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A critical review on the experimental model of animal for antigout drugs

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Abstract

In this era where the pharmaceutical companies and products are hiking in its need and production it is inevitable to document the safety and toxicity along with the indications of the same. This is where the experimental study has a vital role to play. Experimental pharmacology is the science where the drug interaction with different receptors and target sites in living organism are explained. This article reviews about the different aspects of experimental pharmacology and its uses. Gout is an acute inflammatory disease characterized by the presence of uric acid crystals in the joint. This event promotes neutrophil infiltration and activation that leads to tissue damage. Mice received oral administration of BL or saline daily for 7 days and then were injected with MSU in the knee cavity. The present review aims to provide an updated overview on history, types, uses and mechanism of drug treatment for gout (hyperuricemia) with the detail review on information about experimental model of antigout drugs, their operating procedure, these agent works correct overproduction or under excretion of uric acid.

Keywords: Gout; Inflammatory disease; Experimental pharmacology

1. Introduction

Gout distinguished itself in the history of Homo sapiens since time immemorial. It appeared in medical records very early in the history of medical writing, and was also mentioned in the biographies of many famous names. It was depicted as the fate of a life of affluence as much as the challenge to a physician's skill, and truly it was. Modern ages witnessed remarkable progress in managing gout. More recently, thanks to quantum leaps in molecular biology, diagnostic modalities, and pharmacotherapy, we enjoy deeper understanding of the disease and a more sophisticated armamentarium. Gout is a systemic disease that results from the deposition of monosodium urate crystals (MSU) in tissues. Increased serum uric acid (SUA) above a specific threshold is a requirement for the formation of uric acid crystals. Despite the fact that hyperuricemia is the main pathogenic defect in gout, many people with hyperuricemia do not develop gout or even form UA crystals. In fact, only 5% of people with hyperuriceamia above 9 mg/dL develop gout. Accordingly, it is thought that other factors such as genetic predisposition share in the incidence of gout [1],[2].

MSU crystals can be deposited in all tissues mainly in and around the joints forming tophi. Gout is mainly diagnosed by identification of the pathognomonic MSU crystals by joint fluid aspiration or in tophi aspirate. Early presentation of gout is an acute joint inflammation that is quickly relieved by NSAIDs or colchicine. Renal stones and tophi are late presentations. Lowering SUA levels below deposition threshold either by dietary modification and using serum uric acid lowering drugs is the main goal in management of gout. This results in dissolution of MSU crystals preventing further attacks [3], [4].

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1.1. Epidemiology

The general prevalence of gout is 1–4% of the general population. In western countries, it occurs in 3–6% in men and 1–2% in women. In some countries, prevalence may increase up to 10%. Prevalence rises up to 10% in men and 6% in women more than 80 years old. Annual incidence of gout is 2.68 per 1000 persons. It occurs in men 2–6 folds more than women. Worldwide incidence of gout increases gradually due to poor dietary habits such as fast foods, lack of exercises, increased incidence of obesity and metabolic syndrome [5].

1.2. Pathogenesis of Gout

Urate is the ionized form of uric acid present in the body. Uric acid is a weak acid with pH of 5.8. Urate crystals deposition in tissues starts to occur when serum uric acid level rises above the normal threshold. Pathological threshold of hyperuricemia is defined as 6.8 mg/dL [1], [6].

Some factors may affect the solubility of uric acid in the joint. These include synovial fluid pH, water concentration, electrolytes level, and other synovial components such as proteoglycans and collagen. SUA level in the body is determined by the balance between its production either from purine intake in diet or endogenous production by cellular turnover and its excretion by the kidneys and GIT. Increased production of UA is responsible for only 10% of cases of gout while the remaining 90% are caused by its renal under-excretion [7].

