POSSIBLE ANTITHROMBOTIC PROPERTIES OF ROSIGLITAZONE *(A Peroxisome Proliferator Activated Receptor-γ "PPARγ" Activator)* INDEPENDENT OF ITS GLYCEMIC CONTROL IN RATS

Thesis

Submitted In Fulfillment Of The

Master Degree

In

Pharmacology

By

Eman Abd El-Salam Abd El-Azim Badawy

M.B.B.Ch.

Faculty of medicine – Cairo University

Supervisors

Prof. Dr. Mohsen Samy Selim
Professor of pharmacology
Faculty of Medicine –Cairo University

Assis. Prof. Dr. Magdi Ishak
Ass. Professor of pharmacology
Faculty of Medicine –Cairo University

Assis. Prof. Dr. Hind Mohamed Moataz El Sherbini
Ass. Professor of Clinical Pathology

2008

Acknowledgement

First and foremost thanks are to God the most kind and the most merciful, "who taught man what he did not know".

There is no word capable of expressing my gratitude and appreciation to Prof. Dr. / **Mohsen Sami Selim,** *professor of pharmacology, faculty of Medicine, Cairo University, for resolving the most difficult obstacles I met throughout this work. I shall never forget his excellent guidance, continuous encouragement and most valuable suggestions.*

I would like to express my appreciation and gratitude to Dr. / **Magdi Ishak Attalla,** *assistant professor of pharmacology, faculty of Medicine, Cairo University, for his eminent supervision, valuable guidance and patience that made him meticulously revise this work word by word.*

I would like to express my deep appreciation and gratitude to Dr. / **Hind Moataz El Sherbeiny,** *assistant professor of clinical pathology, faculty of Medicine, Cairo University, for her generous assistance and great effort in helping me to investigate the blood samples that I have taken in this study.*

I will always be grateful to Dr. / **Hesham Mohamed Mahmoud,** *lecturer of pharmacology, faculty of Medicine, Cairo University, for his kind guidance, close supervision and valuable instructions.*

My special appreciation and thanks go to Dr. / **Hossam Hussein,** *assistant professor of pathology, faculty of Medicine, Cairo University, who devoted much of his time and a remarkable effort in helping me to comment on the histopathological examination of the samples collected in this work.*

ABSTRACT

Rosiglitazone is a peroxisome proliferator-activated receptor-γ agonist that has an important role in regulating blood glucose and lipid levels, thus, improving insulin resistance in diabetic patients type II.

The present study was to prove the ability of rosiglitazone to reduce platelet function in rats, in comparison to the reducing effect of aspirin. The results proved a significant reduction in platelet aggregation, without affecting platelet count that was nearly equal to that of aspirin, while the combination of both drugs; rosiglitazone and aspirin, resulted in a more significant reduction in aggregation. Therefore, rosiglitazone would be of beneficial effect in reducing risk of cardiovascular disease.

Key words: PPAR- γ, platelets, rosiglitazone, aspirin.

Contents

List of Tables

List of Figures

Figure number *Page*

INTRODUCTION

Cardiovascular disease (CVD) continues to be the leading cause of mortality and disability in the United States and most European countries **(Sans et al., 1997)**. With the prevalence of sedentary lifestyles, western diet, smoking, and an aging population, the scope of this problem has broadened to epidemic proportions. CVD refers to the diseases that affect the heart and the blood vessels. The American Heart Association refers to the CVDs as those which encompass coronary heart disease, stroke, and hypertension. Atherosclerosis, a chronic inflammatory disease characterized by formation of multiple plaques within the arteries, is known to be the underlying cause of CVD **(Lusis, 2000; Ross, 1999)**. Arterial narrowing and plaque rupture, can lead to thrombus formation, leading to unstable angina and myocardial infarction **(Libby et al., 2002).**

High levels of cholesterol and triglycerides in the blood, hypertension, and tobacco smoke exposure, are all risk factors for atherosclerosis, whereas the development and progression of CVD. Also type 2 diabetes mellitus (T2DM) is another risk factor for developing CVD, the leading cause of diabetes-related death **(Nathan, 1993)**.

