Effect OF Sildenafil Citrate on Peripheral Natural Killer Cells in women with Recurrent Pregnancy Loss (A Clinical Trial)

Thesis

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BY

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List of Abbreviations

Abb.	Meaning
AICD	Activation-induced cell death
ART	Assissted reproductive technology
BMI	Body mass index
cGMP	Cyclic guanosine monophosphate
CRP	Complement regulatory proteins
DAF	Decay-accelerating factor
DES	Diethylstilbestrol
DM	Diabetes mellitus
dNK	Decidual naturel killer
FOXP3	Forkhead box p3
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GTP	Guanosine triphosphate
hCG	Human growth hormone
HIV	Human immunodeficiency virus
HLA	Humen leucocyte Antigen
IDO	Indolamine 2,3 dioxygenase
IFN-γ	Interferon gamma
IL	Interleukin
IUGR	Intrauterine growth retardation
IVF	Invitro fertilization
IVIG	Intravenous immunoglobulin
KAR	Killer activating receptors
KIR	Killer immunoglobulin-like receptors
LH	Luteinizing hormone

List of Abbreviations

Abb.	Meaning
MAC	Membrane attack comlex
MCP	Membrane cofactor protein
M-CSF	Macrophage-colony stimulating factor
MHC	Major histocompetability complex
MTHFR	Methylene tetrahydrofolate reductase
NK	Naturel Killer
NO	Nitric oxide
OPN	Osteopontin
PBMCs	Peripheral blood mononuclear cells
PCOS	Polycyctic ovarian syndrome
PCR	Polymerase chain reaction
PDL1	Programed death ligand 1
PMA	Phorbol 12-myristate 13-acetate
RPL	Recurrent pregnancy loss
RSA	Recurrent spontaneous abortion
TGF-α	Transforming growth factor α
Th1	T helper type 1
Th2	T helper type 2
Th3	T helper type 3
TNF	Tumer necrosis factor
uNK	Uterine naturel killer
βhCG	Beta human chorionic gonadotropin

Abstract

Back ground Researchers' defined recurrent miscarriage as three or more pregnancy losses, the available research data denotes that the risk of abortion is 30% and 33% after two miscarriages and three miscarriages, consecutively. Sildenafil citrate (a type 5-specific phosphodiesterase inhibitor, augments the vasodilatory effects of NO. Vagina sildenafil augments the vasodilatory effects of NO. NK cytotoxicity has been reported to be predictive of subsequent pregnancy loss in women who had recurrent spontaneous abortions (RSA).

Aim Is to investigate whether sildenafil citrate affect peripheral natural killer cells successful in women with history of recurrent pregnancy loss.

Methodology An interventional research study, with one arm clinical trial: one group of study subjects were administered the sildenafil citrate without comparative placebo effect, conducted at the Obstetrics and Gynecology Outpatient clinic at Ain Shams University hospital throughout 2017-2018. The sample size consists of 77 women within the reproductive age (from 18 to 35 years old) having a history of recurrent pregnancy loss

Results there was a statistical significant reduction in peripheral blood natural killer cell activity after having vaginal sildenafil .(p value <0.001), there was no statistical significant correlation between peripheral blood natural killer cell activity (%) and other demographic characteristics(age, BMI, parity, miscarriages, time from last miscarriage, p values before =0.204, 0.929, 0.416, 0.302,0.627, consecutively, p values after intervention =0.133,0.709,0.380,0.410,0.559,consecutively,p values of reduction =0.794, 0.458, 0.687, 0.181, 0.944,consecutively)

Conclusions

The current research reveals and displays that vaginal sildenafil citrate is a potentially promising agent in managing cases with recurrent miscarriage and it reduces the natural killer cell activity levels.

