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Research Article
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Free Amino Acids Glycine and Glutamic Acid Inhibit Angiogenesis Induced by AGE in Bovine Retinal Endothelial Cells

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Abstract

Previous studies from our lab have shown that amino acids act as antiglycating agents and can be beneficial in diabetes mellitus. Accumulation of advanced glycation end products (AGE) in uncontrolled diabetes mellitus can induce microvascular complications such as diabetic retinopathy that results in neovascularization in the retina. This study explored the effect of amino acids Glycine and Glutamic acid (0.5–2.5 mM) in mitigating the AGE (100 µg/ml) induced angiogenic effects in primary bovine retinal endothelial cells (BREC) cultured in vitro. Tube formation induced by AGE in the BREC cells were reduced by glycine and glutamic acid (p=0.05, p=0.008). Transwell migration assay revealed signficant inhibition of migration by glycine. Expression of actin cytoskelatal filaments that promotes migration was reduced predominantly by glycine than glutamic acid as seen by immuofluorescence. Leukocyte adhesion promoted by AGE treatment was reduced significantly by glycine (p=0.03) and glutamic acid (p=0.02). The mechanism was delineated in terms of AGE-RAGE/VEGF axis. The receptor for AGE (RAGE) and the VEGF expression was found to be decreased both at protein level and at mRNA level by glycine and glutamic acid treatment. mRNA expression of RAGE by qPCR revealed a maximal decrease of 60% by glycine at 2.5 mM but by glutamic acid at 0.5 mM and VEGF mRNA level showed maximal inhibition by glutamic acid at 2.5 mM. Western blot analysis showed that VEGF expression was predominantly reduced by glycine. This is the first study showing the anti angiogenic potential of the amino acids in an in vitro model of primary bovine retinal endothelial cell implying therapeutic potential in the management of diabetic retinopathy.

Key words: Angiogenesis; AGE; Amino acids; VEGF.

Introduction

In diabetes mellitus (DM) hyperglycemia causes accumulation of the advanced glycation end products (AGE) The reaction of proteins with glucose leads to the formation of AGE. Uncontrolled hyperglycemia for longer duration triggers dysregulation of various pathways such as the protein kinase C pathway, sorbital pathway, PI3K and Akt pathways that result in microvascular complications such as retinopathy. Diabetic retinopathy (DR) is a major complication of DM characterized by the growth of new blood vessels in the retina. The binding of the AGE to the cell membrane receptors (RAGE) mediates signaling pathway downstream resulting in nuclear factor-KB (NFkB) that causes cellular oxidant stress and apoptosis [1], expression of vascular cell adhesion molecule-1 (VCAM-1) that alters vasopermeability and leukocyte adhesion to endothelial cell leading to trans endothelial migration [2-5]. Thus neovascularization, oxidative stress, inflammation, apoptosis and permeability changes are characteristic of diabetic retina in DR patients.

Modulating at the level of AGE-RAGE axis can be a viable therapeutic strategy for the treatment of diabetic retinopathy [6]. sRAGE, PEDF, Telmisartan, and Ramipril have been shown to block the AGE-RAGE axis [7], However, photo coagulation continues to be the major option in treatment of most cases [8]. Nagai et al. recently discussed on the AGE inhibitors that can be obtained from daily meals which are preferable over prescribed drugs like benfotiamine, pyridoxamine and aminoguanidine [9]. Lyzohub et al. have shown that branched-chain amino acids improve antioxidant status, promote mitochondrial biogenesis, anti-aging processes and may play a major role in regulating obesity and diabetes mellitus [10].

Previous studies in our laboratory have revealed the anti-diabetic effect of amino acids [11-13], wherein supplementation of amino acids was shown to decrease the glucose levels and glycosylated haemoglobin level. Further the anti-glycation activity of free Lysine, Alanine, Aspartic acid, or Glutamic acid was demonstrated in human lens proteins, in-vitro [14,15]. In a recent study from our lab the amino acids mixture was found to augment glucose uptake by potentiating insulin signaling in CHO k1 exposed to high glucose condition [16, 17].

In this study, we explore the role of amino acids especially glycine and glutamic acid in mitigating diabetic complications like diabetic retinopathy at the level of AGE-RAGE axis by using an in-vitro model of primary bovine retinal endothelial cells exposed to AGE.

