Indian J Physiol Pharmacol 2018; 62(1) : 87–96

Original Article

Early Adaptive Changes in Transporters of Blood Retinal Barriers and Glutamate Excitotoxicity in Experimental Diabetes

Vetrivel Sharmilee[1], Nath Madhu[2], Halder Nabanita[2] and Velpandian Thirumurthy[2]*

[1]Department of Biotechnology,
All India Institute of Medical Sciences,
New Delhi – 110 029 (India)

[2]Ocular Pharmacology and Pharmacy Division,
Dr. RP Centre for Ophthalmic Sciences,
All India Institute of Medical Sciences,
New Delhi – 110 029 (India)

Abstract

Aim: Transporters are well studied in blood retinal barriers (BRB) to regulate transfer of respective substrates through retina. The present study was undertaken to evaluate the alteration of retinal transporters expression under hyperglycemic conditions in streptozotocin (STZ) induced diabetic rats and its functional consequences in the ocular system.

Method: Diabetes was induced in adult male wistar rats using STZ (45 mg/kg) and hyperglycemia was confirmed by measuring blood glucose. At periodic intervals, retinal function and vasculature was assessed by electroretinography and fundus imaging respectively. Gene expression analysis of 15 transporters of ABC and SLC family-importantly glutamate transporter were studied on day 10, 20, and 30. Levels of glutamate substrate in vitreous were quantified by LC-MS/MS. BRB integrity was also assessed by blood to vitreous ratio of P-glycoprotein substrate-ofloxacin.

Result: In the experimental period of 30 days, significant changes in ERG were observed at 10th day along with concordant significant fold increase in glutamate transporters expression (SLC1A1,SLC1A3) at 20th and 30th day respectively. Abc1b transporter isoform coding for P-glycoprotein was also found to be significantly upregulated at 30th day. Increased vitreous glutamate levels were accompanied by the blood to vitreous ratio of ofloxacin at 30th day.

Conclusion: This study concludes that glutamate induced retinal excitotoxicity precedes vascular complications in the very early stage of diabetes itself. Moreover, the alteration of blood retinal barrier properties in diabetic condition indicates its impact on ocular drug kinetic.

***Corresponding author :**

*Dr. T. Velpandian, Professor, Ocular Pharmacology & Pharmacy Div, Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India, Phone : +91-11-26593162, Fax : +91-11-26588919, Email id: tvelpandian@hotmail.com.

(Received on August 1, 2017)

Introduction

International Diabetes Federation (2014) reports 175 million diabetic populations progressing un-diagnosed towards diabetic complications. As endocrine disorders, diabetes represents complex pathologic state where different bodily systems are involved. Amongst the organs retina has one of the highest metabolic demands basically due to its role in phototransduction. The combination of high metabolic demand and minimal vascular supply limit the retina's ability to adapt the metabolic stress of diabetes (1). Hence efforts are needed to characterize the early diabetic changes before the onset of irreversible micro and macrovascular complications in retina.

In the retina, the importance of transporters in diabetic condition has been illustrated through GLUT1, the glucose transporter at blood retinal barrier playing a major role in the development of diabetic retinopathy (2). Also knockdown studies by Lu et al., (2013) indicate GLUT1 as a promising therapeutic target in diabetic retinopathy (3). But the change in the expression state of different transporters viz. amino acid transporters, peptide transporters have not yet been studied. Beside till date no studies have been done regarding the change in the expression level of important drug transporters viz. OCT, OAT and ABC efflux transporters in diabetic condition in eye. Diabetic induced earlier changes in the transporter expression in the blood ocular barriers are expected to play a vital role in the progression of retinopathy in diabetic conditions. Breakdown of the blood-retina barrier (BRB) is one of the most important pathophysiological aspects in the diabetic retina. The enhancement of retinal vascular permeability and vascular leakage resulting from BRB dysfunction has been observed both in patients with diabetes and diabetic animal models (4). Functional importance of transporters in cornea and retina and their modulation causing altered kinetics of their substrates from blood-to-vitreous or vitreous-to-blood has been studied with the help of externally administered xenobiotics from our laboratory (5, 6). Thus the various molecular and functional aspects of retinal transporters under the context of diabetes need to be revisited.

Therefore, the present study was conducted to understand the earlier changes in the expression of selected transporters in the retina of STZ induced diabetic rats. STZ-diabetic rats have been known to clearly manifest retinal sorbitol pathway, hyperactivity, oxidative stress, neuro retinal apoptosis and glial changes associated with diabetes, providing detailed molecular information, including acute and chronic changes in gene expression and cell signaling (7). Characterization of diabetic animal model studies have been done from a timeline of utmost 2 months during which pericyte loss (8) visual acuity damage have been noted (9). Hence an understanding of the earlier events leading to diabetic retinopathy could help in identifying an optimum time point for intervention in diabetic retinopathy. To serve these purpose, physiological parameters of fasting blood glucose & HbA1c levels and functional & vascular analysis of retina using Electroretinography (ERG) and fundus imaging were correlated with expression changes of selected transporters at time points of 10, 20, 30 days.

