Plasma Endothelin-1 in Pulmonary Hypertension

Thesis
Submitted for Partial Fulfillment of
MD of Clinical Pathology

By
Perihan Hamdi Tawffik

Supervised by
Prof Dr Mahmoud Sabry Sallam
Professor of Clinical Pathology
Faculty of Medicine
Ain Shams University

Dr. Nadia Aly Abd El Sattar
Assisstant Professor of Clinical Pathology
Faculty of Medicine
Ain Shams University

Dr. Dalia Helmy Farag
Lecturer of Clinical Pathology
Faculty of Medicine
Ain Shams University

Dr. Mervat Abou El Maaty Nabih
Lecturer of Cardiology
Faculty of Medicine
Ain Shams University

Faculty of Medicine
Ain Shams University
1995

Acknowledgement

I would like to express my deepest gratitude to Professor Dr. **Mahmoud Ibry Sallam**, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for his valuable support, guidance and encouragement, and for giving me the opportunity to work under his supervision.

I also offer my sincere thanks to Dr. **Nadia Aly Abd El Sattar**, Assistant Professor of Clinical Pathology, Ain Shams University, from whom I've learned a lot, for her generous co-operation, sincere guidance and for her help in the delivery of this work.

I am also very grateful to Dr. **Dalia Helmy Farag**, Lecturer of Clinical Pathology, Ain Shams University, for her continuous supervision, stimulating suggestions, constant advice throughout this work.

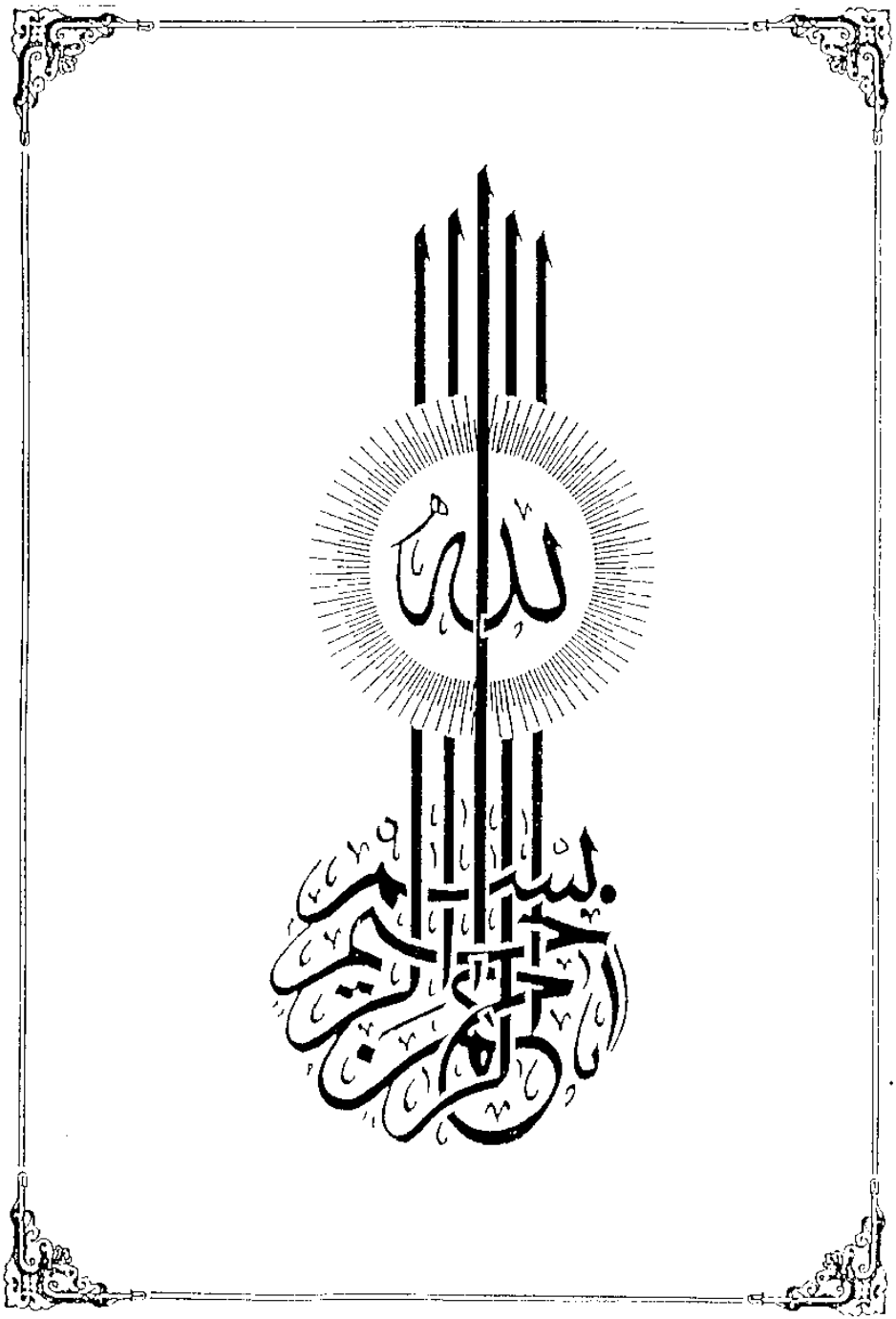
I would like to thank Dr **Mervat Abou El Maaty Nabih**, Lecturer of Cardiology, Ain Shams University, for her generous help, and valuable remarks that lead to this final outcome.