Materials and Method

Cell culture

Primary cultures of Bovine retinal endothelial cells (BREC) were isolated from bovine retina as described in our previous publication [18]. Briefly, homogenization of the capillaries was followed by filtration through a 41µ nylon filter. The cells in the filter were then kept on a petridish coated with 0.1 % gelatin and incubated with endopan media, a commercial media (Genex India Bioscience, India) for growing endothelial cells. Cells were cultured in media containing 20 % FBS in 5 % CO_2 at 37°C, and media was changed once in every 3 days. The primary cells grew to confluence after 10-12 days after which they were trypsinised and subjected to the CD31 antibody coated bead based isolation. This was to prevent contamination with other cells, especially pericytes which are in a 1:1 ratio with the endothelial cells in the retina [19]. Homogeneity of the cell was confirmed by immune reactivity with anti–factor VIII and VE-cadherin. Passages 2 –5 were used for the experiments and all the experiments were conducted in DMEM/F12 medium.

Amino acids: The amino acids were a gift from Tablets India Pvt

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Ltd. The amino acids were reconstituted in DMEM medium and exposed to cells. The concentration of amino acids in the media was 0.5 mM for glutamic acid and 249 nM for glycine. Amino acids namely glycine, glutamic acid, leucine and lysine, representative of neutral, acidic, branched chains and basic respectively, were evaluated for anti-angiogenic effects and it was found that amongst these amino acids, glycine and glutamic acid alone showed an inhibitory effect on tube formation in BREC exposed to high glucose concentration (Supp Figure 1). Therefore the following experiments were done with glycine and glutamic acid from concentration
ranging from 0.5 to 2.5 mM. The concentration of glycine and glutamic used in the study did not show any cytotoxicity when exposed to BREC in MTT assay.

MTT assay

Briefly 1000 cells were plated in a 96-well plate and grown to confluence and exposed to 100 µg AGE alone (Biovision, USA) and along with amino acid for 24-72 h and the untreated cells were used as controls. The formazan crystals formed after treatment with MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) were dissolved in DMSO and read at 570 nm in ELISA reader.

In-vitro Angiogenesis experiments:

To test if the amino acids are pro-angiogenic or anti-angiogenic, the following experiments were done.

Matrigel (tube formation assay): For the matrigel assay, BREC ($25 cm^2$ flask) was initially exposed to 100 µg/mL of AGE for 24 h. The cells were then trypsinised and seeded (1000 cells/well) onto the solidified growth factor reduced matrigel in a 96-well plate. After which amino acids (glycine and glutamic acid) along with AGE (100 µg/mL) were treated for 4-6 h. The cells were fixed, and the tube formation was analyzed by light microscopy. Four random fields were chosen in each well for analysis. Grading and counting of the tube was done according to the guidelines given by the manufacturer (Chemicon International, Inc,USA).

Migration assay: The BREC (10,000cells/well) were grown to confluence in gelatin coated 6-well plates in DMEM/F12 media. A scratch was made with a sterile pipette tip in the middle of the confluent layer of the cells and then they were treated with AGE (100 µg/mL), which enhances the migration of the cells. The inhibition of migration was tested with co treatment with amino acids, glycine and glutamic acid, at different concentrations varies from 0.5 mM - 2.5 mM till 48 h and the results were recorded. The migration was also carried out using cell culture inserts (Millipore) and the number of cells migrated were counted after staining with crystal violet and the results documented.

Actin stress fibers: The BREC cells (1000cells/well) were grown in chamber slides treated with AGE(100 µg/mL) alone, or co-treated with glycine and glutamic acid (2.5mM) fixed with 2% paraformaldehyde, permeabilized with 0.1% Triton X-100, and labeled with FITC-phalloidin for 30 min, at room temperature and was Counter stained with DAPI. The cells were captured using the zeiss fluorescent microscope [13].

Adhesion assay: The Bovine retinal endothelial cells were plated in 6-well plates (10,000cells/well) and allowed to grow to confluence. Subsequently, they were exposed to 100 µg/mL of AGE for 24 h, with and without amino acids glycine and glutamic acid. To this 1500 cells /well, peripheral mononuclear cells were added and incubated for 4 h, the excess cells which were not adhered, was removed by PBS wash. The number of PBMC cells adhered were counted in four different fields and recorded.

IF and mRNA expression of RAGE: The BREC (1000 cells/well) grown in chamber slides were stained for the receptor for advanced glycation end product(RAGE) protein expression with anti RAGE antibody (Santa Cruz, CA) and a secondary antibody with FITC after exposing the cells to AGE(100 µg/mL) for 24 h with and without amino acid . mRNA expression was done under similar condition in cells grown to confluence in 6 well plates using real time: On the basis of the information of the RAGE cDNA sequences (GenBank accession number NM_173982.2) gene-specific primers were designed. The sequences of the RAGE primers are as follows:

F:5'ACCGAGTCCGAGTCTATCAGATTC3'

R:5'CCATCCAAGAGCCAGTTAAGAGTC3'

Fluorescent labeling of VEGF protein in BREC: Briefly the BREC were grown in cover slips in a 24 well plate and exposed to AGE (100 µg/mL) for 24 h with and without amino acid. The cells were fixed in 100 % methanol and stained for vascular endothelial growth factor (VEGF) protein with primary antibody raised in rabbit (Abcam, Cambridge,UK) the anti rabbit conjugated with FITC were used as secondary antibody. The cells were captured under the zeiss fluorescent microscope. Western blotting of the VEGF protein was also done in the cell lysates and the band intensity was normalized to beta- actin and quantified using the Image J software.

