Jakarta, Indonesia). MAG was of analytical grade and obtained from Plamed Science Technology Company (Xi-an, China). RNA isolation, cDNA synthesis and reverse transcription polymerase chain reaction (RT-PCR) kits were purchased from Roche (Jakarta, Indonesia).

2.2. Animals

Study animals used were male Sprague-Dawley rats aged 12–16 weeks weighing about 180–200 g obtained from Badan Pengawas Obat dan Makanan, Jakarta, Indonesia. Rats were kept in a room with constantly-controlled temperature (21°C) and humidity (55%) with a 12 hour light/ dark cycle. They were allowed free access to standard laboratory food and water. The protocol has been approved by Animal Care Committee from Ethics Committee, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.

2.3. DOX and MAG preparation

To prepare DOX, a certain volume of DOX (supplied as 2 mg/mL of DOX in saline 0.9%) was extracted from the original product vial using syringe in a fume hood. No dilution was needed. MAG 60 mg/kg bw was prepared by mixing 200 mg of MAG powder with 10 mL of 0.5% sodium carboxymethyl cellulose (CMC) using mortar and pestle until MAG was perfectly suspended (Arozal *et al.* 2015). MAG 30 mg/kg bw was prepared using the same procedure, except the amount of MAG powder used was 100 mg.

2.4. Experimental design

Dosing and schedule of the study were determined according to our previous study (Arozal *et al.* 2015) with some adjustments. Previously, higher dose of MAG (100 mg/kg bw/day) had been proven to give negative results. Hence, for this study the MAG doses were decreased to 30 and 60 mg/kg bw/day. Dosing and schedule of DOX treatment were selected according to the work of Ibrahim *et al.* (2009), where cumulative dose of15 mg/kg bw of DOX divided in six injections within 2 weeks was able to induce cardiotoxicity in rats.

After 2 weeks of acclimatization, rats were randomly divided into four groups consisting of five rats each. The groups were as follows: normal group (control) which only received CMC 0.5% and saline (vehicles), toxic control group (DOX) which only received DOX, and two MAG groups which received both DOX and MAG (both groups received DOX with the same dose as DOX group; DOX + MAG30 group was given MAG 30 mg/kg bw/day and DOX + MAG60 group was given MAG 60 mg/kg bw/day). MAG was given orally every day for 7 consecutive weeks in CMC 0.5% as vehicle. DOX was given intraperitoneally with a total dose of 15 mg/kg bw divided in six injections with saline 0.9% as vehicle, starting from the beginning of second week until the end of third week.

Throughout the experiment, rats were monitored closely for signs of toxicity and mortality. Rats were weighed every day. At the end of the seventh week, rats were sacrificed by cervical dislocation method. Brains (the whole cerebrum in both right and left hemisphere) were extracted then rinsed with cold 0.9% normal saline. The brains were subsequently froze at −80°C before undergoing biochemical and molecular examinations.

2.5. Total RNA isolation

Brain tissue was homogenized using Ultra Turrax electric homogenizer. Total RNA was isolated from brain homogenate using *Tripure Isolation Reagent* (Roche) according to the manufacturer's protocol. The isolated total RNA concentration and purity were measured spectrophotometrically at 260 nm using Nanodrop 2000 (Thermo Scientific, Wilmington, USA). Only the samples with sufficient purity ($A_{260/280} > 1.8$) were subjected to the next treatment.

2.6. cDNA synthesis

cDNA synthesis reaction was performed using *Transcriptor First Strand cDNA Synthesis Kit* (Roche). The resulted cDNA concentration and purity were measured spectrophotometrically at 260 nm using Nanodrop 2000 (Thermo Scientific).

2.7. Examination of TNF-α mRNA Expression

RT-PCR was conducted using the *FastStart Essential* DNA *Green Master* (Roche) kit on *LightCycler Nano* (Roche). Primers used for amplification are presented on Table 1. The relative quantification calculation of target mRNA was based on the expression of β-actin mRNA as a comparator. Amplification was performed in 45 cycles followed by melting curve analysis. Number of cDNA templates used were 250 ng and the primary concentration used was 0.4 μM. The amplification conditions for each gene were as follows: β-actin (predenaturation: 95°C for 10 minute, denaturation: 95°C for 10 seconds, annealing: 53°C for 10 seconds, elongation: 72°C for 23 seconds) and TNF-α (predenaturation: 95°C for 10 minute, denaturation: 95°C for 10 seconds, annealing: 60°C for 30 seconds, elongation: 72°C for 1 second).

After RT-PCR, the amplification product underwent electrophoresis on 2% agarose gel stained with SYBR Green for band analysis. Only the amplification products that showed one band

Table 1. Gene-specific primer sequences used in RT-PCR

Gene	Primer sequence	PCR product
TNF-α	F: 5' – TCT CAA GCC TCA AGT AAC AAG C – 3' R: 5' – ATG AGG TAA AGC CCG TCA GC – 3'	330 pb
β-actin	F: 5' – TGT TGT CCC TGT ATG CCT CT – 3' R: 5' – TAA TGT CAC GCA CGA TTT CC – 3'	222 pb

RT-PCR = reverse transcription polymerase chain reaction.
Primer sequences used were referred to previous publication by Mohamed *et al.* (2011).

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