I would like to express my gratitude to all members of the chemistry department of Clinical Pathgology, Ain Shams Specialized Hospital and My Special thanks go to all my colleagues in the clinical Pathology department for their impressive help, affectionate support, and advice.

I am obliged to my husband for his devotion, unlimited patience, willing support and understanding.

Last but not least I **am wholeheartedly indebted to my parents**, for their enthusiastic encouragement, perpetual support, and I pray God to reward them in return.

Perihan Hamdi



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List of abbreviations

(L)AP: Left atrial pressure
5HT: Serotonin
Ache: Acetyl choline esterase
ANP: Atrial natriuretic peptide
AVP: Arginine vasopressin
BNP: Brain natriuretic peptide
CCD: Cortical collecting duct
DBP: Diastolic blood pressure
EDHF: Endothelin derived hyperpolarizing factor
EDRF: Endothelium derived relaxing factor
ET-1: Endothelin-1
ETA: Arterial endothelin
ETV: Venous endothelin
GFR: Glomerular filtration rate
HR: Heart rate
IMCD: Inner medullary collecting duct
ir ET-1: Immunoreactive ET-1
KIU: Kallikrein inhibitory unit
LTB4: Leukotriene B4
LTC4: Leukotriene C4
MPP: Mean pulmonary pressure
NO: Nitrous oxide
NOS: Nitrous oxide synthase
O2-: Superoxide anions
OMCD: Outer medullary collecting duct
PADP: Pulmonary artery diastolic pressure
PASP: Pulmonary artery systolic pressure
PDP: Pulmonary diastolic pressure
PGI2: Prostacyclin
PPH: Primary pulmonary hypertension
PSP: Pulmonary systolic pressure
1ry P+: Primary pulmonary hypertension
2ry P+: Secondary pulmonary hypertension
PVR: Pulmonary vascular resistance
PWP: Pulmonary wedge pressure
rhSOD: Recombinant human superoxide dismutase
SBP: Systolic blood pressure

TNF: Tumour necrosis factor
TXA2: Thromboxane

Introduction and aim of the work

Endothelin is a 21 amino acid peptide synthesized by endothelial cells as a prepro-endothelin (≈ 200 amino acids), which is cleaved to an intermediate form of proendothelin or big endothelin (≈ 39 amino acids). Subsequently, an endothelin converting enzyme cleaves the big endothelin between tryptophan 21 and valine 22 to form the mature 21 amino acid endothelin ***(Yanagisawa et al., 1988)***.

Endothelin exists in three different isoforms: Endothelin-1 (ET-1), endothelin 2 (ET-2) and endothelin 3 (ET-3) ***(Itoh et al., 1988)***. Endothelin receptor is widely distributed not only in the vascular system but also in such tissues as kidneys, lungs, adrenal glands and neurons ***(Koseki et al., 1989)***.

Endothelin-1 induces vasoconstriction in a variety of vascular beds, possibly by directly or indirectly modulating vascular smooth muscle dihydropyridine-sensitive calcium channels responsible for influx of exterior calcium ions without depolarization of the membrane, or by activating other pathways of trans-membrane signaling ***(Simonson et al., 1989)***.

Endothelin-1 is quickly eliminated by the lungs and kidneys. In fact, these two tissues have a lot of high affinity binding sites for endothelin-1. These two tissues also exhibited high neutral endopeptidase activity, which may play an important role in degradation of endothelin ***(Vijeyaraghavan et al., 1990)***.

At an early stage of primary pulmonary hypertension, elevated pulmonary vascular resistance has been shown to be amenable to vasodilators, indicating a significant component of vasoconstriction. In addition, migration of fibroblasts into the intima of pulmonary arteries has been reported early in the course of primary pulmonary hypertension ***(Heath et al., 1987)***. Although there is no reliable biochemical marker for primary pulmonary hypertension, endothelin-1 may be implicated in the pathogenesis as it could promote both these processes by virtue of its potent vasoconstrictor effects and smooth muscle proliferative actions ***(Dubin et al., 1989)***.

Therefore, pulmonary production of endothelin-1 might contribute to the progressive vascular narrowing in primary pulmonary hypertension and consequent increased pulmonary vascular resistance ***(Steizner et al., 1990)***.

Measurement of arterial and venous endothelin-1 may identify a subgroup of patients with pulmonary hypertension who share a common basic abnormality.

Direct assessment of the functional importance of endothelin-1 to the manifestations of pulmonary hypertension may lead to the development of antagonists to endothelin-1 or inhibitors of its production.

Aim of the work

The aim of this work was to study the level of endothelin-1 in arterial and venous blood in patients with primary and secondary pulmonary hypertension, and to evaluate its role in the pathogenesis of this condition.