Expression of VEGF in BREC by RT-PCR and real time PCR: RNA was extracted (Sigma Genelute mammalian total RNA mini prep kit) according to the manufacturer's instructions. RT-PCR (reverse transcription polymerase chain reaction) was performed, wherein, 1 µg of RNA was treated with DNAse I (Invitrogen,USA) followed by CDNA conversion using random hexamer (Thermoscript, Invitrogen,USA) based on manufacturer's protocol. For this assay, 0.5 mM and 2.5 mM concentration of the amino acids glycine and glutamic acid were used. The primers which were used are as follows:

VEGF Forward primer: 5'-CGAAACCATGAACTTTCTGC-3'

Reverse primer: 5'-CCTCAGTGGGCACACACTCC-3' forming a 299 bp product

GAPDH Forward primer: 5'-TGTTCCAGTATGATTCCA CCC-3'

Reverse primer: 5'-GTCTTCTGGGTG-GCAGTGAT-3' forming a 424 bp product

Quantitative real-time PCR was performed using the SYBR Green chemistry, in MicroAmp Optical 96-Well Reaction Plates. PCR runs and fluorescence detection were carried out in a 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The increase in fluorescence emission (R_n) was measured during the course of PCR amplification, and the difference (ΔR_n) between the fluorescence emission of the product and the baseline was calculated by the Sequence Detection System software and plotted versus the cycle number. On the basis, of the information of the VEGF and TUBB cDNA sequences (GenBank accession number: NM_174216.1 and NM _001003900.1, respectively), two pairs of gene-specific primers were designed for real time PCR. The sequences of real time PCR and RT-PCR primers are given below

Bovine VEGF :

Forward primer:5'CATAGCCGCCGCCACCAC3'

Reverse primer: 5'CGCACAGCCTCCTCTTCCTTC3'

Bovine Beta TUBBULIN:

Forward primer: 5'GCTGGTGGAGAACACCGATGAG3'

Reverse primer: 5'GGTCAGTTTCAGGGTGCGGAAG 3'

Measurement of nitrate/nitrite: The cells were grown to 90% confluence in 96 well plate serum starved and exposed to 2.5 mM amino acids glycine and glutamic acid with AGE (100ug). The nitric oxide produced in the media by the endothelial cells after exposure was measured using the greiss method in which the first step is the conversion of nitrate to nitrite using nitrate reductase. The second step is the conversion of nitrite to the deep purole azocompound by greiss reagent which is read at 540nm. The amount of the azochromophore accurately reflects nitric oxide levels in the samples (Nitric oxide assay kit, Biovision,USA)

Statistical analysis: All the experiments were done in triplicates and the results are expressed as mean ± SD unless otherwise indicated. Statistical analysis was conducted using the unpaired Student's t test and a P value of <0.05 was considered statistically significant.

Results

Cytotoxicity assay: The cytotoxic effect, of glycine and glutamic acid in BREC was studied up to 72 h at 5 mM concentrations along with AGE treatment (100µg/mL). The amino acids were not cytotoxic to the cells as determined by MTT assay. They also did not show any proliferative effect (Supp. Figure 1).

In vitro angiogenesis assays

In order to see if glycine and glutamic acid show anti angiogenic properties in BREC, matrigel based tube formation assay, migration and adhesion assays were done.

Tube formation

The cells treated with AGE (100µg/mL) showed increased tube formation within 6-12 h of treatment. The number of tubes formed in the AGE treated were counted and compared with AGE co-treated with amino acids. There was a significant inhibition of tube formation by glycine at 1mM concentration (p=0.012) and 2.5mM concentration (p=0.008). Glutamic acid at 1mM concentration showed a significant inhibition of tube formation (p= 0.05) and 2.5 mM concentration showed a significant inhibition (p =0.008) (Figure 1A & B). Thus a dose dependent effect was seen in both the amino acids.