Kewords: Osteopontin, Polycyctic ovarian syndrome, T helper type 2, Beta human chorionic gonadotropin

INTRODUCTION

Recurrent pregnancy loss (RPL) refers to the consecutive loss of three or more clinically recognized pregnancies prior to the 20th week of gestation (excluding ectopic, molar and biochemical pregnancies). RPL is classified into two categories: primary RPL, which consists of repeated miscarriages in which a pregnancy has never been carried to viability and secondary RPL in which a live birth has occurred at some time. Secondary RPL confers a better prognosis than primary. About 10–15% of all clinically recognized pregnancies end in miscarriage. Approximately2% of women experience two and 0.4–1% of women experience three consecutive losses. At less than 6 weeks gestation the risk of miscarriage ranges from 22 to 57%, it declines to 15% at 6–10 weeks and 2–3% after 10 weeks of gestation ***(Christiansen, 2008)*.**

The etiology of RPL is multi factorial, it includes uterine abnormalities (15-27%), maternal diseases (20-50%) including reproductive tract infection ,parental chromosomal abnormalities (5%), drug and toxin exposure, endocrine abnormalities like luteal phase defect ,hyperprolactinemia, diabetes mellitus, or immunological factors like anti-phospholipid syndrome, thrombophilia, antithyroid antibodies ***(Jaslow, 2010)***.

Despite extensive endocrine, chromosomal, immunologic and an anatomical evaluation, 30-40% of cases of RPL remain unexplained ***(Warren, 2008).***

Natural killer cells and miscarriage: NK cells comprise about 10–15% of peripheral blood lymphocytes. Two distinct subsets of human NK cells are possible, depending on the cell surface density of the CD56 molecule. Approximately 90% of peripheral blood human NK cells are CD56dim and express high levels of FcγIII (CD16) as well as perforin ***(Ntrivalas, 2005).***

In contrast, a minority (approximately 10%) of NK cells are CD56bright and CD16dim. These CD16dim cells are the primary source of NK cell derived cytokines and thought to be an important regulatory subset ***(Thum, 2004).***

In summary, despite a few contradictory studies a significant amount of data points to increased peripheral or local NK cell activity contributing towards the pathogenesis of recurrent miscarriage. An abnormal increase in peripheral blood NK cell parameters (either in NK cell absolute values or in proportion (%) prior to conception or during early pregnancy) is associated with recurrent miscarriage and infertility with multiple implantation failures ***(Emmer and Nelen, 2000).***

Sildenafil citrate (VIAGRA), a type 5-specific phosphodiesterase inhibitor, augments the vasodilatory effects

of NO. Vagina sildenafil augments the vasodilatory effects of NO. Vaginal sildenafil improves uterine artery blood flow and sonographic endometrial thickness. While improving uterine blood flow in the proliferative phase, NO may have detrimental effects at the level of the endometrium during the implantation window. The NO- mediated release of cytokines such as tumor necrosis factor- from activated natural killer cells has been implicated as a cause of implantation failure ***(Barroso, 1998).***

NK cytotoxicity has been reported to be predictive of subsequent pregnancy loss in women who had recurrent spontaneous abortions (RSA). Therefore, the purpose of the study was to establish the effect of sildenafil on NK cell activity in women with a history of RSA (after natural or IVF conception).

AIM OF THE WORK

The aim of this study is to prove whether sildenafil citrate affect peripheral natural killer cells successful in women with history of recurrent pregnancy loss.

Chapter 1

SILDENAFIL CITRATE

Sildenafil citrate, is an agent sold in market as the brand name **Viagra** implemented in practice to manage erectile disorders and pulmonary arterial hypertension. Its efficiency for managing sexual disorders in females still requires research efforts to elucidate its impact ***(Taylor et al., 2013).***

Common medical side effects involve headaches and heartburn, and skin flushing and its used with caution in cases with cardiovascular diseases. Rare but serious issues involve prolonged erections, which could damage the penis, and sudden-onset of hearing loss. Sildenafil is contraindicated in cases taking nitrates e.g nitroglycerin (glycerin trinitrate), since this may cause a severe and possibly fatal fall in blood pressure ***(Wang, et al., 2014; Laties et al., 2009).***

Sildenafil citrate is a phosphodiesterase-5 (PDE-5) inhibitor. It acts by preventing the degradation of the second messenger cyclic guanosine 3 ′,5 ′-monophosphate by the enzyme PDE-5. This causes an increased nitric oxide synthesis and subsequent vascular smooth muscle relaxation and an increase in vasodilation. Due to its preferential vasodilatory effects on pelvic vasculature, sildenafil citrate is now a well-established agent for erectile disorders in males and this stays its most frequent indication for use **(*Linnemann et al., 2016).***

