ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Establishment Of Hyperuricemia Mouse Model With Oxonic Acid Potassium Salt And Essence Of Chicken.

Dian Ratih Laksmitawati*, Liliek Nurhidayati, Nining Novita Ningsih.

Laboratorium Biochemsitry and Pharmacology Faculty of Pharmacy Pancasila University, Jalan Srengseng Sawah Jagakarsa Jakarta 12640

ABSTRACT

Nowadays the exploration of anti-hyperuricemia herbs becomes more interesting, but making an animal model of hyperuricemia is still facing a problem due to a different uric acid metabolism between mice and human. The aim of this study was to investigate the effects of essence of chicken (EC) and urease inhibitor, oxonic acid potassium salt (PO) used singly or in combination on the concentration of serum uric acid in mice, to determine the optimal procedure, time of blood withdrawal and induction period and to evaluate the feasibility of this method to establish a mouse model of hyperuricemia. Determination of blood withdrawal time was as follows: DDY mice was given PO 250 mg/kgBW intra peritoneally and the blood was collected per hour to measure the plasma uric acid concentration. Determination of inductor combination and period of induction time were conducted by dividing mice into 6 groups: normal, PO 1 day, PO 3 days, PO and EC 1 day, PO and EC 3 days and EC 3 day and PO only on the third day. On the third day, the blood was collected and plasma uric acid was reacted with EHSPT (N-Ethyl-N-(2-hydroxy-3-Sulfopropil) *m*-Toluidine) and 4-AAP(4-Aminoantipyrine). The absorbance of color formation were measured by using spectrophotometer at 546 nm. The results showed that plasma uric acid was the highest at 2 hours after OA injection. The administration of both PO and EC could raise the uric acid compared with singly OA. Administration of EC and PO combination each day for 3 days' consecutively could increase plasma uric acid in mice. In conclusion, the administration of chicken essence (EC) with oxonic acid potassium salt (PO) for 3 days significantly increased the plasma uric acid. Thus, this procedure could be used as a suitable method to establish a mouse model of hyperuricemia.

Keywords: hyperuricemia model, potassium oxonic acid, essence of chicken.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

In recent years, there has been an increase in the prevalence of hyperuricemia. Hyperuricemia, characterized by high serum uric acid level, is a very common conditionusually caused by an unhealthy lifestyle mainly represented by a poor diet exceeding in purine nucleotides, protein, alcohol, and carbohydrates intake (1,2). Hyperuricemia is also caused by endogenous purine production associated with cell and tissue catabolism during forced diet or tumour cell lysis or decline in kidney function due to ageing, disease or drugs, such as diuretics and aspirin, impairment of the appropriate excretion of uric acid. (3)

The solubility of uric acid in water is low, and in humans, the average concentration of uric acid in blood is close to the solubility limit (6.8mg/dL).When the level of uric acid is higher than 6.8mg/dL, crystals of uric acid form as monosodium urate (MSU). Chronic hyperuricemia may cause uric acid precipitation in joints, some tissues and develop clinical manifestations such as erosive and deforming arthritis, nephrolithiasis and chronic nephropathy. MSU crystal formation and deposition is the key pathogenic process of gout. MSU crystals are rapidly recognised and ingested by human phagocytes then develop into an inflammation process.(3).

Uric acid is the final product of purine nucleotide catabolism. Many enzymes are involved in the conversion of the two purine nucleic acids, adenine and guanine, to uric acid. The last enzyme in purine nucleotide catabolism pathway in human is xanthine-oxidase (XO)(4). Inhibition of this enzyme may reduce production of uric acid. Xanthine oxidase inhibitors (XOIs) still remain the first line of treatment as recommended by all guidelines. Among these, allopurinol is the first-line agent in all but the American College of Rheumatology (ACR) guidelines, which recommend allopurinol or febuxostat interchangeably (5).

Fixed doses of 100 and 300 mg of daily allopurinol based on renal function, showed that only 39– 41% achieved a target SU of 6 mg/dl [Schumacher et al. 2008]. Another trial that titrated allopurinol up to 300 daily mg showed that only 56% of patients reached a target SU of 6 mg/dl [Reinders et al. 2009]. Therapy with allopurinol may cause gastrointestinal problems, development of rashes, Steven– Johnson's syndrome, and a rare case of allopurinol hypersensitivity syndrome (AHS)(5).

Due to side effect of chemical drug therapy such allopurinol and febuxostat, many researchers had interests in searching alternative substance as anti-hyperuricemia, especially from herbal. Some herbal had been screened for in vitro xanthine oxidase inhibitor activity and flavonoid was the most bioactive substance acting as inhibitor to that enzyme so it may be called hypouricemic agent. (6)(7). To verify the activity in biology system in reducing serum uric acid, it required in vivo testing in animal model of hyperuricemia. (8).

