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Efficacy and Safety O-desmethyl Quinine Compare to Quinine for Nocturnal Leg Cramp

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Quinine is an alkaloid which isolated from the bark of *Cinchona* sp., native plant from South America, especially in Andes Mountains. Beside the function as an anti-malaria, quinine is used as anti-pyretic, analgesic, treatment for myotonia and nocturnal leg cramps. Nocturnal leg cramps is common clinical problem seen most frequently in the elderly. Quinine therapy was reported to be effective in relieving nocturnal leg cramps. However, due to side effects that appear limited this used. O-desmethyl quinine ($C_{19}H_{22}N_2O_2$) is one of quinine active metabolites that lost its methyl group. Therefore, this study was aimed to determine the efficacy and safety O-desmethyl quinine compared to quinine in treatment nocturnal leg cramps. Mice model were established by triction and rota rod method. Rats model were established through spinal cord injury and be evaluated by swimming test for 6 weeks. Test animals of spinal cord injury method were divided into three group consisted of control group, group treated with quinine orally at a dose of 26 mg kg^{-1} b.wt. and group treated with O-desmethyl quinine orally at a dose 26 mg kg^{-1} b.wt. On the last day, blood was taken to evaluate hematological profile. O-desmethyl quinine showed reduction frequency of cramps in rats with spinal cord injury. Improvement of quinine group was seen at week 6 and on O-desmethyl quinine group began from week 3 O-desmethyl quinine group showed lower blood toxicity compared to quinine group.

Key word: Quinine, O-desmethyl quinine, nocturnal leg cramps, spinal cord injury, blood profile

INTRODUCTION

Quinine is an alkaloid obtained from the bark *Cinchona* sp. It is a native plant from South America, especially in Andes Mountains. Indonesian people cultivated quinine in middle of 18th century. For almost 400 years, quinine have been used for anti-malaria (Achan *et al.*, 2011; Padua and Bunyapraphatsara, 1999). Beside the function as anti-malaria, quinine is used as anti-pyretic, analgesic, treatment for myotonia and nocturnal leg cramps. The bark of cinchona is also used in softdrinks, beverages containing alcohol and liquor due to content of its bitter substance. Due to fetal and abortifacient effects, quinine should not be used in pregnancy (Nair, 2010).

The development of quinine synthesis is still going until now. Bannon *et al.* (1998) explained that O-desmethyl quinine is the one of metabolite which found in the urine after oral administration. O-desmethyl quinine ($C_{19}H_{22}N_2O_2$) is active metabolite of quinine which synthesized by losing its methyl (Asnawi *et al.*, 2011). Because of having similar structure, O-desmethyl quinine is suspected to have similar pharmacology effect as quinine.

Nocturnal leg cramps are clinical problem in the form of involuntary muscle contractions that occur at night which is common in older people. Although there have been many hypotheses to explain the cause of these night cramp, the pathophysiology is still poorly understood. Cramp is caused by inadequate blood supply compared to blood demand (Gootnick, 1943). Many therapeutic approaches are offered. Some patients may benefit from measures such as raising the foot of the bed or dorsal flexing the feet. The most commonly prescribed medication is quinine sulfate. Some clinicians think that it is effective and prescribe it on an empiric basis, even though there is no scientific consensus concerning its efficacy or mechanism of action. Nocturnal leg cramps was reported to be effective treated with quinine therapy in early studies from the 1930-1940s. Unfortunately, these studies were based on uncontrolled data and suffered from inadequacies in their designs. More recent controlled studies have shown contradictory results. Jones and Castleden (1983) reported that in quinine treated patients with night cramp showed superior effect compared to placebo. In the other hand, it is found that effect of quinine showed no significant difference compared to placebo. In view this controversy, researcher studied quinine therapy in a prospective, double-blind, placebo-controlled cross over study in patients with nocturnal leg cramps and here in report the result.

Because of the anti-cramps effect, quinine oral has used as first line medication for this disease. However, FDA banned the use of quinine as an anti-cramps therapy because of its side effects (Katzberg *et al.*, 2010). Therefore, this study was aimed to evaluate the efficacy and safety O-desmethyl quinine compared to quinine to treated nocturnal leg cramps.