Materials and Methods

Streptozotocin HCl, L-glutamate, Ofloxacin were purchased from Sigma Aldrich (USA). Insulin (Humulin 70/30), xylazine and ketamine were procured from C.B Pharma, India. Sodium citrate dihydrate, citric acid, were purchased from Merck, Germany. RNA later was purchased from Ambion, USA. Glucometer was acquired from AccuCheck Active, USA. All other solvents and chemicals were of the highest analytical grade available. Primers were obtained from Oligo IDT Technologies, USA.

Induction of diabetes using streptozotocin and sampling

Male Wistar rats (225-250 g) were procured from Central Animal Facility and the study protocol was approved by the standing Animal Ethics Committee of All India Institute of Medical Sciences, New Delhi. The experiments were conducted according to Association for Research in Vision and Ophthalmology (ARVO) guidelines. The animals were housed in polypropylene cages and were maintained under standard laboratory conditions with 12 hour

light/dark cycle having free access to water and food *ad libitum.*

Diabetes was induced by streptozotocin (i.p.) in the overnight fasted rats using the vehicle containing 0.1 M citrate buffer having pH 4.5 at the dose of 45 mg/kg body weight by using the procedure adopted by Ward et al (2001). Control rats received only vehicle. Two days after the injection of streptozotocin, all rats were subjected for screening of their diabetic state by determining the blood glucose levels using calibrated glucometer (Accuchek, Roche Diagnostics, USA) by sampling blood from tail vein by prick method. Rats showing blood glucose levels more than 250 mg/dl were grouped under diabetic rats. Their body weight was measured weekly while fasting blood glucose was measured every three days.

Diabetic rats (n=30) were randomly divided into four groups. They were coded and their body weight was recorded before and end of the experiment. Group 1 served as control, Group 2, 3 & 4 were diabetic and were sacrificed after the functional investigations on the day 10, 20 & 30 respectively. After sacrificing the animals using carbon-di-oxide, blood was collected by cardiac puncture and was subjected for the analysis of glycated haemoglobin (HbA1c) by using the method of Park et al*.,* (2009) with the help of HbA1c Assay Kit (Biosystems, Spain). Two hours before sacrificing the rats, ofloxacin (35 mg/kg) was administered orally to all the groups for the calculation of blood to vitreous ratio and to evaluate the condition of blood retinal barrier permeability.

Electroretinography & Fundus Imaging

Retinal function in the diabetic model was assessed by using image guided electroretinogram (ERG) in anesthetized rats. After at least 40 minutes of dark adaptation the animals were anesthetized using ketamine (75 mg/kg B.W) and xylazine (5 mg/kg B.W). The ERG and fundus images were taken using the method described by the Aron et al., (2016) according to (International Society for Clinical Electrophysiology of Vision) ISCEV guidelines with the help of MICRON III rodent imaging system (Phoenix laboratory, USA) (10). For ERG analysis the 'a' and 'b' wave amplitude and latency were measured in all groups, using the inbuilt algorithm of Labscribe software. The oscillatory potentials were also extracted out from the obtained ERG by narrowing down the frequency bandwidth to 30-250 Hz. The fundus images were analysed in terms of tortousity index as method described previously by Liu et al. (2006) (11).

RNA Isolation and quantification of gene expression

The rats were euthanized by carbon-di-oxide and sacrificed at different time intervals of 10, 20 and 30 days. Enucleated eye balls were quickly subjected for the removal of iris and lens through transverse incision on cornea. With calibari forceps vitreous was removed followed by retina with choroid. The pooled retinal samples (n=6) were immediately placed in RNA*later* (Ambion, USA) and subsequently RNA purification was done using RN-easy Mini Kit (Qiagen, USA). The contaminating genomic DNA was digested using the RNase-Free DNase Kit (Qiagen, USA). Isolated RNA samples were quantified by using NanoDrop 1000 Spectrophotometer (ThermoScientific, USA). RNA samples of sufficient purity (A_{260}/A_{280} ratio of 1.9–2.1) were used for the synthesis of cDNA (Thermoscientific, USA). The cDNA were synthesised from an RNA concentration of 200 ng. The synthesized cDNAs were used for QPCR analysis (Thermocycler, Biorad CFX 96, USA). Relative Gene Expression Analysis was done by 2-Delta Delta C (T) method. The geometric mean of two reference genes, including phospholipase (*Ywhaz*) and *18S* was used as a normalization factor between the control (normal) and reference samples (obtained from 10^{th}, 20^{th} and 30^{th} diabetic rats). Vitreous and blood samples were stored at –80°C for the quantification of ofloxacin and glutamate levels by tandem mass spectroscopy.