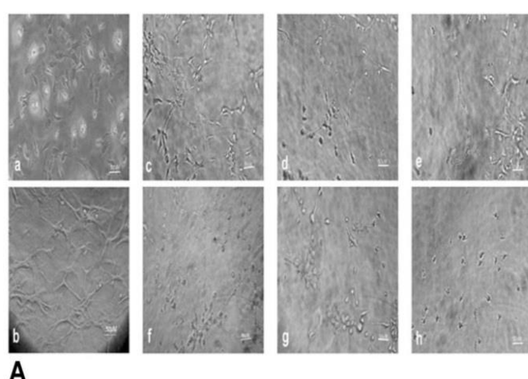
Migration assay

The AGE (100 µg/mL) was able to induce the migration of BREC in scratch assay, but when the cells were treated with amino acids, there was a dose dependant inhibition of migration by both the amino acids, glycine and glutamic acid (0.5 to 2.5 mM) (Figure 2B). To quantify the inhibition of migration a transwell assay was also done with 2.5 mM amino acids in which the cells were allowed to pass through the filter, keeping AGE as the chemoattractant. There was a significant inhibition of migration on supplementing with 2.5 mM glycine (p=0.033). However glutamic acid did not show any significant effect in transwell migration assay Figure 2C (i&ii).

Actin stress fibers: The stress fibers are involved in the migration of the cell. The active fibers can be stained with phalloidin. In this experiment, we observed an enhanced active actin stress fibers formation after treatment with AGE. When the cells were co-treated with amino acids glycine and glutamic acid (2.5mM) there was a decrease in the expression of actin cytoskeleton filaments, as seen by immunofluorescence, However the decrease was more prominent with glycine treatment (Figure 2A).

Adhesion of mononuclear cells to retinal endothelial cells: Since AGE has been reported to induce inflammation, adhesion of PBMC to the endothelial cell surface was tested. The number of mononuclear cells adhered to the BREC surface was counted and was found to be increased by 2 fold with AGE treatment compared to control. The number of adhered mononuclear cells was found to be significantly reduced by more than 50% when amino acids glycine (p=0.03) and glutamic acid (p=0.02) were co-treated (Figure 4A&B).

RAGE protein and mRNA expression: Receptor for AGE (RAGE) mediates the signaling of AGE. As glycine and glutamic acid showed inhibition of the angiogenic assays in AGE induced condition, RAGE expression was tested by immunofluorescence (IF). Both glycine and glutamic acid at 2.5mM concentration decreased the RAGE protein expression in BREC (Figure 5A). qPCR revealed a 3.3 fold increase in the mRNA expression of RAGE with AGE treatment. This was found to be dose dependently decreased by glycine treatment with 60% and 67% reduction at 0.5 mM and 2.5mM concentration respectively compared to the AGE treated. Glutamic acid showed a maximal decrease of 60 % at 0.5mM and 34% for 2.5 mM concentration (Figure 5B).



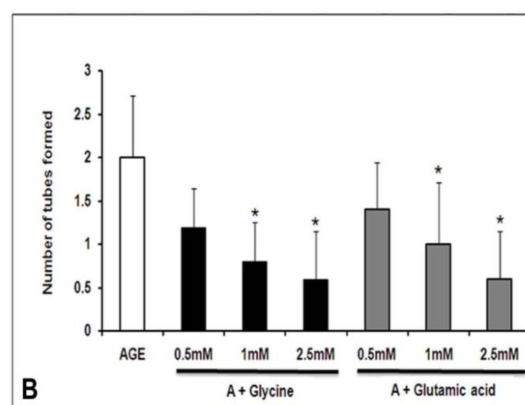


Figure 1: *In vitro* matrigel tube formation assay.
A; a: Control (untreated), b: AGE (100 µg/ml) treated cells c-e: Glycine+AGE (0.5, 1 and 2.5 mM), f-h: Glutamic acid+AGE (0.5, 1 and 2.5 mM).
B: Bar graph representing the tube formation in AGE and amino acid treated condition. Three different fields were counted and the number of tubes formed and inhibited was recorded. The mean ± SD values were plotted.

