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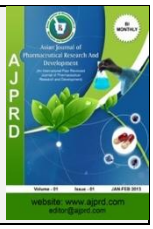

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Review Article

Review: Drug Discovery and Development of Warfarin

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ABSTRACT

Background: The history of the discovery of Warfarin started from the plains of North America in Canada in 1920. Livestock in that area died from bleeding. Warfarin was first used in 1948 as a rodenticide, and in 1954 the US Food and Drug Administration (FDA) approved it for medical use as an anticoagulant.

Purpose: This review article aims to discuss the history of the discovery of warfarin starting from the presence of blood clotting disorders to the point that researchers worked to find drugs that can inhibit blood clotting, namely the anticoagulant group.

Research Methods: The method used is the study of relevant literature which is accessed through online sites such as Google Scholar, Research Gate, Science Direct, Springer Link, and NCBI.

Conclusion: In its development, several trials such as in silico, preclinical, and clinical trials have shown significant results but are always associated with bleeding.

Keywords: Warfarin, Drug Discovery, Anticoagulants.

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INTRODUCTION

Researchers in Indonesia and abroad have discovered a variety of new drugs with different properties. Drug discovery and development is one of the most important activities that contribute to human health and well-being in the world[1].However, low efficacy, poorly targeted delivery, inefficient time consumption, and high costs pose challenges that impact the discovery of new drugs[2]. Drug discovery begins because of a disease or condition of a drug product that is not in accordance with clinical needs[3].

Coagulation (clotting) in the blood is a common disorder that is often causes obstruction of the blood supply[4]. To overcome this, research has been carried out on the prevention and treatment of blood clots. The results showed that there is a class of drugs used to inhibit blood clotting usually called as anticoagulantdrug class. Anticoagulants are a class of drugs used to inhibit blood clotting[5]. The most widely used anticoagulant is warfarin.

Warfarin is an anticoagulant used worldwide for the treatment of blood clots, one of which is the prevention of thromboembolic disease[6].Warfarin belongs to the class of vitamin K antagonists of anticoagulants and has activity by inhibiting the vitamin K epoxide reductase (VKOR) cycle[7]. Some health problems that can cause blood clots include hyperglycemia[8], obesity[9] and hypercholesterolemia[10] and the symptoms that are often caused are swelling, pain, weakness, a warm sensation and changes in skin color to bluish. Therefore, it is necessary to review articles on warfarin which is useful as an anticoagulant drug.

The steps taken in the review of warfarin drug compounds include testing the action of the drug, followed by a determination that explains the process of the compound based on its chemical structure (in silico), then continued with preclinical testing (in vitro and in vivo) and clinical trials to see the reaction of the drug to the human body. When the testing stage has been passed, then the registration stage is the final stage to obtain a distribution permit from

the authorized party in order to strengthen the drug safety statement[3].

METHODS AND DATA COLLECTION

The author chose the study methods relevant to the purpose of the review. Sources of information from international journals were accessed through online sites such as Google Scholar, Research Gate, Science Direct, Springer Link and NCBI. The keywords used to search the journal were Warfarin, Anticoagulants, Toxicity.

DISCUSSION

Overview of Warfarin

The history of the discovery of Warfarin dates back to the plains of North America in the country of Canada in 1920. At that time, healthy cattle suddenly became dying of internal bleeding and the cause was unknown[11].Karl Link and his students started the isolation of the active substance from the coumarin that caused the bleeding and found 3,3'-methylene-bis-[4-hydroxycoumarin], naturally oxidized coumarin was found in hay fed to livestock[12]. The bleeding disease is also known as "sweet clover disease" because livestock eat sweet clover that has been infected with the fungus[13].

In research, it was found that natural coumarin is oxidized in moldy hay, to form a substance better known as dicoumarol[13]. The discovery of the bioactive properties of dicoumarol resulted from the investigation of a mysterious livestock disease in the 1940s and later developed as a drug in the 1950s as warfarin[14]. Warfarin was first used in 1948 as a rodenticide before the US Food and Drug Administration (FDA) approved it for medical use for anticoagulants in 1954. Warfarin became increasingly popular in 1955, at that time US President Dwight D. Eisenhower who had a myocardial infarction was prescribed warfarin as a therapy. The name warfarin comes from the Wisconsin Alumni Research Foundation (WARF) which funded the initial study of coumarins in Karl Paul Link's laboratory and arin is derived from coumarin[12].

Warfarin with the chemical formula $C_{19}H_{16}0_4$ has a structural formula that can be seen in Figure 1 below.