Quantitative gene expression study was conducted for 15 transporters viz. *Slc1a1, Slc1a3, Slc1a6, Slc1a7, Slc6a6, Slc15a1, Slc15a2, Abcb1a, Abcb1b, Abcc1, Abcc2, Abcc3, Abcc4, Abcc5, Abcc6* (shown in Table I). The forward and reverse primers used for this study is shown in the Table I.

TABLE I : Primer sequences of different transporters along with their Ct values and reported substrates. The represented Ct is the average of threshold cycle (Ct) values of normal group from three different experimental runs. NCBI accession refers to the gene sequence used to design the primers.

Nomenclature	*Gene*	*Substrate*	*NCBI Accession No.*	*Forward Primer*	*Reverse Primer*	*Ct*
SLC6A6	*Taut*	Taurine	NM_017206.1	AGA GCA AGG GGT GGA CAT TG	GAA TGG ACC AAA AGG TGG GC	26.72
SLC1A1	*Eaat-3*	Glutamate	NM_013032	TCT CAT CTA GCT CGG CAA CC	TCT TGT GAT CCT CTT GTC CAC G	31.28
SLC1A3	*Eaat-1*	Glutamate	NM_019225	AAG TAT CAC AGC CAC AGC CG	CCC ACA GAT GTC AGC ACG A	32.32
SLC1A6	*Eaat-4*	Glutamate	NM_032065	CCT TTC CCT TCA TCG GTG G	CTC CAG GCA TCG GAA AGT GA	32.2
SLC1A7	*Eaat-5*	Glutamate	NM_001108973	CCG ACC GAT GAC ATC AAC C	ATC GCC CAG CAC GTT AAT CA	27.98
SLC15A1	*Pept-1*	β-lactam antibiotics, renin inhibitors	NM_057121	GGC CCC AAT CTA TAC CGT G	TCT CGT TAA GGG TGC TGA CG	37.51
SLC15A2	*Pept-2*	β-lactam antibiotics, renin inhibitors	NM_031672	TCA TTG TGC TTG TCG TGG C	CAT GAC GGA GAA GAT CAG GCA	N/A
ABCB1a	*Abcb1a*	Steroids, Glycosides, Glucocorticoids, Ofloxacin	NM_133401	AC TCG CAA AAG CAT CCG TG	GGA GGT ACG TCG TCA TCC AG	38.67
ABC1b	*Abcb1b*	Steroids, Glycosides, Glucocorticoids, Ofloxacin	NM_012623	CCA CAG AAG ACA AGA CCA GGA G	CTG CCA AAA GGA AAC CAT AGG C	40.02
ABCC1	*Abcc1*	Anticancer drugs, nucleoside and nucleotide analogs, leukotriene C(4)	NM_022281	TCT GAA ACG GAG AAG GAG GC	CTC TAC ACG GCC TGA ATG GG	36.11
ABCC2	*Abcc2*	Anticancer drugs, bilirubin, glucuronides	NM_012833	CCA TTG GAC TGC ACG ACC	TCA TCC TCA GAC TCC CCG AG	N/A
ABCC3	*Abcc3*	Anticancer drugs, nucleoside and nucleotide analogs, bilirubin, glucuronides	NM_080581	CTC CAT GAC CTG CGT TCA CA	AGT AAC GGC CAA AGG GAT CG	40.05
ABCC4	*Abcc4*	Anticancer drugs, nucleoside and nucleotide analogs, arsenical prostaglandins E1 and E2, cGMP	NM_133411.1	TCG GAC ACA TGG ACG ACT TG	CCA CGG CGA TCA CAC TTA CA	N/A
ABCC5	*Abcc5*	Anticancer drugs, nucleoside and nucleotide analogs, arsenical cGMP	NM_053924	TAC CCA CGA GGA GCT GAT GA	TTA ATC TCG ACC GGG GGT G	44.05
ABCC6	*Abcc6*	Anticancer drugs, nucleoside and nucleotide analogs	NM_031013	GAG GAT CAG TTT CCC GAG GC	CAT GTA GCG GCC ACA AAC AC	N/A

Quantification of ofloxacin and glutamate using tandem mass spectroscopy

Samples were thawed and the levels of ofloxacin and glutamate in vitreous and blood samples were estimated using liquid chromatography (Surveyor, Thermo, USA) coupled with tandem mass spectroscopy (4000QTrap, Absciex, USA). Briefly, the analytical separation of both the compounds were achieved using phenyl-hexyl column (Merck, Germany) using a gradient elution with water (0.1% formic acid) and methanol (0.1 formic acid) at flow rate of 200 µl/min. Sulfadimethoxine (SDM) was used as the internal standard. Analytes were quantified in multiple reaction monitoring mode and transitions 362.2/261.3, 148.1/84.2 and 311/129 were used for ofloxacin, glutamine and sulfadimethoxine respectively. All other source and compound dependent parameters were optimized using the inbuilt algorithm to get maximum ions intensity in

the analysis to reach required sensitivity.