There was a problem when using mice or rat as an animal model of anti-hyperuricemia due to different uric acid metabolism between rat or mice and human. In human, uric acid as final metabolite of purine catabolism was excreted through renal as an urine but the water solubility of uric acid is rather low so its tended to deposit in the body especially in soft tissue such as bones joints. In rodents, there was an uricase enzyme, an uric oxidase which converting uric acid into an allantoin which was very soluble in water, so uric acid can be excreted more easily. (9).

In order to manufacture the rodent model of hyperuricemia, the theory of increasing the source of the uric acid, reducing uric acid excretion and inhibiting uricase were used. Potassium oxonic is a substance which blocks the effect of hepatic uricase and to produce hyperuricemia in rats, rabbits, dogs, mice, and pigs. (10). High purine diet can be conducted to increase the level of uric acid in rodent model of hyperuricemia. Chicken essence is one of high purine feed. There is a commercial product of chicken essence in liquid form that is practically found and ready to use.

The aims of this research was to investigate the effects of essence of chicken (EC) and urease inhibitor, oxonic acid potassium salt (OA) used singly or in combination on the concentration of serum uric acid in mice; to determine the optimal procedure, time of blood withdrawal and induction period to evaluate the feasibility of this method to establish a mouse model of hyperuricemia.

ISSN: 0975-8585

MATERIAL AND METHOD

Chemical

Essence of chicken (Brand's) as high purine diet, potassium oxonic (Sigma 156124) as inhibitor of uric acid, Uric Acid Mono SL (Ellitech Clinical System), standardized feed BR 512, generic allopurinol powder (PT. Indofarma), l CMC Na 0,5%.

Animal

Male DDY Mice, 2-3 months old, 20-30 g body weight obtained from Laboratorium non-ruminantia Satwa Harapan IPB Bogor were maintained on a 12-h light/dark cycle in a temperature- and humidity-controlled room for 1 week prior to the experiment. The animals were fed with a laboratory pellet chow (BR-12) and water ad libitum during the experiment. This study was conducted in accordance with the general animal laboratory standards.

Determination of blood withdrawal time

To know the time that mice have highest uric acid after intra peritoneally injection of oxonic acid, one group of mice (n=6) were given suspension of 250 mg/kg BW oxonic potassium in CMC Na 0,5% . Blood were collected at 0, 2, 4 and 6 hour after injection and uric acid plasma concentrations were determined in comparison with untreated group.

Induction Period

This research was a post only control group design experimental study. Mice were classified into 6 groups consisting of 5 mice. The first group (normal) was a normal healthy group . The second (PO 1) was a control group in which mice were injected with potassium oxonic only and blood samples were collected after 1 hour. The third group (PO 3), mice were treated with potassium oxonic for 3 days and on the third days 1 hour after last treatment, blood was collected. In the fourth group ((PO+ ES) 1), mice were injected with potassium oxonic and one hour later fed orally with essence of chicken 28 ml/kg BW. Mice were only treated for 1 day. The fifth group ((PO + ES) 3) followed the same as the treatment of fourth group but for 3-day period. The sixth group (ES 3 +PO1) mice were fed with essence of chicken for 3 days, but intraperitoneally injected with oxonic potassium only on the last/ third days.

Blood (0.5 ml) was collected in tubes containing K2-EDTA as anticoagulant. Every time blood collection was conducted, mice were fasted in 12 hour previously. Start from second night, mice were fasted. On the third day in the morning, mice was injected with PO , followed by EC at one hour later. Two hours after EC, blood samples were collected from vena orbitalis. The blood was then centrifuged for 15 minutes at the rate of 3.000-3.500 rpm.

Determination of Plasma Uric Acid

Plasma uric acid levele were determined using enzymatic assay measured by Microlab 300 photometric according to Uric Acid Mono SL (Ellitech Clinical System) manufacturer instruction. Briefly, uric acid in the sample was oxidized by uric oxidase to allantoin, CO2 and H2O2. The forming hydrogen peroxide reacted with EHSPT (N-ethyl-N-(2-hydroxy-3-Sulfopropil) m-Toluidine) dan 4-AAP (4-Aminoantipyrine) to form purple quinoneimine. This reactions were catalized by peroxidase. The intensity of the formed color was equivalent with the level of uric acid in plasma.

RESULT AND DISCUSSION

As shown in Fig. 1, intraperitoneal injection of potassium oxonate (250 mg/kg) markedly increased the serum uric acid levels, and reached C max to 1,33 ± 0,08 mg/dl at 2 h followed by slow decrease in serum uric acid level until 6 h after injection. In this research the uric acid level in normal mice was only 0,67±0,10 mg/dl.