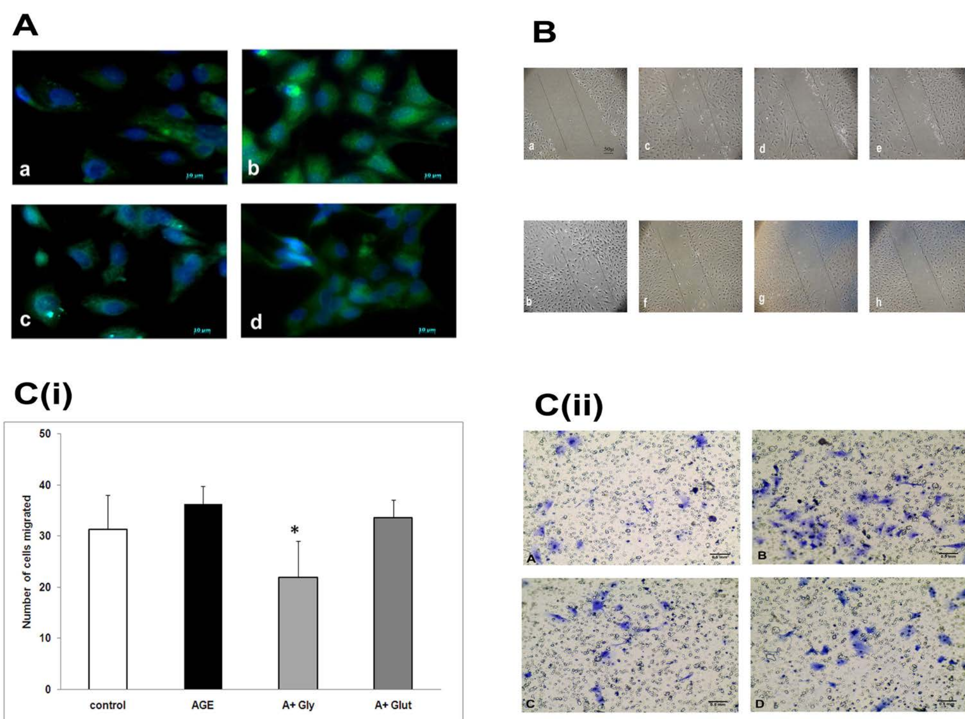
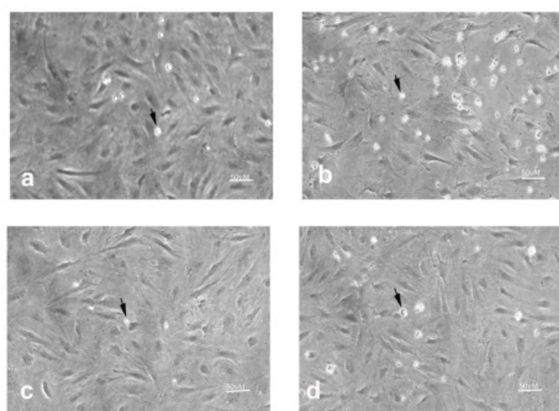


Figure 2:
A: Actin stress fibers
a: Control (untreated), b: AGE (100 µg/ml) treated c : Glycine (2.5 mM)+AGE (100 µg/ml),d: Glutamic acid (2.5 mM)+AGE (100 µg/ml).
The green fluorescence represents the phalloidin stained active actin stress fbers. Nucleus is stained blue with DAPI
The Stress fiber formation showed an increase with AGE compared to control. Glycine and glutamic acid decreased the actin stress fiber formation when added along with AGE (100 µg/ml).
B : Scratch assay
a: Control (untreated), b: AGE (100 µg/ml) treated c-e : Glycine (0.5,1 and 2.5 mM)+AGE, f-h: Glutamic acid (0.5,1 and 2.5 mM)+AGE (100 µg/ml),There is inhibition of migration by both the amino acids.
C: Transwell migration assay:
C (i) Bar graph of the cells counted in transwell assay is represented here and there was a signifcant inhibition of migration by glycine.
C (ii) A: control (untreated), B: AGE (100 µg/ml) treated C: Glycine (2.5 mM)+AGE, d: Glutamic acid (2.5 mM)+AGE.



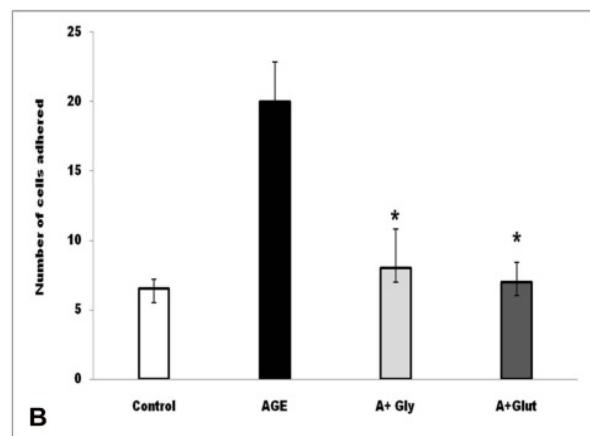


Figure 3: Adhesion assay
A: a: control (untreated), b: AGE (100 µg/mL) treated c: Glycine (2.5mM) + AGE (100 µg/mL), d: Glutamic acid (2.5mM) + AGE(100 µg/mL)
The figure shows there is an increased adhesion of PBMC to endothelial cells when treated with AGE compared to control (Mononuclear cells are indicated with an arrow). There was a 50% decrease with treatment with amino acids glycine and glutamic acid at 2.5 mM concentration.
B: The number of cells adhered were counted in three different fields and plotted as a bar graph