Statistical analysis

The obtained values are represented as mean±SEM. Statistical analysis was done between normal vs 10 day diabetic ,normal vs 20 day diabetic and normal vs 30 day diabetic using unpaired t-test in Sigma Plot version 11.p value of <0.05 has been considered as statistically significant and marked with an asterisk (*). Statistical analysis of gene expression data was done using REST 2009 software.

Results

Assessment of hyperglycemia and body weight changes

Animal showing blood sugar levels more than 250 mg/dL were randomly assigned under different groups (n=6 in each group). The percentage reduction in their body weight between the start and end of the experimental period was found to be statistically significant at 20th day and 30th day. A sustained and increasing hyperglycemic condition was found in the experimental animals through the experimental period of 30 days. The increase in blood glucose levels was found to be statistically significant from 20 days. The HbA1c levels found in all the experimental groups were found to be within the range of 7.5.

Effect on Electroretinography

ERGs in different experimental groups were found to be appreciably different. Altered levels of the a-wave and b-wave for the different days were observed along with the increased amplitude of the oscillatory potentials post diabetic induction (Fig. 1A & 1B).

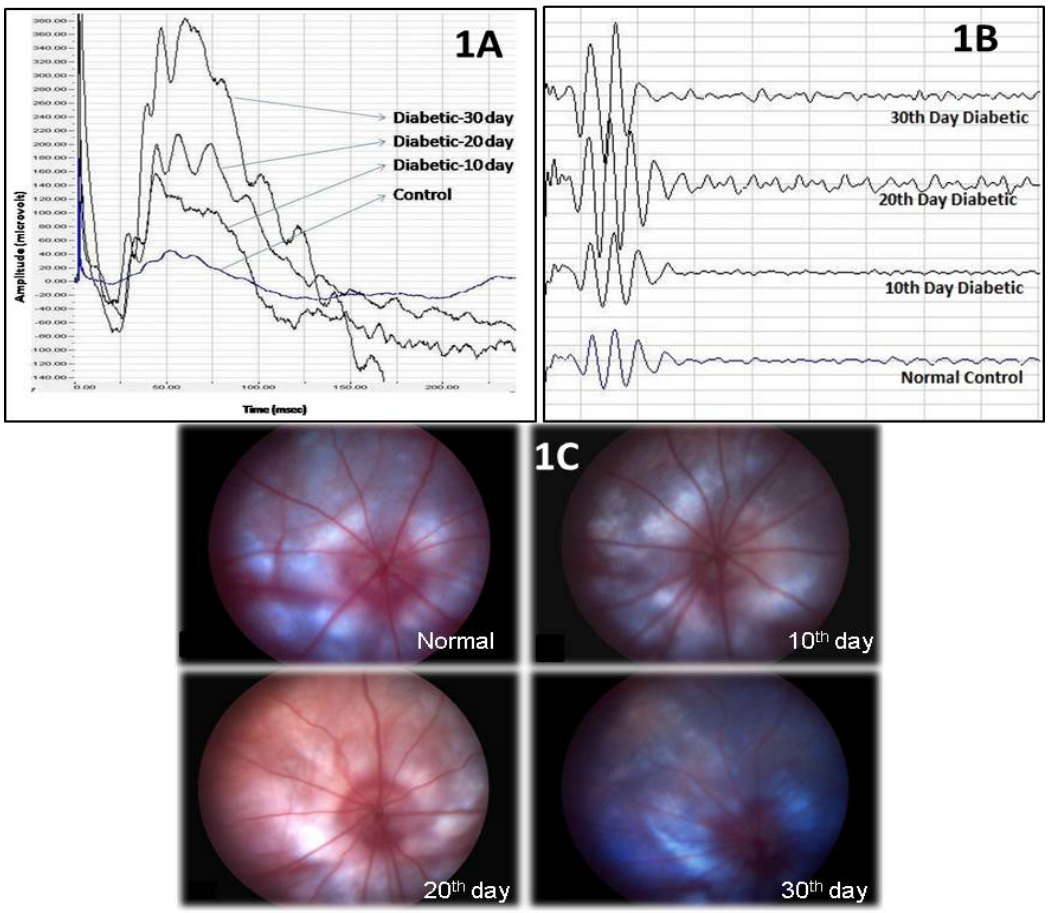

Fig. 1 : Fig. 1 depicts the diabetic changes happened in retina. Fig. 1A shows the altered electroretinography waves of 10th, 20th and 30th day when compared to normal. Same trend of altered oscillatory potentials was observed in the Fig. 1B. The representative images of retina of normal, 10th, 20th and 30th shown in Fig. 1C.

