ORIGINAL ARTICLE

Vasorelaxant Effect of Metformin in Human Umbilical Artery In Vitro

Sidrah Ikram[1], Naseem Saud Ahmad[2], Waqar Ahmed Siddique[3], Mahwash Malik[4], Ayesha Zahoor[5], Ayesha Iqbal[6]

ABSTRACT

Objective: To evaluate the effect of metformin on the vasomotion of human umbilical artery (HUA) in vitro.

Study Design: Experimental study (Ex-vivo Pharmacodynamic study).

Place and Duration of the Study: Department of Pharmacology of Azra Naheed Medical College - Superior University, and Combined Military Hospital, Lahore from Nov 13, 2024 to Apr 30, 2025.

Materials and Methods: A total of 24 HUA samples were obtained from 24 different donors to ensure biological independence, and were divided into 4 groups with a sample size of 6 in each. Fresh HUA tissues with intact-endothelium, and without endothelium were suspended in Kreb's solution, pH 7.4. A force transducer (MLT0420) connected with an amplifier (AD Instruments Bridge Amps) was used to record isometric tension. The vasorelaxant responses of HUA to metformin (1-20 µM/l) were noted. In another setup the responses of metformin in KCl (60µM) precontracted HUA rings were recorded. The response of KCl + PGE_2 were taken as standard.

Results: The effect of metformin (1-20 µM/l) on HUA with intact endothelium showed significant relaxation ($p<0.0001$) IC_{50} was 7.031. The HUA ring with intact endothelium presented with better relaxation than rings without endothelium. The IC_{50} were 7.031 and 0.6821 respectively. In case of KCl (60µM) pretreated HUA rings, the findings of metformin and PGE_2 were comparable showing IC_{50} 5.739 and 6.248 respectively.

Conclusion: Metformin with KCl exhibited the strongest vasorelaxation followed by PGE_2. Metformin alone without endothelium had comparatively weaker relaxation. These findings highlight the significant efficacy of metformin under vasoconstrictive stress. It has potential to protect fetoplacental circulation during GDM pregnancies.

Key Words*: Endothelium, Human Umbilical Artery, Metformin, PGE2, Vasorelaxation, Verapamil.*

Introduction

Diabetes mellitus is primarily classified into two distinct etiologies: Type 1 (T1DM), characterized by an autoimmune-mediated destruction of pancreatic beta-cells leading to absolute insulin deficiency, and Type 2 (T2DM), a complex metabolic disorder involving peripheral insulin resistance and progressive secretory dysfunction.[1] Gestational diabetes mellitus (GDM) is the form of diabetes in which there is abnormal or uncontrolled rise in blood sugar levels during pregnancy, compromising the health of both fetus and mother. 2 In the past, diabetes was diagnosed in adulthood but it is now increasingly observed in children as well.[3]

During pregnancy, placental hormones induce insulin resistance that is most pronounced in the last trimester. [4] Glucose Tolerance Test (OGTT) with glycated hemoglobin (HbA1c) are used as diagnostic tests. The normal level of HbA1c is < 5.7%, whereas the value ≥ 6.5% is indicative of GDM. [5]

The global prevalence of GDM is estimated at approximately 14% according to a World Health Organization (WHO) report. The prevalence of GDM in Pakistan is 16.7% and, the Middle East countries have about 27.6%.[6,7] Family history, advanced maternal age, low socioeconomic status, illiteracy, multiparity and obesity are the risk factors of GDM. [8]

The growing fetus has a high energy demands and utilizes glucose at an average rate of 6 mg/kg/min, which is nearly three times the adult requirement of 2 mg/kg/min. The increased fetal requirement is regulated by transplacental transfer from the mother to the fetus during normal pregnancy.