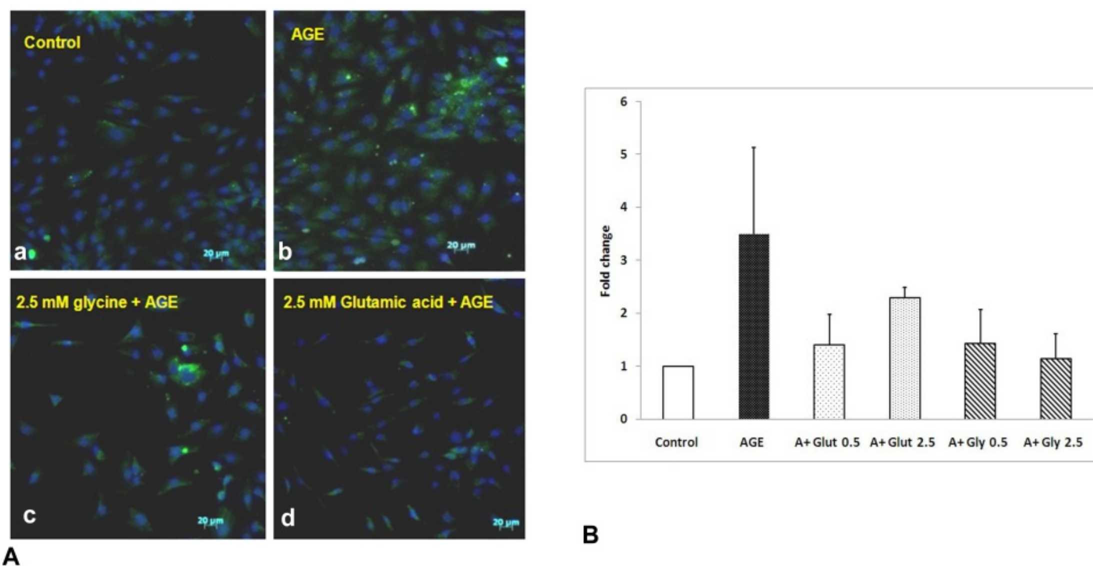


Figure 4:
RAGE immunofluorescence and mRNA expression:
A: a: Control (untreated), b: AGE (100 µg/mL) treated, c: Glycine (2.5mM) + AGE(100 µg/mL),d: Glutamic acid (2.5mM)+ AGE(100 µg/mL)
RAGE immunofluorescence is shown by the dotted green fluorescence and the nucleus is stained blue with DAPI. The cells treated with AGE showed increased RAGE expression compared to control and there was a decrease in the RAGE fluorescence after co-treatment with 2.5mM glycine and glutamic acid.
B: The fold change in the mRNA expression of RAGE using real time PCR for Glutamic acid and glycine treated condition was plotted as a bar graph keeping untreated control as 1.

VEGF expression: The key factor, which ultimately leads to angiogenesis, is the increased expression of vascular endothelial growth factor. AGE treated BREC showed increased protein expression of VEGF, but when co-treated with 2.5mM glycine and with 2.5mM glutamic acid, the protein expression as seen by IF was found to be decreased (Figure 6A). Western Blot analysis further confirmed the decrease in the protein expression and it was found that glycine decreased the VEGF expression. (Figure 6B).

VEGF mRNA expression was done by RT-PCR, which showed an increased VEGF expression on AGE treatment and a decrease in expression when co treated with glycine and glutamic acid at 2.5mM concentration (Figure7A&B).

A quantitative real time PCR for the VEGF mRNA showed a 2.3 fold increase in VEGF expression with AGE treatment compared to control. Co-treatment with glycine showed nearly 30 % decrease at 2.5 mM concentration and nearly 80 % decrease at 2.5 mM concentration with glutamic acid compared to AGE treated (Figure 7C).

Discussion

AGE can bind the receptors on the membrane of the endothelial cells resulting in increased VEGF in cells [20].VEGF promotes tube formation of the endothelial cell and the migration of the endothelial cells is promoted by the increased expression of the actin cytoskeleton filaments [21]. Glycine has been shown to inhibit VEGF induced cell proliferation and migration by blocking the influx of Ca^{2+}, through the glycine gated chloride channels as studied in bovine pulmonary artery endothelial cell line [22]. The inhibition of endothelial cell migration is indicative of the anti-angiogenic property of the compound [13,23]. This study in BREC shows reduction of tube formation by both glycine and glutamic acid and reduction in actin expression with glycine which may inhibit the migration as observed by the scratch assay and transwell migration assay. However actin expression and migration were not significantly influenced by glutamic acid.

We also observed a decrease in the adhesion of PBMC to the BREC. Inflammation is said to be the initial trigger in the progression of DR which leads to angiogenesis [24]. PBMC adhesion was reduced by both glycine and glutamic acid. In this study, we observed that the amino acids glycine and glutamic acid exhibit anti-angiogenic properties in AGE induced angiogenesis in BREC cells However, no reports are available on the role of glutamic acid in the regulation of angiogenesis. This is the first study showing amino acid glutamic acid with anti-angiogenic property.