Fundus Imaging and Tortuosity Index

In the diabetic rats, along with the progression of diabetes, no significant increase in retinal tortuosity was observed through the experimental period. Representative fundus image of respective days of the experiment are shown in Fig. 1C.

Quantitative transporter gene expression analysis

Amongst the 15 transporters analysed in the samples of retina choroid, expression of 3 transporter proteins were found to be significantly altered in QPCR analysis (Table I). Glutamate transporters (Slc1a1, Slc1a3) were found to be upregulated in the retinal tissue samples. Particularly the expression of Slc1a1 was found to become more prevalent with increasing time intervals- the 20day diabetic group exhibited fold expression levels of 5 while the 30 day diabetic group exhibited fold expression levels of almost 20 (Fig. 2). While the change in fold expression levels were found to be significant for Slc1a1,Slc1a3 transporters, no significant expression changes were found in the isoforms of Slc1a6, Slc1a7 (Fig. 2).

In case of xenobiotic transporters Abc1b transporter protein coding for P-gp transporter was found to be significantly upregulated at 30th day (Fig. 3A). While other transporter proteins- Slc6a6, Slc15a1, Abcb1a, Abcc1, Abcc3, Abcc4, Abcc5 showed no significant expression changes during the experimental period (Fig. 3B & 3C). Moreover the transporters Slc15a2, Abcc2, Abcc6 were not found to be expressed in the retinal samples (Unpublished data).

Assessment of blood retinal barrier Integrity

The blood to vitreous ratio of ofloxacin and concentrations of glutamate in vitreous, blood were estimated by LC-MS/MS. The values of ofloxacin in the normal groups ranged from 4.8±0.7 and increased significantly to a value of 12.9±5.2 on the 30 day in diabetic group (Fig. 4A). Moreover the glutamate levels in the normal groups which were at a concentration of 6457.1±508 ng/ml increased significantly to a concentration of 9892±927 ng/ml in diabetic animals on 30th day. (Fig. 4B). While the glutamate levels in the blood did not vary significantly between the groups (Fig. 4C).

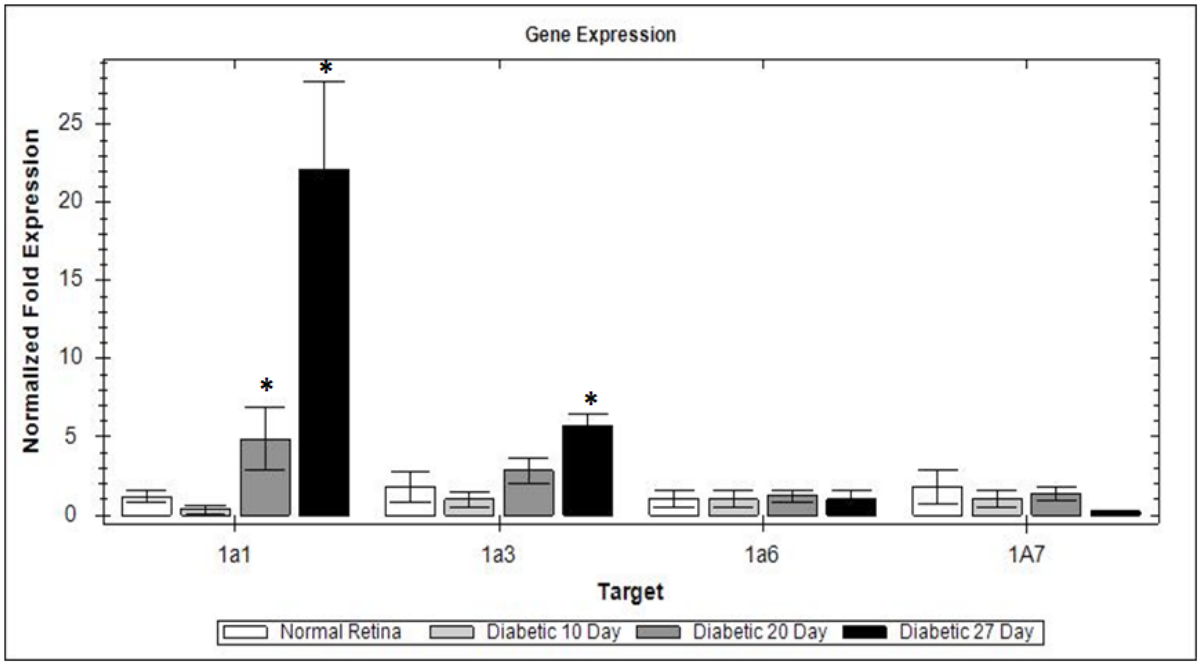

Fig. 2 : Fig. 2 depicts the gene expression of glutamate transporters at various day of normal, 10th, 20th and 30th day. Slc1a1, Slc1a3 were found to be significantly upregulated at day 30th. No significant expression changes were found in the isoforms of Slc1a6, Slc1a7. Statistical analysis was done using REST software, *p≥0.05.