MATERIALS AND METHODS

This study was conducted at the Laboratory of Pharmacology, School of Pharmacy, Bandung Institute of Technology from September 2011 to June 2012. All experimental procedures were approved by animal ethics committee of Hasan Sadikin Hospital, Bandung, Indonesia.

Female mice of Swiss Webster strain two to three month old and weighed 20-25 g which were used for triction and rota rod test. Male rats of Wistar strain two to three month old and weighed between 175-200 g were used for spinal cord injury method. Rats were divided into five groups, each consisting of five rats. Before surgery, rats were adapted for one week in a cage with room temperature ($\pm 25^\circ\text{C}$) and food-drink facilities.

Drugs: Quinine (Sinkona Indonesia) and O-desmethyl quinine (Laboratory of Chemical Pharmacy, Bandung Institute of Technology, Bandung, Indonesia), dissolved in carboxy methyl cellulose 0.5%, was orally in mice and rat with a single dose $26 \text{ mg kg}^{-1} \text{ b.wt.}$ Control group were orally with carboxy methyl cellulose 0.5%.

Triction test: Placing the forepaws of the mice in a small twisted wire rigidly supported above the bench top did the screening of animal. Normally the mice grasp the wire with the forepaws, at least one hind foot on the wire for 5 sec when allowed to hang free. The test was conducted on three group of animal ($n = 8$) that were previously screened, 15 min after the administration of test drug or control vehicle, the same test was repeated every 15 min for 6 h (Kumar *et al.*, 2008).

Rota rod: Animal remaining on Rota-rod (10-16 rpm) for 4 min successive trials were selected for testing; 45 min after administration of test drug or control vehicle, the same test was repeated every 45 min for 4.5 h. The fall off time from rota rod was noted (Kumar *et al.*, 2008).

Spinal cord injury: all surgical procedures were performed under Urethane ($100 \text{ mg kg}^{-1} \text{ b.wt.}$) as anaesthetic. Lesi was performed in sacrum by needle. After surgery, rats were heated under the lamp. Povidone

iodine was administered for 1 week after surgery. The test was conducted on 3 groups of animals ($n = 5$) which consisted of quinine (26 mg kg⁻¹ b.wt.), O-desmethyl quinine (26 mg kg⁻¹ b.wt.) and carboxy methyl cellulose 0.5% as control. All of these substances were administered for 6 week start from week 2.

Scoring of muscle spasm: Rats had to swim in a rectangular 80×10×40 cm which was filled with water (18°C) for 4 min. Angle of lower trunk and water surface serves as the measure for spasms score.

Blood profile: on the last day, all rats were tested for its blood profile by hematology analyser.

Statistical analysis: Statistical analysis was performed with SPSS 15.0. Mean Witney non parametric was used to compare total score of spasm in rats with SCI.

Student t-test was used to compared blood profile quinine and O-desmethyl quinine.

RESULTS AND DISCUSSION

Motor and sensory deficits are an immediate consequence of Spinal Cord Injury (SCI), caused by damage to ascending and descending fiber tracts. In addition, signs and symptoms of spasticity, such as muscle hypertonia, hyperreflexia, clonus and spontaneous muscle spasm, develop gradually over weeks and months in 65-78% of patients with spinal cord injury. Painful muscle spasms are a common manifestation of the spastic syndrome. This experiment characterized muscle spasm that readily occurred during swimming in spinal cord injured rats. The occurrence of spasm in SCI rats was characterized by a delayed onset, similar to the situation in human. Frequency and severity spasm reached a stable plateau at 4-5 weeks, compared to 2-6 months in human. Lesion size did not correlated with severity of muscle spasm, this is similar in human (Gonzenbach *et al.*, 2010).

In this study the efficacy and safety of O-desmethyl quinine could be compared to quinine. Figure 1 showed the structure of O-desmethyl quinine and quinine. Because of having similar structure, O-desmethyl quinine

is suspected to have similar pharmacology effect as quinine. Moreover, losing its methyl group can decrease the lipophilicity and can reduce the side effects. Figure 2 showed that 1 week after surgery, there was an increasing a total score of spasm. This condition indicated that there was development spasm condition 1 week after surgery (acute phase).