Factors affecting SUA levels include age and gender. SUA is low in children. After puberty, SUA levels start to increase to reach their normal levels. In men, levels are higher than in women. However, SUA levels in postmenopausal women increase to reach men's levels. This explains why gout is usually a disease of middle aged and older men, and postmenopausal women. Rarely, it may happen in children and young adults in some rare inborn errors of purine metabolism. These enzymatic defects result in increased SUA with consequent production of UA crystals in kidneys and joints [8].

1.2.1. Overproduction of uric acid

Deficiency of enzymes involved in purine metabolism leads to overproduction of UA. For example, Lesch Nyhan syndrome is an inborn error of metabolism resulting from deficiency of an enzyme involved in UA metabolism named hypoxanthine–guanine phosphoribosyl transferase.

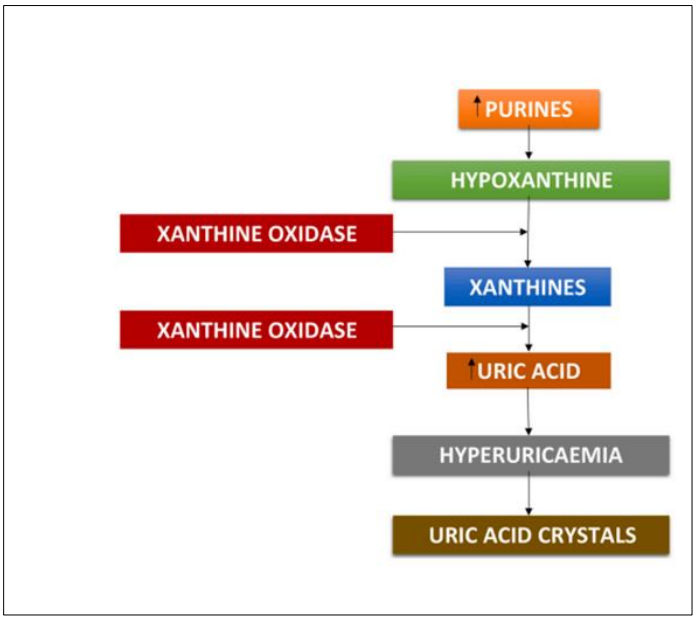

Figure 1 Enzymatic pathway of purine to uric acid end product and risk factors of elevated uric acid level

It is a genetic X-linked recessive disorder with varying degrees of severity according to the type of mutation. The clinical picture of this disease involves neurological abnormalities such as dystonia, chorea, cognitive dysfunction, compulsive injurious behavior, self-mutilation and articular manifestations (early onset gout) in addition to renal stones. If left untreated, it may lead to tophi formation and renal failure [9]. Another enzymatic abnormality that causes gout in the young is the super activity of phosphoribosyl pyrophosphate synthetase. It is an X-linked dominant inherited disorder. The syndrome has two clinical forms, a severe early onset form in children and a mild late juvenile or early adultonset form. Clinical picture includes neurological abnormalities such as sensorineural hearing loss, hypotonia and ataxia in the severe form. The mild form manifests as uric acid renal stones and arthritis. However, these enzymatic disorders constitute only less than 10% of cases of overproduction of urates [10]. Purine is converted to hypoxanthine, which is then oxidized by XO (xanthine-oxidase) to form xanthine). Uric acid is formed when xanthine is oxidized again by xanthine oxidase. Fig. 1 shows the enzymatic pathway of purine to the uric acid end product [11].

2. Animal Model

2.1. Experimental *in-vivo* models for gout

Several animal models of hyperuricemia and gouty arthritis have been reported, for in vivo assessment of hypouricemic, anti-inflammatory as well as renal protective effects of the compound. The injection of MSU-crystals into various anatomical structures to induce crystal-induced inflammation has been proposed; however, only a few of these models accurately reflect the joint microenvironment in which an acute gouty attack occurs.