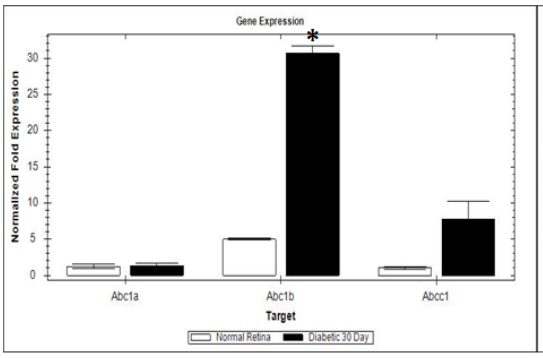

Fig. 3A

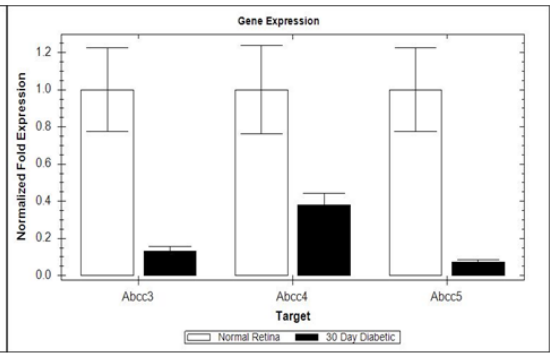

Fig. 3B

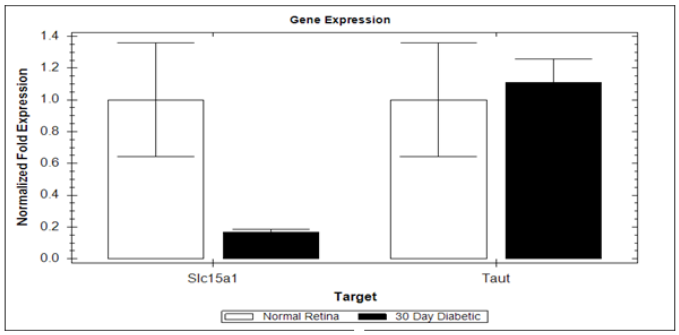

Fig. 3C

Fig. 3 : Xenobiotic transporters Abc1b transporter protein coding for P-gp transporter was found to be significantly upregulated at 30th day (Fig. 3A). While other transporter proteins-, Abcb1a, Abcc1, Abcc3, Abcc4, Abcc5 (Fig. 3B) and Slc15a1 (Fig. 3C) showed down regulation as compare at 30th day but the change was not statistically significant. Statistical analysis was done using REST software, *p≥0.05.

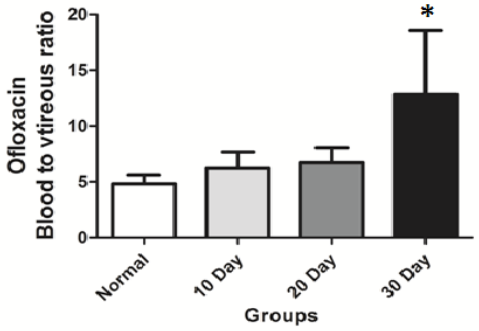

Fig. 4A

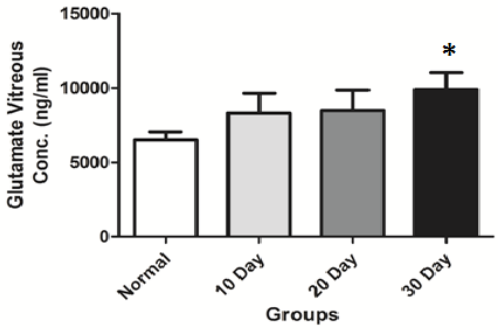

Fig. 4B

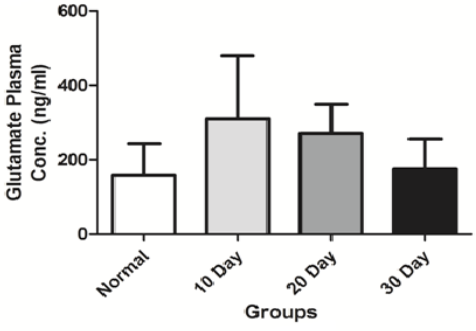

Fig. 4C

Fig. 4 : Figure 4 shows the blood to vitreous ratio of ofloxacin and concentrations of glutamate in vitreous, blood were estimated by LC-MS/MS. The ofloxacin ratio increased significantly at the 30 day in diabetic group (Fig. 4A). The glutamate levels were also significantly high at 30th day post diabetes as compare to normal (Fig. 4B). While the glutamate levels in the blood did not vary significantly between the groups (Fig. 4C). Statistical analysis was done using student t-test, *p≥0.05, **p≥0.01.