Figure 3 showed that quinine and O-desmethyl quinine could reduce spasm frequency which decreased score of spasms. In quinine group the improvement began in week 5-6.

The improvement in O-desmethyl quinine group began to be seen in week 2-3. Figure 4 and 5 showed the result from triction and rotarod method. This figure showed that O-desmethyl quinine could delay onset of muscle cramps in hiperexercise condition. In triction method O-desmethyl quinine showed better strength than quinine especially in min 270 compared to control. The same result showed in rotarod method that O-desmethyl quinine and quinine showed better strength in min 270 compared to control but O-desmethyl quinine showed the significant strength earlier than quinine in 225 min. The exact mechanism of O-desmethyl quinine is still not understood but quinine may act by decreasing the excitability of the motor end plate region so that response to repetitive nerve stimulation and to acetylcholine are reduced. Quinine also increases the refractory period of muscle contraction so that response to tetanic is decreased (Fung and Hokbrook, 1989). Quinine was decreased the frequent of muscle spasms but not influence the duration and severity of spasms (Miller and Layzer, 2005).

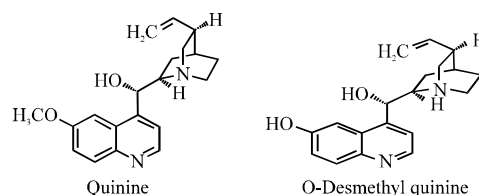


Fig. 1: Chemical structure of quinine and o-desmethyl quinine

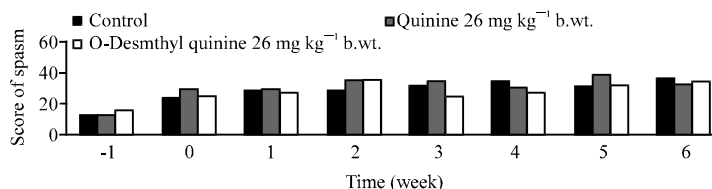


Fig. 2: Result of swimming test analysis o-desmethyl quinine treated group compared to quinine

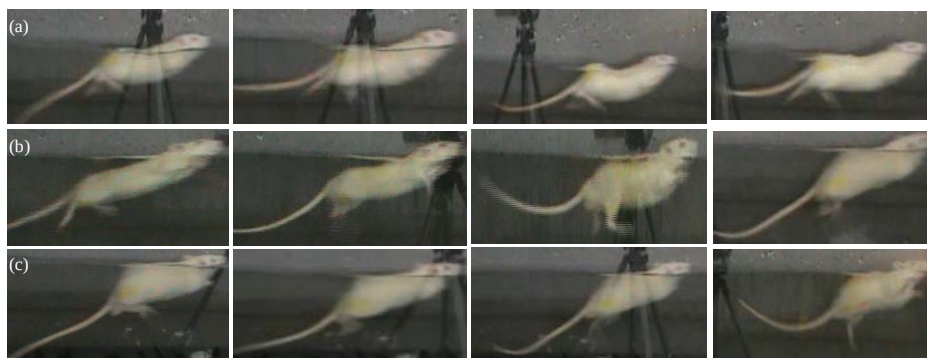


Fig. 3(a-c): Spasm condition in spinal cord injury rat (a) No spasm, (b) Minor spasm and (c) Major spasm

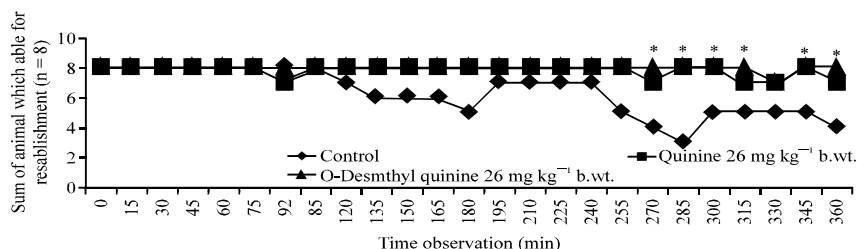


Fig. 4: Triction test of o-desmethyl quinine treated group compared to quinine, *Significant different compared to control group ($p<0.05$) treatment groups