2.2. Potassium oxalate induced hyperuricemia model

Potassium oxalate, a competitive uricase inhibitor, produces hyperuricemia in rodents [12]. The end product of purine nucleotide catabolism is Uric acid. Adenine and guanine, two purine nucleic acids, are converted to uric acid with the help of the xanthine oxidase enzyme. (ASC: Apoptosis-associated speck-like protein, IL-1 β: Interleukin 1 β, MSU: Monosodium urate crystals, NLRP3: Nod-like receptor protein. Xanthine oxidase is the last enzyme in the purine nucleotide catabolism pathway of humans [13]. The purine metabolism pathway is different in rodents and humans. Metabolites of purine are excreted through renal as urine and water solubility of uric acid is low so it tends to deposit in the body especially in joints. In animal uricase, an enzyme is present instead of a xanthine oxidase enzyme. The uricase enzyme, also known as uric oxidase, transforms uric acid to allantoin, a water-soluble material that allows uric acid to be excreted more readily through the urine [14]. The basic principle of increasing the source of uric acid, reducing uric acid excretion, and inhibiting uricase is used to establish the rodent model of hyperuricemia. Potassium oxonate is the agent that inhibits the role of uricase and produces hyperuricemia in rats, mice, rabbits, dogs, and pigs [13].

An experimental animal model of hyperuricemia initiated by the administration of potassium oxonate and used to determine the antihyperuricemic effect of test compounds. To summarise, per animal, except those in the normal control group, received 250 mg/kg potassium oxonate dissolved in 0.9% saline solution intraperitoneally 1 h before oral administration of test compounds, once a day, for 3 days of the experiment.1 h after the final drug administration, mice are anesthetized with ketamine and xylazine (100 mg/kg and 20 mg/kg, respectively) to collect blood from the abdominal aorta. The blood is enabled to clot for around 1 h at room temperature before being centrifuged for 10 min at 2500 rpm (revolutions per minute). The serum is isolated and deposited at 20 ◦C before the uric acid assay is performed [15].

2.3. MSU crystals induced gouty arthritis animal model

The formation of MSU crystals is the first critical step in the progression of gout. MSU crystals trigger inflammatory cellular responses. Monosodium urate crystals formation in synovial joints was first discovered in 1961. The presence of MSU crystals is believed to be a requirement for diagnosing gout [16]. Synovial cells, monocytes-macrophages, and neutrophils are stimulated by MSU crystals, which allow them to release cytokines such as IL-1 (interleukin-1), TNF (tumor necrosis factor), and iNOS (inducible nitric oxide synthase) [17]. Many of these molecules are implicated in the production of acute inflammation in gout flares [18]. The oxidative stress caused by iNOS can affect synoviocyte survival by controlling mitochondrial functionality [20] and inhibits chondrocyte proteoglycan synthesis through PGE2(Prostaglandin E2) inhibition of chondrocyte apoptosis [20–22]. Suppression of these pro-inflammatory mediators has been shown to reduce the intensity of gouty arthritis inflammation

- Advantages These agent works correct overproduction or under excretion of uric acid
- It is long term control of gout
- It is also effective against formation of uric acid from purine

2.4. Experimental design

Mice were randomly divided into four group equal group (6 mice per group) .In group 1, the normal group ,each animal received only water as vehicle .Group 2 ,the hyperuricemia group ,PO (150 mg/kg) was administrated intra-peritoneal .In group 3,each animal was first injected intra-peritoneal the same dose of PO 1 hour before administration of test compound and after 3 hour received 0.5 mg/kg coumarin .The group 4,each animal was first injected intra-peritoneal the same dose of PO 1 hour before administration of test compound and after 3 hour received 5 mg/kg Allopurinol. The group 5, each animal was injected intra- peritoneal the same dose of PO and the same dose of coumarine in the same time. The freshly prepared samples were administered to the corresponding groups for 3 days [22]

2.4.1. Advantages

- It is provide researchers with a high level of control
- Experimental research provide conclusion that are specific

2.5. Experimental in-vitro models for gout

In vitro xanthine oxidase inhibitory (XOI) activity Xanthine oxidase is the enzyme that catalyzes the metabolism of hypoxanthine to xanthine and then xanthine to uric acid in the presence of molecular oxygen to yield superoxide anion and hydrogen peroxide [23] that contribute to oxidative damage of living tissues [24]. It has been shown that XO inhibitors may be useful for the treatment of hepatic diseases, gout, which are caused by the generation of uric acid and superoxide anion radical [25]. In this study, the extracts of 9 different plants belonging to different families were investigated as potential XO inhibitors. The selected plants and their XO inhibition assay results are summarized in Table The degree of XO inhibition was evaluated for all extracts at concentration of 100 µg/ml.