Discussion

Hyperglycemia induced early diabetic changes accompanied by the alteration in the transporter expression in the blood ocular barriers (BRB) are of interest in understanding the initiation of cascade of events leading towards neuronal dysfunction and vascular changes in the retina. Therefore, the present study was conducted to identify the earlier changes in the expression of selected retinal transporters and to correlate the changes with the retinal functions using ERG in experimentally induced diabetes in rats.

To achieve the above objective, diabetes was induced in the experimental group by using streptozotocin (45 mg/kg) as a single i.p injection to identify the earlier changes in BRB, where control rats received saline. After the induction of diabetes, only rats showed blood sugar levels more than 250 mg/dl on day 3 were included to avoid variation in their diabetic state. Along with the time, progressive loss of body weight was observed in hyperglycemic rats as compared to the control (data not shown). These rats were randomly selected for retinal function studies at different days and were sacrificed to enable the quantitative gene expression of transporters in retina choroid. Before sacrificing the rats at different days, fundus photography was documented after analysis their retinal functions by recording electroretinography (ERG) and oscillatory potentials (Fig. 1A & 1B).

Retinal vascular tortuousity has been documented as an early indicator of microvascular damage in diabetes (12, 13). In this study, fundus images of the diabetic rats revealed no significant increase in tortousity indices as compared to control implying that the vascular complications were yet to begin till the 30th day (Fig. 1C). In the blood, glycated haemoglobin has been considered as an indirect indicator of diabetic microvascular (14). Although, a positive correlation is known to exist between blood glucose levels and glycated hemoglobin with time (15), in the present study at the end of 30th day, the Hba1c was not found to increase. This could be due to the lack of complete change in RBC within the study period owing to its lifespan (rat RBCs life span is 60±3.2 days) (16).

In this study, quantitative gene expression analysis was performed on 15 transporters from two super families such as Solute Linked Carriers (Slc1a1, Slc1a3, Slc1a6, Slc1a7, Slc6a6, Scl15a1, Slc15a2) and ATP Binding Cassettes (Abcb1a, Abcb1b, Abcc1, Abcc2, Abcc3, Abcc4,Abcc5, Abcc6). Among the studied transporters, it is evident that hyperglycemia induced significant gene alteration was seen in Slc1a1 and 1a3 within the study period of 30 days (Fig. 2). More than 20 fold increase in the expression of Slc1a1 (EAAC1) and 5 fold increase in levels of 1a3 (GLAST) was seen in the rats on day 30. Slc1a1 and Slc1a3 are reported as high-affinity glutamate transporters mediating cellular uptake of glutamate by the mechanism involving co-transport of three sodium (Na+) with one proton (H+), along with the counter-transport of one potassium (K+) (17).

A significant upregulation of Slc1a3 was found at 30th day together with progressive increase in glutamate levels in the vitreous of the diabetic rats as compared to normal (Fig. 2). Slc1a3 upregulation was also reported in the study of Lau et al (2013) in diabetic Long-Evans rat retina after 4 weeks of STZ injection (18). Also corroborating evidence of increasing vitreous glutamate levels and BRB leakage at 5th week after induction of diabetes has been reported by Kusari et al (2010) (19).

This study characterized the initial excitation of ERG wave forms, oscillatory potentials and Slc1a1 transporter upregulation at 10th & 20th day respectively before the increasing vitreous glutamate levels at 30th day. An increase in vitreal glutamate levels was found without any increase in blood glutamate level pointing to an endogenous source of glutamate in the ocular system (Fig. 4B & 4C). Under conditions of severe depolarization Slc1a3 in Muller cells has been reported to undergo reversal releasing glutamate and thereby increasing extracellular glutamate concentration to excitotoxic levels (20, 21). In our study increase in b-wave pointing to an increased depolarisation of the contributing cells types such as muller cells, bipolar cells and

horizontal cells was found in the initial time points of 10th and 20th day. Thus through the experimental timeline of 30 days this study demonstrates the initial excitation of ERG wave forms, the associated upregulation of the glutamate transporters in RGC and muller cells preceding increasing glutamate levels and BRB leakage.

The results of the current study indicated that the gene coding P-gp efflux transporters- Abc1b was showing the trend of significant upregulation through the experimental period of 30 days (Fig. 3A). In contrast, blood to vitreous ratio of ofloxacin at the end of 30 days was found to be higher as compared to normal rat's eye. Both paracellular pathway involving endothelial cell tight junctions, and the endothelial transcellular pathway mediated by endocytotic vesicles (caveolae) have been reported to be affected in blood retinal barriers as far as vascular permeability is concerned (22-24). In our study the increased blood to vitreous ratio of ofloxacin found along with increased expression of the efflux transporter validates the findings of Reichel et al (2011) (Fig. 4A) (25).