ISSN: 0975-8585

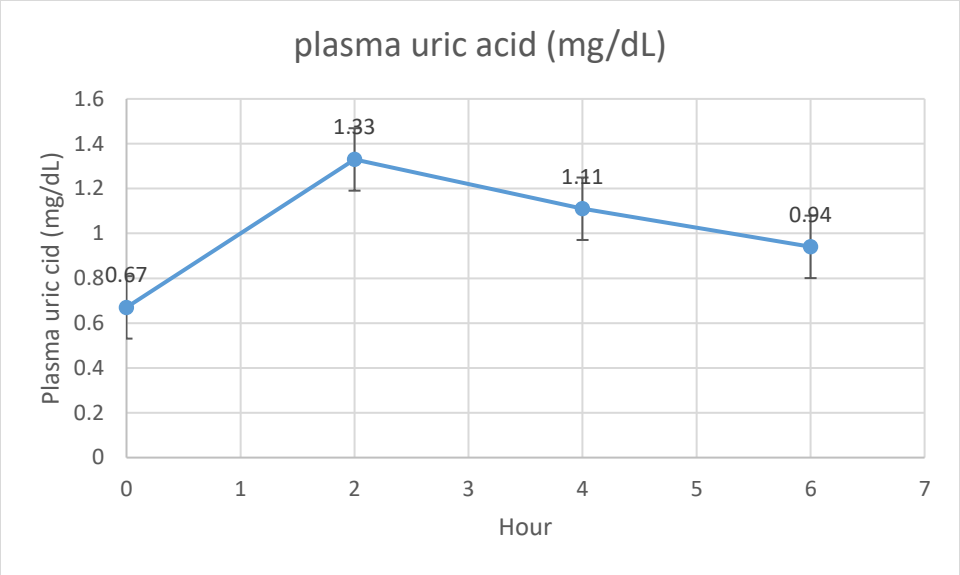

Figure 1. Plasma uric acid level after intraperitoneally injection of potassium oxonic 250 mg/kg BW.

Mice excrete much more allantoin than uric acid because they have active uricase which efficiently converts uric acid to allantoin. Allantoin is the major end product of purine metabolism.(11)

The UA levels increased in most tissues of mice during aging. At 3 months of mice age the plasma UA level was ± 60µM equivalent with 1.01 mg/dL(12)

Determining of Optimum Induction Period

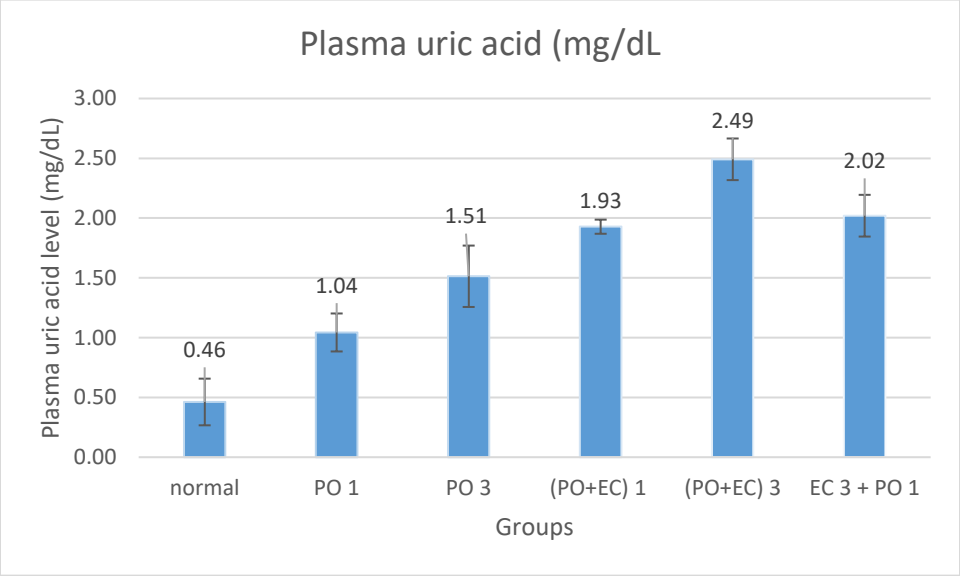

Fig 2. Plasma uric acid level (mg/dL) in different composition and length of induction periode. PO 1 : potassium oxonic for 1 day, PO 3 : potassium oxonic for 3 days, (PO+EC)1 : essence of chicken and potassium oxonic for 1 day, (PO+EC)3 : essence of chicken and potassium oxonic for 3 days, EC 3+PO1 : essence of chicken for 3 days and potassium oxonic 1 times at the third day.

As shown in Figure 2, the composition of combination and the length of induction period increased the serum uric acid levels in hyperuricemic rats in significantly difference level.