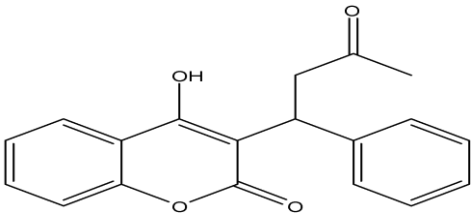

Figure: 1. Structure of warfarin[15].

Warfarin is an anticoagulant drug with a narrow therapeutic index[16] and is widely used in the prevention and treatment of thrombosis, treatment of chronic atrial fibrillation, pulmonary embolism, and dilated cardiomyopathy. Colorless and tasteless warfarin is also used as a poison in animals such as rats and mice in the form of rodenticide products[17].Warfarin is soluble in acetone and dioxane and moderately soluble in solutions of methanol, ethanol, isopropanol and freely soluble in alkaline aqueous solutions (forming water-soluble sodium salts)[18].

WARFARIN SYNTHESIS

Synthesis is a chemical reaction to obtain a chemical product involving one or more reactions. The pathway of warfarin synthesis is done through several processes, the following figure represents the warfarin synthesis pathway

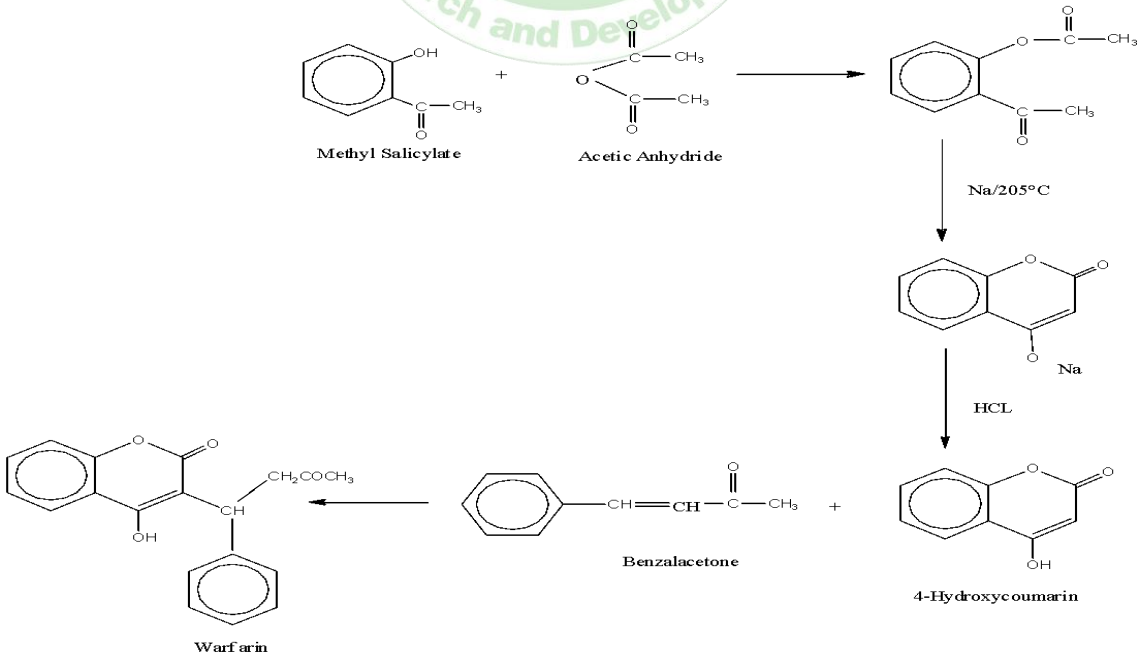

Figure 2: Synthesis of Warfarin

Methyl salicylate, on treatment with acetic anhydride in the presence of a sodium salt, followed by acidification produce 4-hydroxycoumarin.4-hydroxycoumarin that reacts with benzalacetone produce warfarin[19].

PRECLINICAL DEVELOPMENT

Pharmacology

Pharmacological process that occurs in experimental animals after being given warfarin is that warfarin acts in the liver by inhibiting the synthesis of clotting factors that depend on vitamin K, which include factor II (prothrombin), factor VII (proconvertin), factor IX (prothromboplastin beta) and factor X (prothrombinase)[20]. In the gastrointestinal tract, warfarin is rapidly absorbed and has high bioavailability and reaches a maximum blood concentration at 90 minutes after oral administration. Warfarin has a half-life of 36 to 42 hours, circulates bound to plasma proteins (mainly albumin), and accumulates in the liver[21].