[1,2,4,5,6]*Department of Pharmacology*
Faculty of Medical & Dental Sciences,
The Superior University, Lahore, Pakistan
[3]*Department of Pharmacology,*
CMH Medical College & Institute of Dentistry, Lahore
Correspondence:
Prof. Naseem Saud Ahmad
Department of Pharmacology
Faculty of Medical & Dental Sciences,
The Superior University, Lahore, Pakistan
E-mail: naseem.saud@superior.edu.pk
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Consequently, the fetal blood glucose levels are directly proportional to maternal glycemic levels. [9]

GDM can lead to serious complications such as obesity, increased rate of pre-eclampsia, pregnancy induced hypertension (PIH), antepartum hemorrhage, cesarean births in mothers as well as results in newborns, such as large for gestational age, macrosomic babies and hypoglycemia in neonates. Infants of mothers with GDM are much prone to have severe chemical imbalances, such as low serum calcium and magnesium levels.[10]

In cases where lifestyle modifications, including medical nutrition therapy and physical activity, fail to achieve glycemic targets, insulin therapy is indicated to optimize maternal-fetal outcomes and mitigate gestational complications.[11,12]

Metformin, a biguanide is used for T2DM. Its main mechanism is to activate AMP activated protein kinases, which in turn reduces hepatic glucose production. Metformin is preferred over insulin in GDM because it passes through placental barrier via organic cation transporters.[13] In addition to antidiabetic effects, metformin has well established antioxidant role in preclinical animal models. [14,15] However, a significant knowledge gap persists regarding the translatability of these pleiotropic effects in human clinical cohorts. This study was designed to bridge this gap by evaluating the vasoactive effect of metformin on human umbilical artery (HUA). The aim of this study was to compare the vasodilator effect of metformin against potassium chloride (KCl) induced vasoconstriction whereas the vasorelaxant effect was compared against standard vasodilator on HUA of pregnant patients.

Materials and Methods

Ex-vivo experimental study was carried out in the Department of Pharmacology of Azra Naheed Medical College - Superior University, CMH Medical College and Institute of Dentistry, Lahore from Nov 13, 2024 to Apr 30, 2025. Ethical approval was taken from the Board of Advanced Studies & Research (BASR) Superior University vide Ltr. no. Acad/BASR/42/2024 dated 13th November, 2024; and Ethical Review Committee, CMH Lahore Medical College vide Ltr. No. 73/ERC/CMH/LMC dated 23rd September 2024.

A sample size of 6 blood vessels for each group was calculated using data of similar studies where sample size varied from 6-8 blood vessels.[16] Fresh samples were taken from the Obstetric Units of CMH Lahore, and CMA Teaching Hospital and Research Institute, Azra Naheed Medical College. A total of 30 healthy pregnant women of 18-35 years having full term pregnancy were enrolled, out of which 24 best samples were included in the study. Pregnant females, having PIH, cardiovascular, respiratory, renal & liver diseases were excluded. Consent was taken during admission according to Declaration of Helsinki. A 10 cm piece of umbilical cord from the portions for biological disposal were taken. The samples were placed in modified Kreb's solution at a temperature of 37°C and instantly transported to the Laboratory of Pharmacology Department. A piece of 3cm was taken, Wharton's jelly, connective and adipose tissues were cleaned from the HUA rings. The HUA ring was used with intact endothelium. A piece of HUA ring was denuded by passing a thread through the lumen.[17]

For the solutions for tissue bath system, the research grade chemicals were procured from the market. Kreb's solution was used in the organ bath where HUA ring was placed. All the salts were dissolved in distilled water and the following concentration (mM) were achieved: KCl 4.7, NaCl 118.3, KH2PO4 1.2, NaHCO3 25, glucose 11, MgSO4 1.2 and CaCl2, 2.5mM. The solutions were held at < 40 ºC, pH was adjusted at 7.4 with NaOH and HCl.[18]

The tension exerted on the HUA rings was determined by the HUA ring, that was dipped in the organ bath and suspended between two parallel stainless-steel wires. The tension exerted by the rings was measured through isometric transducers (MLT0420) to record isometric tension. An analog digital converter platform installed on a computer was connected with an amplifier (AD Instruments Bridge Amps) linked with a transducer. The organ bath solution was replaced after every 15 min during the rest periods. A 10 ml of Kreb's solution with a continuous oxygen in a carbon-oxygen mixture (95% O2;5% CO2) at a temperature of 37°C was used. After every 15 minutes of suspended artery rings, the new solution was added. Contractions were recorded after stabilization of human umbilical artery rings. Isometric contraction of the tissue was recorded using the force displacement transducer connected