Material and Methods:

This study included 23 patients with pulmonary hypertension (primary and secondary) as well as 10 healthy controls.

The subjects in this study were subjected to:-

1- Full history and clinical examination.
2- Cardiac catheterization for patients only.
3- Routine laboratory investigations including hepatic, renal function tests and electrolytes.
4- Plasma endothelin-1 determination by radioimmunoassay.

Review of Literature

Endothelin

I-Endothelium-derived vasoactive factors:

To maintain the patency of the blood vessels, the endothelial cells synthesize many vasoactive substances such as prostacyclin, endothelium-derived relaxing factor and endothelin-1 ***(Vane et al., 1990)***.

A- Prostacyclin:

Prostacyclin (PGI2) was discovered by ***Moncada et al. 1976*** as a major member of prostaglandins produced by endothelial cells. Mechanical or chemical perturbation of cell membranes results in the formation and release of prostacyclin without storage in the cells. Prostacyclin generation is stimulated by pulsatile pressure, a number of endogenous mediators, and some drugs. Endogenous chemical stimulants include substances derived from plasma, such as bradykinin and thrombin, and those liberated from stimulated platelets such as serotonin, platelet derived growth factor, interleukin-1 and adenine nucleotides ***(Forsberg et al., 1987)***. Prostacyclin synthesis is initiated by the enzyme phospholipase A2 which liberates arachidonic acid from membrane phospholipids. The enzyme cyclooxygenase converts arachidonic acid into prostaglandin endoperoxides. Prostacyclin synthase subsequently forms prostacyclin

from the endoperoxide prostaglandin H2 ***(Vane et al., 1990)***.

Physiologically, prostacyclin is a local hormone rather than a circulating one. The release of prostacyclin by endothelial cells affects the local environment on the abluminal side of the vessel where it causes relaxation of the underlying smooth muscle. In the lumen it prevents platelets and perhaps other blood cells from clumping onto the endothelium ***(Vane et al., 1990)***.

In the lung pulses of prostacyclin reverse sustained vasoconstriction initiated by prostaglandin F2α or thromboxane A2. It has been suggested that endothelium responds to vasoconstriction by release of PGI2 thus modulating vasoconstriction ***(Higgenbottam, 1994)***.

B-Endothelium-derived relaxing factor (EDRF):

Stimulation of muscarinic receptors on endothelial cells trigger the release of EDRF which causes relaxation of the underlying smooth muscle ***(Furchgott, 1983)***.

Ignarro et al. (1988) suggested that EDRF might be nitrous oxide NO or some closely related unstable radicle species. Similarly, ***Furchgott (1988)*** proposed that EDRF is the free radical (NO). The assumption was based on

the fact that, similar to EDRF, (NO) is labile as indicated by the transiency of the relaxation that it induces, its relaxing effect is blocked by hemoglobin and by generation of superoxide anions (O_2^-), and it is markedly potentiated by superoxide dismutase (a scavenger of superoxide anions) ***(Furchgott, 1989)***.

Is there more than one EDRF?

De Mey et al. (1982) first made the suggestion that certain endothelium-dependent dilators in the canine femoral artery may release different relaxing factors when they found that certain inhibitors could block the relaxation elicited by some of the relaxants but not by others. The most compelling evidence for the existence of two relaxing factors comes from electrophysiological studies. Acetylcholine causes endothelium-dependent hyperpolarization in various arteries due to a diffusable factor (endothelium-derived hyperpolarizing factor EDHF) that is distinct from (NO) ***(Higgenbottam, 1994)***.

The elaboration of (NO) requires the substrate molecules of L-arginine and dioxygen. An enzyme nitrous oxide synthase (NOS) is responsible for this step. A number of isoforms of (NOS) have now been identified. The genes for endothelium and brain NOS have considerable homology but differ from macrophage NOS. In endothelium, the major isoform of NOS is a constitutive or continuously expressed enzyme. It has a heme associated

moiety and requires NADPH, tetrahydrobiopterin, and calcium/calmodulin as cofactors. Activation of the enzyme involves a rise in cytosolic calcium concentration ***(Higgenbottam, 1994)***.

EDRF activated guanylate cyclase of vascular smooth muscle and the resulting increase in cyclic GMP has a causal role in the relaxation of the muscle ***(Ignarro et al., 1984)***.

Hemoglobin is a rapidly acting inhibitor of endothelial relaxation ***(Martin et al., 1985)***. It inhibits the increase in cyclic GMP associated with such relaxation. Hemoglobin also inhibits endothelium-dependent relaxation by reacting with EDRF before the latter gains access to the guanylate cyclase of the smooth muscle cells ***(Palmer et al., 1987)***. (NO) has a particularly high affinity for heme, some 280 times the affinity oxygen shows to heme. Binding of nitrous oxide to the heme moiety of NOS activates guanylate cyclase leading to increase intracellular cyclic GMP ***(Higgenbottam, 1994)***.

There is a flow dependent release of EDRF that is related to shear stress on the luminal surface of the endothelial cells ***(Rubanyi et al., 1986)***. ADP and thrombin were the first blood constituents shown to trigger endothelium-dependent responses ***(De-Mey et al.,***