Sildenafil originally innovated for management of various cardiovascular disorders. Since 1998, sildenafil have been a common agent for erectile disorders **(*Duarte et al., 2013).***

Medical uses

Male Sexual dysfunction

The primary indication of sildenafil is management and improvement of erectile disorders in which there failure to maintain an acceptable erectile function to complete the physiological process of normal sexual intercourse. Its is considered as a standard management protocol for cases suffering erectile disorders, involving men having DM ***(El-Far et al., 2014; Von Dadelszen et al., 2011).***

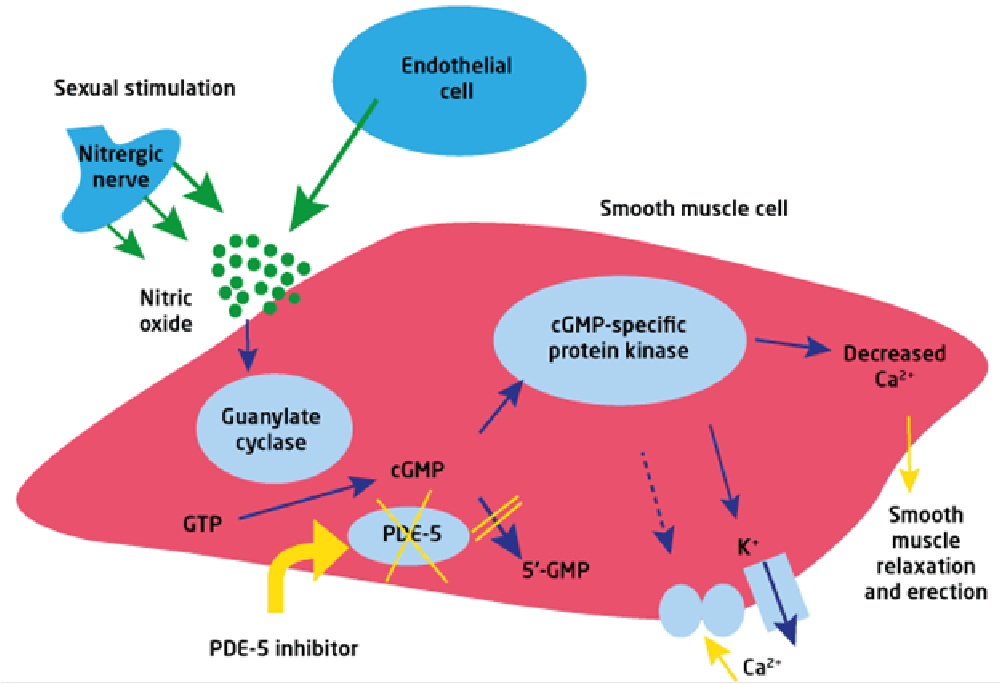

Figure (1): Mechanism of action of sildenafil citrate

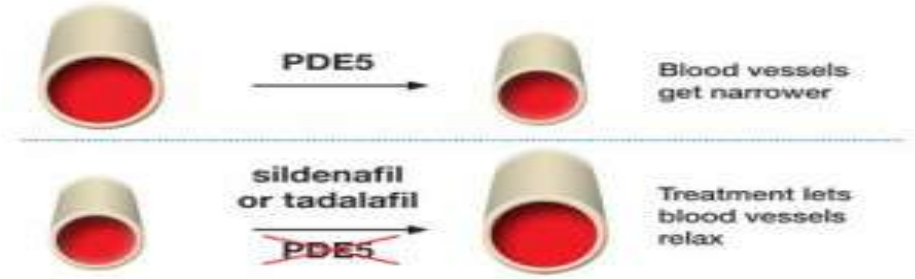

Figure (2): Phosphodiesterase inhibitor impact on blood vessels

Antidepressant-linked sexual functional disorders

Uncertain research evidence denotes that sildenafil could aid males experiencing antidepressant-correlated erectile disorders ***(Duarte et al., 2013).***