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to a Power Lab data acquisition system. [18]

Total number of patients were divided into four groups. For Group I, tissue was stabilized in normal Kreb's solution and metformin (1-20 µM) was used to develop graded dose-response relationship with HUA (intact endothelium) rings. For Group II, tissue was stabilized in normal Kreb's solution and vasorelaxant effect of metformin (1-20 µM) was observed on HUA (denuded endothelium) rings. For Group III: Tissue of HUA (intact endothelium) ring was pretreated with KCl (60mM) and vasorelaxant effect of metformin was observed. For Group IV the HUA rings were pretreated with KCl (60 µ M) and graded dose response relationship of prostaglandin E_2 was observed.

GraphPad Prism version 9.0 was used for statistical analysis. After analyzing the data, through non-linear regression, fit curves were achieved. The effects of metformin, and PGE_2 on HUA ring were evaluated by applying row statistics to get IC_{50} values. Degree of freedom explored R-squared (R^2) for Good Fit values showing the reliability of concentration-response relationship.

Results

Metformin had graded dose response relationship in HUA ring and reduced basal tone by up to 76.34% ($p<0.0001$). The R-squared value was 0.5906 (Table I & II). Nonlinear regression analysis showed value of 7.031 with 95% confidence interval (CI). When metformin (1-20 µM) was used in HUA ring with denuded endothelium; the vasorelaxation response was 28%, and IC_{50} of 0.6521. The R-squared value was 1.787, showing good curve fit on GraphPad Prism (Table II)

To get the response of metformin with intact endothelium for pharmaco-mechanical excitation-contraction coupling (ECC), the smooth muscle contraction of HUA was induced with KCl (60µM). Metformin showed relaxation response on HUA when its increasing concentrations were added in the presence of high KCl (60µM) showing CI 95% value of 7.135%, ($p<0.0001$) and R squared value of 0.8291 (Table.II).

Graded dose response relationship was carried out on isolated HUA ring. PGE_2 produced vasorelaxation under precontracted KCl (60µM). One-way ANOVA followed by post hoc Tukey's multiple comparison test was used. There was significant difference between baseline and higher doses of PGE_2 ($p<0.05$). The maximum vasorelaxation achieved with metformin and PGE_2 in precontracted vessels with KCl were almost the same (Figure.1).

Table I: Dose-response relationship of drugs on isolated human umbilical artery (n=24)

µM/l	Metformin (intact endothelium) (n=6)	Metformin (without endothelium) (n=6)	KCl + metformin with (intact endothelium) (n=6)	KCl + PGE_2 (intact endothelium) (n=6)
1	11.07914	2.56712	7.36435	8.93425
4	24.18774	13.08751	22.67973	22.7087
8	36.98836	20.14684	37.9108	45.32477
12	46.85018	20.79666	53.09538	50.41856
16	61.74670	21.89781	71.6646	63.60850
20	76.34016	28.4789	78.98972	78.13234

Table II: Non-linear regression analysis for comparison of effects of metformin (intact and denuded endothelium) with KCl and PGE_2 on HUA in vitro (n=24)

Parameters	Metformin with Intact Endothelium (n=6)	Metformin without Endothelium (n=6)	KCl + Metformin with Intact Endothelium (n=6)	KCl + PGE_2 with Intact Endothelium (n=6)
95% CI* IC_{50}	7.031	0.6821	5.739	6.248
P-Value	<0.0001	<0.0001	<0.0001	<0.0595
R-Squared	0.5906	-1.787	0.8291	0.6185
* CI: Confidence Interval				