In Silico Test

This test is a method that utilizes computational technology and databases to develop further research, as follows:

The results of research using haplotype-based computational methods show that the Cyp2c29 proteinassists in the biotransformation of warfarin in the liver[22].

In Vitro Test

This test is a preclinical test on isolated cell cultures or isolated organs, some of the results are as follows:

1. L929 murine fibroblastic cells were placed in 96-well culture plates and incubated with Dulbecco's Modified Eagle's Medium (DMEM)/ Ham's F12 supplemented with 10% serum. The results showed that dilution with warfarin at 24 and 48 hours had significantly lower cell viability[23].
2. Mtln3 tumor cells were tested using three techniques, namely clonogenic assay, growth curve analysis, and thymidine labeling index. The results showed that warfarin at an average concentration of 1.63µm could inhibit tumor metastasis[24].

In Vivo Test

This test is a preclinical test conducted on experimental animals, some of the research results are as follows:

1. Warfarin at a dose of 0.25 mg/kg-day was given orally to mice with arterial thrombosis. The results of the study using data analysis method using Dunnett's multiple comparison test found that warfarin at this dose produced an effective anticoagulation[25].
2. Male Sprague–Dawley rats (350–400 g) with venous thrombosis treated with warfarin that were prepared in 0.2 M NaHCO3 solution at a stock concentration of 1 mg/mL and administered orally. The results of the study using the statistical software version 6 as the data analysis methods found that the therapeutically relevant dose of warfarin (international normalized ratio; INR 3.0) achieved 65% inhibition of thrombus formation[26].

Toxicity Test

This test is a test to detect the toxic effect of a substance on biological systems, some of the results of the research are below:

1. Warfarin 0.005% given to wild boars causes toxic effects, namely vomiting, bleeding, abnormal breathing, and limping[27].
2. Warfarin sodium (0.3 mmol/kg) administered to anesthetized dogs by intravascular infusion caused a toxic effect, namely a 2-fold increase in ventilation (VE) and oxygen consumption (VO_2)[28].
3. Rats with arterial thrombosis and cancer metastases were given warfarin at a dose of 0.50 mg/kg per day causing a toxic effect, namely death after two weeks of treatment[26].
4. Administration of dietary warfarin on lymphatic metastases from PAIII prostate adenocarcinoma in male Lobund Wistar rats after tumor implantation in the tail. Initial study showed that 0.500 mg/kg dose resulted in death at +14 days[29].
5. It was found that for rats that were given warfarin 2 mg/kgBW per 24 and 30 hours, the hemorrhagic blood volume was 2.5 times greater in W-24 mice and 3.1 times greater in W-30 mice[30].
6. DBA/2 mice receiving diets containing warfarin concentrations (0.03, 0.3, and 3 mg/g) with vitamin K1 at time intervals (1, 4, and 7 weeks) experienced cardiovascular damage[31].
7. Mice (Mus musculus) given 0.025% warfarin for 1 to 21 days caused a toxic effect, namely 5-75% death on the first to fifth day and 5% survival for 21 days[32].

Hepatotoxicity Test

Hepatotoxicity is a condition in which liver cells are damaged by toxic chemicals, such as the results of the warfarin study below:

1. Warfarin was given orally at a dose of 0.8 mg/kg daily for 5 days to a group of rats weighing 250-300 g and fed wheat groats. The results showed that liver damage occurred in rats fed with wheat groats[33].
2. A total of 27 wistar rats were divided into three treatment groups, namely 2 treatment groups were given warfarin doses of LD-50 200 mg/kgBW and LD-100 400 mg/kgBW. The results showed that liver damage occurred, namely bleeding, necrosis and signs of inflammation in Wistar rats[34].

Teratogenicity test

This examination aims to obtain information on fetal abnormalities that occur due to warfarin administration during the formation of fetal organs (organogenesis period), such as:

Pregnant Sprague-Dawley rats were given oral warfarin at a daily dose (100 mg/kg). The results showed that from day 9 to day 20 the administration caused bleeding in the rat fetus[35].

CLINICAL TESTING

Phase I

Phase I is the phase in which the drug is tested on healthy volunteers to see if the traits observed in experimental animals are also seen in humans. Healthy volunteers were given warfarin at doses (0.2 mg and 1 mg daily) for three weeks. Experimental results showed that there was a significant prothrombin time (0.9 s) prolongation after daily administration of 1 mg warfarin, but no significant change in prothrombin time after daily administration of 0.2 mg warfarin[36].