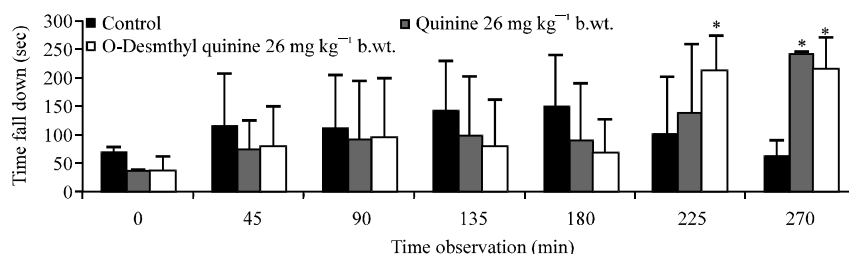


Fig. 5: Result of rota rod analysis o-desmethyl quinine treated group compared to quinine, *Significant different compared to control group ($p<0.05$) treatment groups

Blood profile: One of the most concerning side-effects quinine is blood toxicity (Mintzer *et al.*, 2009), consist of agranulocytosis (Sutherland *et al.*, 1977), thrombocytopenia (Bateman and Dyson, 1986; Dawson, 1979), hemolytic anemia (Miller and Layzer, 2005). This study showed that quinine decreased white blood cell that indicated agranulocytosis, The decreasing white blood cell in o-desmethyl quinine was slightly lower than quinine.

Agranulocytosis is caused by the failure of the bone marrow to form sufficient white blood cells (neutrophils). Bone marrow is the soft tissue inside the bones that helps the formation of blood cells.

Due to losing the methyl, the lipophilicity of o-desmethylquinine also decreased. So the capability of a substance to penetrate the central nervous system decreased and the side effects was also decreased. The side effects of O-desmethyl quinine were lower than quinine.

This result showed that O-desmethyl quinine had lower toxicity in blood profile than quinine.

Figure 6 showed that, quinine and o-desmethyl quinine group also showed the decreasing count of Red Blood Cell (RBC), Hemoglobin (Hb) and Hematocrit (HCT) that indicated occurrence of hemolytic anemia.

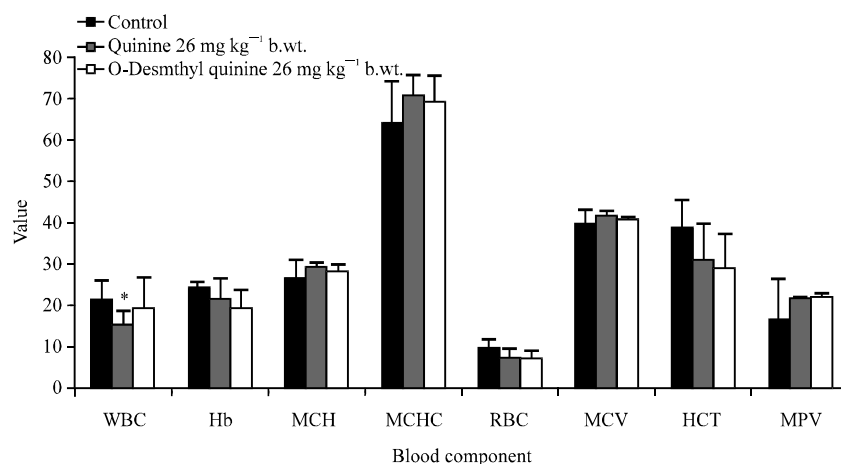


Fig. 6: Blood profile quinine and o-desmethyl quinine, *Significant different compared to control group ($p < 0.05$), WBC: White blood cell, Hb: Hemoglobin, MCH: Mean cell hemoglobin, MCHC: Mean cellular hemoglobin concentration, RBC: Red blood cell, MCV: Mean corpuscular volume, HCT: Hematocrit, MPV: Mean platelet volume

CONCLUSION

O-desmethyl quinine and Quinine showed the improvement with reduced score of spasms. O-desmethyl quinine showed lower toxicity blood than quinine.

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