There are published reports on the vitreous levels of the amino acids glycine and glutamic acid [25,26]. In certain studies, increased glutamate levels in vitreous has been associated with retinal detachment and retinopathy [27-29]. While one of the study reveals no change in glutamate levels in vitreous [30], yet another shows glutamate increase and associated increase in nitric oxide levels, both in the animal model of DR and in cultured retinal endothelial cells [31].

Dietary glycine reduced inducible nitric oxide synthase (iNOS) expression and has been reported as a potent anti-angiogenic agent by CAM assay [32]. In our study the NO levels were significantly decreased by both the amino acids glycine and glutamic acid. AGE signaling leads to the induction of iNOS expression through p38 MAP kinase pathway [33]. iNOS has been shown to play an important role in leukostasis by increasing the ICAM expression in endothelial cells and thereby inducing changes in permeability [34]. Increased levels of nitric oxide (NO) have been shown to cause nitration and nitrosylation of retinal proteins like neural growth factor that leads to neural degeneration [35]. There are reports which have shown that decreasing NO in DR is beneficial. A study has shown that Oral supplementation with losartan reduces the iNOS expression and reestablishes the redox status, in a Diabetic SHR rat model [36]. Mishra et al. have shown that inhibition of iNOS reverses the loss of functional hyperemia in DR [37]. Our study shows that the amino acids decrease NO and thereby inhibit migration and tube formation of BREC. (Supp. Figure 3).

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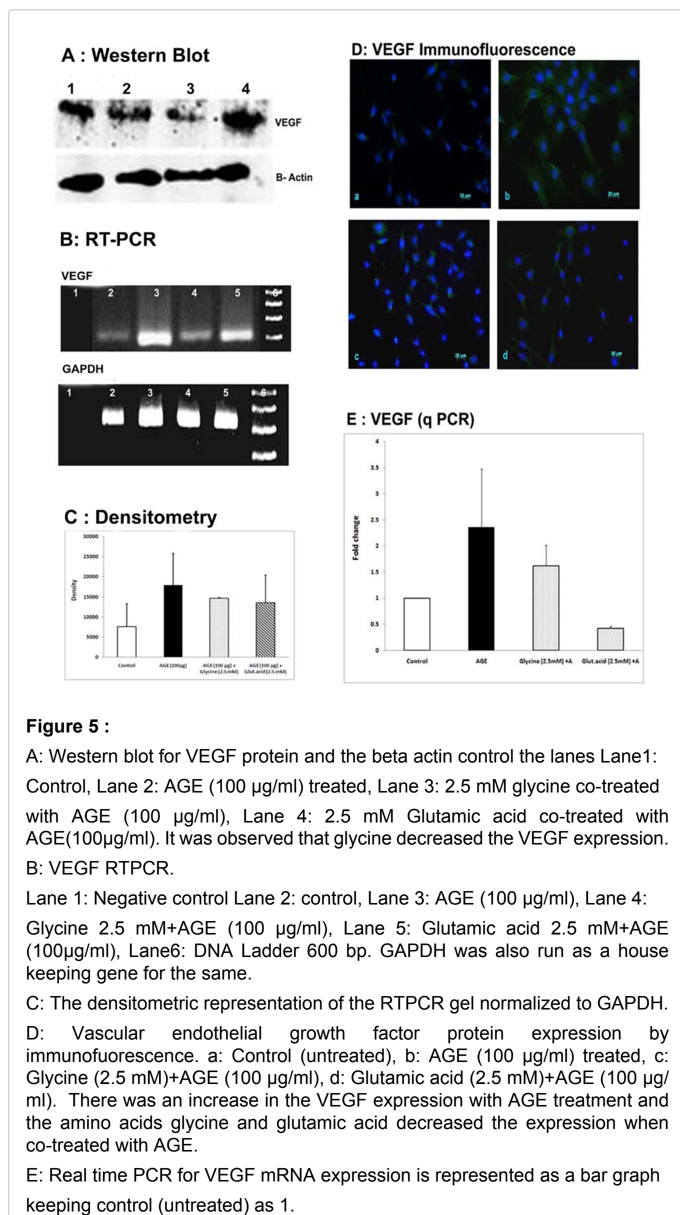

Figure 5 :

A: Western blot for VEGF protein and the beta actin control the lanes Lane1: Control, Lane 2: AGE (100 µg/ml) treated, Lane 3: 2.5 mM glycine co-treated with AGE (100 µg/ml), Lane 4: 2.5 mM Glutamic acid co-treated with AGE(100µg/ml). It was observed that glycine decreased the VEGF expression.