Phase II

Phase II is the phase in which the drug is tested on about hundreds of patients, observing its efficacy in the disease being treated.

1. Warfarin in phase II involved 140 male patients with hypertension with atrial fibrillation. Based on the results of the study, treatment with warfarin did not result in an increase in blood pressure in men with atrial fibrillation[37].
2. Warfarin in phase II involved 115 patients who used warfarin for heart valve replacement and were divided into two groups, namely as group I (INR > 3.5) and group II (INR ≤ 3.5), it was found thatgroup I has higher major bleeding[38].
3. Warfarin in phase II involved 109 patients with antiphospholipid syndrome (APS) and thrombosis who were given high-intensity warfarin (range INR 3.0-4.5).The results obtained showed that recurrent thrombosis occurred in six patients receiving high-intensity warfarin[39].
4. Warfarin in phase II involved 100 patients, namely 64 who underwent Implantable cardioverter defibrillator (ICD) and 36 who underwent a permanent pacemaker (PPM) of which fifty patients were assigned to continue warfarin. The results obtained are that there is a tendency to decrease complications in patients randomized to continue warfarin[40].

Phase III

Phase III involves large groups of patients consisting of about thousands of people and comparing their effects and safety with known comparison drugs.

1. In this phase, 14,264 patients with nonvalvular atrial fibrillation received rivaroxaban (at a daily dose of 20 mg) and dose-adjusted warfarin. Results from the trial showed that in patients with atrial fibrillation, rivaroxaban was not inferior to warfarin for the prevention of stroke or systemic embolism[41].
2. In this phase, a total of 1,216 patients with myocardial infarction received warfarin, 1,206 patients received aspirin (160 mg daily) and 1,208 received aspirin (75 mg daily) in combination with warfarin. The results obtained were death, nonfatal reinfarction or cerebral thromboembolic stroke that occurred in 241 patients who received aspirin, 203 patients who received warfarin and 181 patients who received a combination of warfarin and aspirin. It was concluded that warfarin in combination with aspirin was superior in reducing myocardial infarction but was associated with a higher bleeding risk[42].
3. In this phase, patients undergoing arthroplasty were given fixed dose of oral ximelagatran (36 mg twice daily) 982 and 967 patients were given warfarin for prevention of venous thromboembolism.The results showed venous thromboembolism and mortality in 22.5% of patients treated with ximelagatran and 31.9% treated with warfarin. Thus, it can be concluded that the efficacy of ximelagatran is better than that of warfarin in the prevention of venous thromboembolism[43].
4. In this phase as many as 18,201 patients with atrial Fibrillation (AF) we given apixaban and warfarin, the results showed that the risk of stroke, death, and major bleeding is lower when using apixaban than using warfarin[44].

Phase IV

After the drug is marketed, post-marketing surveillance studies are still being conducted in patients with various conditions, ages, and races. This research was conducted over a long period of time to see the therapeutic value of a drug. In this phase, laboratory data was monitored involving 107 patients and patients were given warfarin 2 mg daily for 2 weeks. The results obtained showed that overall, 60% of patients were within a narrow target range (INR 2.0-3.0) in stable condition, 5 patients with INR >4.0 at each visit after the second week required dose adjustment and 4 patients with INR <1.5 were at steady state[45].

Formulation

Warfarin formulation from several studies:

1. The efficacy of warfarin is higher when the drug is given as a powder than when it is given as a tablet[46].
2. Warfarin injection and tablet formulations showed decreased solubility when combined with acid (HCl). The warfarin solubility was higher when enteral formula was added[47].
3. A mixture of warfarin (1 mg/mL) was prepared in triplicate, namely from Coumadin tablets, Marevan tablets and warfarin sodium clathrate using APF hydroxybenzoic compound solution as preservative. The results showed that all mixtures maintained at least 98% of their original concentrations during the study period of 28 days[48].

Stability Test

This test aims to determine the ability of a warfarin product to survive within the specified limits during storage and use, some research results are as follows:

1. 1 mg/mL sodium warfarin free of preservatives have stable taste in amber glass bottles for at least 30 days stored at 25° C and 45 days stored at 4°C[49].
2. The crushed warfarin tablets were then dissolved in water and syrup and stored in a stable refrigerator for 24 hours[50].

CONCLUSION

Warfarin is used as an anticoagulant drug that works by inhibiting the action of vitamin K in the blood. The use of warfarin drug therapy has limitations such as a narrow therapeutic index where this causes interactions between food and drugs, therefore periodic laboratory monitoring is needed.

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