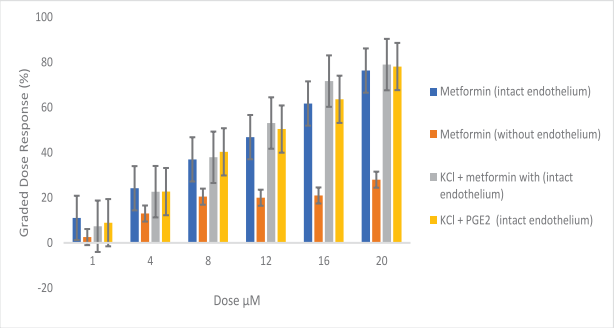

Figure 1: Vasorelaxant Effect of Drugs on Human Umbilical Artery Ring (n=24)

Discussion

It was found that metformin produced dose dependent direct vasorelaxation in isolated HUA (In-Vitro). Metformin has marked vasodilator effect on systemic blood vessels as well as coronary blood vessels. Current studies show that metformin reduces the burden of ischemic heart diseases (IHD) by its beneficial effect on vascular smooth muscle cells (VSMCs). [20,21]

Metformin administered alone produced dose-dependent vasorelaxation with an IC_{50} of 7.03 µM and a moderate goodness of fit ($R^2 = 0.59$). In another

study on human internal mammary arteries from cardiac patients, metformin at 10 µM reversed angiotensin 2-induced endothelial dysfunction. This effect of metformin is comparable to the effect of metformin on HUA in the present study.[22] Relaxation of HUA rings in the presence of intact endothelium is caused by multiple endothelial mediators such as NO, PGI_2, and endothelium-derived hyperpolarizing factors (EDHFs). Metformin improves vascular endothelial function by activating 5-adenosine monophosphate activated protein kinase (AMPK), increase nitric oxide synthesis, and inhibit cardiac remodeling and cardiac fibrosis. Nitric oxide causes vasodilation through cGMP-dependent pathway. [23] It also works through cGMP independent pathway by S-nitrosylation of proteins and activate calcium pumps. Prostacyclin I_2 released from vascular endothelial cells causes vasodilation by reducing Ca^{2+} concentration in the vascular smooth muscle cells.[24]

The metformin induced relaxation in the present study suggests the modulation of ion channels and reducing intracellular Ca^{+2} in smooth muscle through voltage-gated Ca^{+2} channels. [25] Another possibility is metformin has physical action on L-type Ca^{+2} channels and reduce calcium influx as we have seen with nifedipine, verapamil, and diltiazem. These findings suggest metformin has both direct action through the release of endothelial mediators as well as indirect action by modulation of calcium and potassium channels. These findings are consistent with those reported by Sahinturk S et al., [26] in a study demonstrating effect of metformin on thoracic artery in rats.

Metformin in the presence of KCl (60 µM) exhibited more potent and dose dependent vasorelaxation response, replicated with IC_{50} value of 5.74 µM and a markedly higher R^2 (0.83). These findings are suggestive of therapeutic effectiveness in relieving pathophysiological vasoconstrictive ailments such as GDM. This effect of metformin is similar to the vasorelaxant effect of nifedipine in HUA of pre-eclampsia patients.[27]

Higher extracellular KCl changes the membrane potential of cells, and causes persistent depolarization. Depolarized membrane inhibits the influx of Ca^{++} as well as disrupt the internal release and sequestration system that controls contractile proteins such as actin and myosin. In the present setup metformin activated membrane depolarization and voltage gated calcium channels activation; consequently, the stronger relaxation seen with metformin is suggestive of interference with calcium influx or calcium dependent contractile mechanism.[28] A similar hypothesis is proven by a recent study on human and mouse mesenteric arteriole, where metformin induced vasorelaxation through endothelium-dependent hyper-polarization.[29]

Conclusion

Metformin revealed more vasorelaxation with intact endothelium than denuded HUA rings suggesting endothelial-dependent mechanisms are involved. In the presence of KCl induced contractions with intact endothelial tissues of HUA, metformin exhibited the strongest vasorelaxation followed by PGE_2. Metformin has the potential to protect fetoplacental circulation during GDM pregnancies.

The study limits in elaborating direct measurements of biochemical markers like endothelial mediators (NO, PGI_2, K^+ channels, L-type Ca^{++} channels, etc.) to assess NO levels, gene expression, oxidative stress markers, prostaglandin levels etc.

Conflict of Interest: None

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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