B: VEGF RTPCR.

Lane 1: Negative control Lane 2: control, Lane 3: AGE (100 µg/ml), Lane 4: Glycine 2.5 mM+AGE (100 µg/ml), Lane 5: Glutamic acid 2.5 mM+AGE (100µg/ml), Lane6: DNA Ladder 600 bp. GAPDH was also run as a house keeping gene for the same.

C: The densitometric representation of the RTPCR gel normalized to GAPDH.

D: Vascular endothelial growth factor protein expression by immunofuorescence. a: Control (untreated), b: AGE (100 µg/ml) treated, c: Glycine (2.5 mM)+AGE (100 µg/ml), d: Glutamic acid (2.5 mM)+AGE (100 µg/ml). There was an increase in the VEGF expression with AGE treatment and the amino acids glycine and glutamic acid decreased the expression when co-treated with AGE.

E: Real time PCR for VEGF mRNA expression is represented as a bar graph keeping control (untreated) as 1.

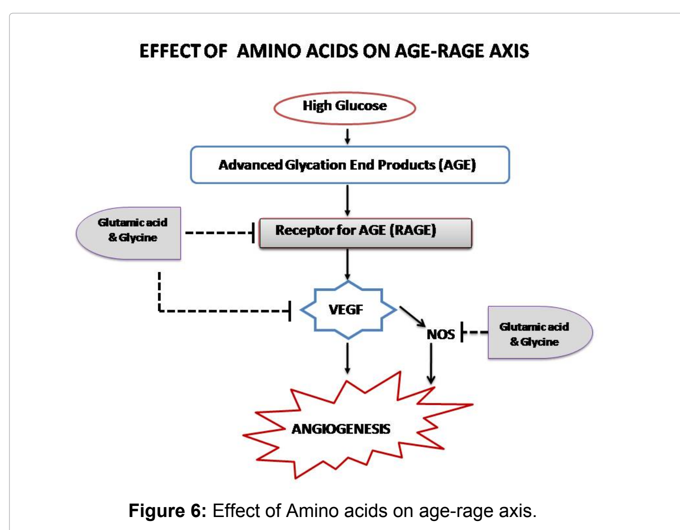

Figure 6: Effect of Amino acids on age-rage axis.

Further, in this study, it was observed that the amino acids glycine and glutamic acid decrease the RAGE expression both at protein and mRNA levels. AGE-RAGE activation results in elevated levels of VEGF [38]. VEGF expression was also found to be reduced by glycine and glutamic acid at protein and mRNA level. Since the study reveals decrease in mRNA expression of both RAGE and VEGF with glycine and glutamic acid treatment, it is possible that these amino acids can influence the downstream signaling of AGE/RAGE that results in lowered NFkB activation through MAP kinases resulting in decreased VEGF expression. Further studies are needed to see if the glycine and glutamic acid act via MAP Kinase activation.

VEGF antagonists such as bevacizumab has been used widely. Many are in phase II or Phase III clinical trials. Apart from photocoagulation, either triamcinolone or bevacizumab are being used presently for PDR and DME treatment. However, triamcinolone has been associated with cataract and raised IOP. Moreover some patients have shown nonresponsiveness to bevacizumab [39]. Angiogenesis can also be addressed at the level of AGE-RAGE interaction that can elicit VEGF elaboration. Previous studies have identified several downstream signaling pathways responsive to RAGE binding [40-42]. Therapeutic strategies such as inhibition of formation of AGE, blockade of the AGE–RAGE interaction, suppression of RAGE expression and downstream of the same, can be promising for the treatment of vascular complications in diabetes. AGE blockers like aminoguanidine [43], Pyridoxamine [44], and Alagebnum [45], have undergone clinical trials that were unsuccessful. The blockade of the AGE–RAGE axis by the administration of sRAGE has been shown to ameliorate neuronal dysfunction and reduce the development of acellular capillaries and pericyte ghosts in hyperglycemic and hyperlipidemic mice [46]. Kaji and colleagues have shown that attenuation of the RAGE axis with soluble RAGE inhibits retinal leukostasis and blood–retinal barrier breakdown in diabetic C57/BJ6 RAGE-transgenic mice, which are accompanied by decreased expression of VEGF and ICAM-1 in the retina [47]. This study reveals that the amino acids, glycine and glutamic acid exhibit anti angiogenic property by decreasing the expression of RAGE and VEGF in bovine retinal capillary endothelial cells (Schematic representation of the hypothesis). They also decrease the NO levels. These amino acids inhibited migration of BREC, tube formation as well as adhesion of the PBMC. These amino acids however have to be evaluated for their therapeutic efficacy in animal models in attenuating neovascular changes in retina.

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Conflict of Interest: NIL

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