Tabel 1. Plasma Uric Acid on different goups

Groups	Plasma Uric Acid (mg/dL)
Normal	0.46 ± 0.22^{a}
PO 1	1.04 ± 0.17^{b}
PO 3	1.51 ± 0.28^{c}
(PO + EC) 1	1.93 ± 0.07^{d}
(PO + EC) 3	2.49 ± 0.19^{e}
EC 3+PO 1	2.02 ± 0.20^{d}

*Note: Data is presented as average of ± SD from 5 replications. Different superscript letter a, b, c, d and e, in a column indicates significance different among concentrations based on Duncan post hoc test with $p < 0.05$ is considered as significantly different.

PO along with EC can increase UA significantly compared to PO alone. Period of induction time influenced the level of UA. Three days induction with PO+EC increased plasma uric acid rather than only one day. Three days giving EC and only on the third day giving PO did not not significantly increase compared with (PO+EC). It seems that PO played important role in rising plasma uric acid significantly.

The oxonate-treated rat can serve as a useful animal model not only in investigation of the uric acid nephropathy, but also in a number of other toxicologic evaluations connected with uric acid. This model has been used to evaluate drugs affecting uric acid excretion, to determine which dietary factors affect serum urates, or to evaluate possible therapeutic agents in certain disorders associated with uric acid. The ideal uricase inhibitor for induction of hyperuricemia would be one which is irreversible, noncompetitive, and relatively nontoxic, so that its activity would be independent of high levels of uric acid, and effective inhibition could be attained at low dosage levels. Oxonic acid is not an ideal uricase inhibitor because it is competitive and eliminated from the body relatively rapidly. Although relatively nontoxic, oxonic acid and its salts are foreign substances that could interfere with some other metabolic systems. The possibility exists that an ideal, or at least a better inhibitor, could be developed by appropriate substitutions on the molecule of oxonic acid or by introducing different types of compounds such as derivatives of diazohypoxanthines, barbiturates, or similar substances. Until such improvements on the uricase-inhibited rat models are available, potassium oxonate, which is readily obtained, can be used as an effective inhibitor of uricase *in vivo*.(10).

CONCLUSION

The administration of chicken essence (EC) with oxonic acid potassium salt (PO) for 3 days significantly increased the plasma uric acid. This procedure could be used as a suitable method to establish a mouse model of hyperuricemia.

ACKNOWLEDGMENT

This work was supported in part by Pharmacy Faculty of Pancasila University's incentive Research Program 2015, Jakarta, Indonesia.

REFERENCES

[1] Torralba KD, De Jesus E, Rachabattula S. The interplay between diet, urate transporters and the risk for gout and hyperuricemia: Current and future directions. Vol. 15, International Journal of Rheumatic Diseases. 2012. p. 499–506.

[2] Yamanaka H. Essence of the revised guideline for the management of Hyperuricemia and gout. Vol. 55,

Japan Medical Association Journal. 2012. p. 324–9.

[3] Schett G, Schauer C, Hoffmann M, Herrmann M. Why does the gout attack stop? A roadmap for the immune pathogenesis of gout. RMD open [Internet]. 2015;1(Suppl 1):e000046. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4632150&tool=pmcentrez&rendertype=abstract

[4] Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. Int J Cardiol. 2016;213(August):8–14.

[5] Sattui SE, Gaffo AL. Treatment of hyperuricemia in gout: current therapeutic options, latest developments and clinical implications. Ther Adv Musculoskelet Dis [Internet]. 2016;8(4):145–59. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27493693\nhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4959626

[6] Kostić DA, Dimitrijević DS, Stojanović GS, Palić IR, Dordević AS, Ickovski JD. Xanthine oxidase: Isolation, assays of activity, and inhibition. J Chem. 2015;2015.

[7] Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, et al. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J Nat Prod. 1998;61(1):71–6.

[8] Mo S-F, Zhou F, Lv Y-Z, Hu Q-H, Zhang D-M, Kong L-D. Hypouricemic action of selected flavonoids in mice: structure-activity relationships. Biol Pharm Bull. 2007;30(August):1551–6.

[9] Werner AK, Witte CP. The biochemistry of nitrogen mobilization: Purine ring catabolism. Vol. 16, Trends in Plant Science. 2011. p. 381–7.

[10] Stavric B, Nera E a. Use of the uricase-inhibited rat as an animal model in toxicology. Clin Toxicol [Internet]. 1978;13(1):47–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20685310

[11] Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL. The mouse in biomedical research. Normative Biology, Husbandry, and Models. Vol. 3, American College of Laboratory Animal Medicine Series. 2007. p. 809.

[12] Iwama M, Kondo Y, Shimokado K, Maruyama N, Ishigami A. Uric acid levels in tissues and plasma of mice during aging. Biol Pharm Bull [Internet]. 2012;35(8):1367–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22863939