In conjugation with the insulin resistance and hyperlipidaemia, that characterizes T2DM, diabetics are often times hypertensive, overweight, and physically inactive **(Vivian, 2006)**. The propensity to acute thrombosis in diabetics has been variably associated with enhanced platelet reactivity, elevated levels of circulating procoagulant factors, and diminished fibrinolytic capacity **(Ferroni et al., 2004)**.

The current goal in the treatment of diabetes is not only to enhance the glycemic control, but also to improve the associated cardiovascular risk factors **(Sudhir et al., 2006).** Great effort has been expended towards developing new methods of treating atherosclerosis, with considerable success, such as coenzyme A reductase inhibitors (statins), angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and aspirin, which have been found to provide significant clinical benefits **(Laufs et al., 1998)**. Activation of the nuclear receptor, peroxisome proliferator-activated receptor (PPAR) may be an appealing candidate for modulating transcription to achieve clinical benefits **(Wilson et al., 2000).**

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, belonging to the nuclear receptor superfamily, which includes; the steroid and thyroid hormone receptors **(Marx et al., 2004).** The PPAR family comprises three members; α, γ and β/δ **(Han et al., 2005).** The term PPAR is derived from observations that certain ligands could induce the growth or proliferation of peroxisomes; which are subcellular organelles in rodents **(Issemann and Green, 1991).**

Upon activation, PPARs regulate gene function; which may repress or activate gene expression, and also may interfere with other signaling pathways such as protein kinase-C (PKC), in a mechanism termed transrepression **(Blanquart et al., 2004).**

PPAR is virtually present in all vascular and atheroma-associated cells seen in the vessel wall, including endothelial cells, monocytes, T-lymphocytes and human atherosclerotic lesions**(Bishop-Bailey, 2000)**.

Work by many laboratories has established that PPAR-regulated target genes are relevant to atherosclerosis and that PPAR agonists may limit atherosclerotic changes through transcriptional effects and providing anti-inflammatory actions **(Bishop-Bailey, 2000).**

Akbiyik et al., (2004), reported that PPAR-γ is also present in platelets. This gives us a clue that PPAR-γ ligand treatment may prove to be useful for dampening unwanted platelet activation especially in cardiovascular diseases **(Jamie et al., 2007)**.

The thiazolidinediones, anti-diabetic insulin sensitizers including; pioglitazone and rosiglitazone, activate peroxisome proliferator-activated receptor- gamma (PPAR-γ), regulating glucose and lipoprotein metabolism, inflammation, reactive oxygen species generation, cellular proliferation and differentiation, and the coagulation cascade **(Kunhiraman et al., 2005)**. However, it should be stressed that PPAR-γ activators may induce decompensated heart failure in some diabetic patients, possibly due to salt and water retention **(Rhian et al., 2005)**

The present study focuses on the role of PPARs on platelet function, beyond their regulatory effects on glucose and lipid metabolism, and discusses the potential therapeutic role of these ligand-activated transcription factors in the management of cardiovascular diseases.

AIM OF THE WORK

As the development of hyperlipidaemia is considered as one of the important risk factors in causing hypercoagulability and thrombus formation, the present work was to demonstrate the effect of *rosiglitazone*; a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist and an insulin sensitizer, on the platelet functions, and its possible pharmacodynamic interaction with *aspirin;* as an example of antiplatelet drugs, in rats with normal platelet functions and in others with experimentally induced hyperlipidaemia and exaggerated platelet functions.

The parameters investigated included; serum levels of cholesterol, triglycerides and glucose. In addition, platelet count and aggregation were measured, as well as bleeding time test was done.

Histopathological examination of the thoracic aorta was done in the different rat groups to detect any antithrombotic or atheroprotective effect of *rosiglitazone*.