2.6. Complement and soluble mediators of inflammation

MSU crystals also promote inflammation by indirect effects on activation of inflamatory cells, as exemplified by MSU crystal-induced activation of the classical pathway of complement in vitro [26]. Th is classical complement activation process does not require immunoglobulin, but is amplified by both C-reactive protein and IgG [27]. MSU crystals also activate the alternative pathway in vitro and, in this process, direct cleavage of C5 to C5a and C5b is triggered by the formation of a stable C5 convertase on the MSU crystal surface [28]. Th e formation of leuco trienes and arachidonic acid metabolites induced by MSU has been extensively studied in the past, and is largely accounted for by the effects of MSU crystals on neutrophils and platelets recruited to the inflammatory site. Pre-treatment of animals with a nonsteroidal anti-inflammatory drug blocked the generation of inflammatory prostaglandins and exudate formation, but did not inhibit local neutrophil accumulation [29].

2.6.1. Advantages

- Allopurinol is medicine used to lower level of uric acid
- Corticosteroid is intraarticular of soluble steroids supress symptoms of acute gout
- Corticosteroid decrease the pain , swelling , redness and inflammation
- Colchicine is best drug to control as acute attack of 1 mg orally followed by 0.25 mg 1-3 hour control attack
- Proben acid also use for second line or adjuent drugs to alkopurinol

3. Conclusion

In conclusion, our rabbit knee model of MSU crystal induced effectively an acute joint inflammatory process, and accurately depicted the early morpho structural changes observed by US during an acute gouty attack. US, SF, and histological analyses provide a working hypothesis: the early presence of deposits and aggregates of MSU crystals is critical in the acute disease phase, challenging the traditional paradigm that this process was limited to the chronic disease stages. The rabbit knee model of MSU crystal-induced arthritis serves as an ideal temporal, spatial, and multimodal platform for further study of the inflammatory process and for detecting structural changes at the joint level during an acute episode of gouty arthritis. Additionally, it affords the opportunity for testing of different pharmacological strategies in the management of gout. Xanthine oxidase activity GCs are undoubtedly amongst the hormones with pleiotropic actions. GCs constitute a class of steroid hormones with important therapeutic applications in disorders involving inflammatory, allergic, and immunologic responses. Despite their efficacy as an anti-inflammatory and immunosuppressive agent, GCs' side effects are not negligible. The knowledge concerning GCs' potential and limitations in clinical practice have permitted the use of safe treatments, when taking into account their potential adverse effects. However, some patients cannot always be free of GC side effects and, among them, glucose

intolerance may prevail. The knowledge about GCs' implications on glucose metabolism is well known, but the molecular mechanisms by which GCs affect such tissues have not been fully elucidated. For instance, how much do the adverse effects depend on genomic or non-genomic GC actions? Although it seems difficult to translate the findings from animals to humans, many aspects are reproducible among them (e.g., GC-induced glucose intolerance), which made these rodent models suitable for mechanistic studies. The cross-talk between peripheral tissues involved in the control of glucose metabolism is becoming even more consistent in the way of scientific knowledge advances, and GCs affect all of these peripheral tissues. In this sense, which tissues are more or less affected? And which tissue is affected by GCs' therapies first? What about these adverse effects in repetitive treatments? These questions merit further investigation. There is also preclinical evidence pointing to the fact that GC-mediated side effects on metabolism are partially dependent on endocannabinoids.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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