HEMOSTASIS AND BLOOD COAGULATION

Hemostasis is the spontaneous arrest of bleeding from damaged blood vessel**.** Whenever a vessel is severed or ruptured, hemostasis is achieved by several mechanisms: Vascular constriction, formation of a platelet plug, formation of a blood clot as a result of blood coagulation, and eventual growth of fibrous tissue into the blood clot to close the hole in the vessel permanently **(Rosenberg et al., 1999).**

Immediately after a blood vessel has been cut or ruptured, the smooth muscle in the wall contracts. However, vasoconstriction probably results from local myogenic contraction of the blood vessels, initiated by direct damage to the vascular wall. While for the smaller vessels, the platelets are responsible for much of the vasoconstriction by releasing a vasoconstrictor substance, *thromboxane* A_2. If the cut in the blood vessel is very small, it is often sealed by a platelet plug, rather than by a blood clot **(Roberts et al., 2004)**

Platelets, or thrombocytes, are minute discs 1 to 4 micrometers in diameter. They are formed in the bone marrow from megakaryocytes, which are extremely large hematopoietic series in the marrow. The normal concentration of platelets in the blood is between 150.000 and 300.000 per microliter **(Brass, 2003).**

Platelets have many functional characteristics of whole cells, even though they do not have nuclei and cannot reproduce. In their cytoplasm are such active factors as: a*ctin* and *myosin* molecules, also *thrombosthenin that* can cause the platelets to contract, e*ndoplasmic reticulum* and the G*olgi apparatus* that store large quantities of calcium

ions; *mitochondria and enzyme systems* that are capable of forming adenosine triphosphate (ATP) and adenosine diphosphate (ADP) ; enzyme systems that synthesize *prostaglandins*, which are local hormones that cause many vascular and other local tissue reactions; an important protein called *fibrin-stabilizing factor* and a gr*owth factor* that causes vascular endothelial cells, vascular smooth muscle cells, and fibroblasts to multiply and grow, thus causing cellular growth that eventually helps repair damaged vascular walls **(Solum, 1999**).

The cell membrane of the platelets is also important. On its surface is a coat of *glycoproteins* that repulses adherence to normal endothelium, yet causes adherence to injured areas of the vessel wall, especially to injured endothelial cells, and even more, adherence to any exposed collagen from deep within the vessel wall. In addition, the platelet membrane contains large amounts of *phospholipids* that activate multiple stages in the blood-clotting process **(Brass, 2003).**

Thus, the platelet is an active structure. It has a half-life in the blood of 7 to 10 days. Then, it is eliminated from the circulation mainly by the tissue macrophage system. More than one half of the platelets are removed by macrophages in the spleen, where the blood passes through a network of tight trabeculae **(Geddis et al., 2004).**

The platelet plug formation begins when platelets come in contact with a damaged vascular surface, especially with collagen fibers in the vascular wall, the platelets themselves immediately change their own characteristics. They begin to swell, they assume irregular forms with numerous irradiating pseudopods protruding from their surfaces,

their contractile proteins contract forcefully and cause the release of granules that contain multiple active factors, they become sticky so that they adhere to collagen in the tissues *(platelet adhesion).*They secrete large quantities of ADP, and their enzymes form *thromboxane* A_2. The ADP and thromboxane in turn act on nearby platelets to activate them as well, and the stickiness of these additional platelets causes them to adhere to the original activated platelets (*platelet aggregation)* **(Roberts et al., 2004**).

Therefore at the site of any opening in a blood vessel wall, the damaged vascular wall activates successively increasing numbers of platelets, that themselves attract more and more additional platelets, thus forming a *platelet plug.* This is at first a loose plug, but it is usually successful in blocking blood loss if the vascular opening is small. Then, during the subsequent process of blood coagulation, *fibrin threads* form. These attach tightly to the platelets, thus constructing an unyielding plug **(Tsai, 2003 a)**.

Within 3 to 6 minutes after rupture of a vessel, if the vessel opening is not too large, the entire opening or broken end of the vessel is filled with a clot, in which, platelets play an important role in the conversion of prothrombin to thrombin. After 20 minutes to an hour the clot retracts, which closes the vessel still further; in which, platelets also play an important role in this clot retraction. Thus, the edges of the broken blood vessel are pulled together, contributing still further, to the ultimate state of hemostasis **(Tsai, 2003 b).**