In conclusion, our work has characterized the initial changes in the rat retina of streptozotocin induced model of diabetes. The raised levels of glutamate in the vitreous, increased amplitude of ERG signals, increased expression of glutamate transporter showed the glutamate excitotoxicity predominating in the diabetic retina. While the upregulation of Pgp efflux transporters in the blood ocular barriers observed along with the increased blood to vitreous ratio of its substrate suggests the alteration of blood retinal barrier integrity at initial stages. Moreover the upregulation in the P-gp isoform expression and increased blood to vitreous ratios of its substrates in diabetic groups implicates the altered ocular kinetics following hyperglycemia. Thus the importance of transporters both in the pathophysiology as well as in ocular pharmacokinetics remain a vital point to be investigated in diabetes.

Acknowledgements

We acknowledge Department of Biotechnology for stipend and CSIR-UGC for providing Junior Research Fellowship for Sharmilee Vetrivel (F.No.045/UGC-22/RS). This paper has been presented in European Molecular Biology Organization 2014 conference on 'Frontier in Metabolism', held at Heidelberg, Germany (2014) by availing travel support from Department Biotechnology, Government of India (DBT/CTEP/02/201400856).

Conflict of interest :

None

References

1. Antonetti DA, Barber AJ, Bronson SK, Freeman WM, Gardner TW, Jefferson LS,Kester M, Kimball SR, Krady JK, LaNoue KF, Norbury CC, Quinn PG, Sandirasegarane L, Simpson IA. (2006). JDRF Diabetic Retinopathy Center Group. Diabetic retinopathy:seeing beyond glucose-induced microvascular disease. *Diabetes* 55(9): 2401–2411.
2. Kumagai AK, Glasgow BJ, Pardridge WM. GLUT1 glucose transporter expression in the diabetic and nondiabetic human eye. *Invest Ophthalmol Vis Sci* 1994; 35(6): 2887–2894.
3. Lu L, Seidel CP, Iwase T, Stevens RK, Gong YY, Wang X, Hackett SF, Campochiaro PA. Suppression of GLUT1; a new strategy to prevent diabetic complications. *J Cell Physiol* 2013; 228(2): 251–257.
4. Xu HZ, Le YZ. Significance of outer blood-retina barrier breakdown in diabetes and ischemia. *Invest Ophthalmol Vis Sci* 2011; 52(5): 2160–2164.
5. Nirmal J, Velpandian T, Singh SB, Biswas NR, Azad R, Thavaraj V, Mittal G, Bhatnagar A, Ghose S. (2012). Evaluation of the functional importance of organic cation transporters on the ocular disposition of its intravitreally injected substrate in rabbits. *Curr Eye Res* 37(12): 1127–1135.
6. Senthilkumari S, Velpandian T, Biswas NR, Bhatnagar A, Mittal G, Ghose S. Evidencing the modulation of P glycoprotein at blood-ocular barriers using gamma scintigraphy. *Curr Eye Res* 2009; 34(1): 73–77.
7. King JF. The use of animal models in diabetes research. *British Journal of Pharmacology* 2012; 166: 877–894.
8. Hammes HP, Lin J, Wagner P, Feng Y, Vom Hagen F, Krzizok T, Renner O, Breier G, Brownlee M, Deutsch U. Angiopoietin-2 causes pericyte dropout in the normal retina: evidence for involvement in diabetic retinopathy. *Diabetes* 2004; 53(4): 1104–1110.
9. Aung MH, Kim MK, Olson DE, Thule PM, Pardue MT. (2013). Early visual deficits in streptozotocin-induced diabetic long evans rats. *Invest Ophthalmol Vis Sci* 15; 54(2): 1370–1377.

10. Neelima Aron, Madhu Nath, Shorya Vardhan Azad, Parijat Chandra, Raj Vardhan Azad, Thirumurthy Velpandian. Pharmacological Interventions for Vascular Targeting In Retinopathy of Prematurity: An Experimental Study. *Ind J Physiol Pharmacol* 2016; 60(3): 282–290.
11. Liu K, Akula JD, Falk C, Hansen RM, Fulton AB. The retinal vasculature and function of the neural retina in a rat model of retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 2006; 47(6): 2639–2647.
12. Sasongko MB, Wong TY, Nguyen TT, Cheung CY, Shaw JE, Wang JJ. Retinal vascular tortuosity in persons with diabetes and diabetic retinopathy. *Diabetologia* 2011; 54(9): 2409–2416.
13. Noda K, Nakao S, Zandi S, Sun D, Hayes KC, Hafezi-Moghadam A. Retinopathy in a novel model of metabolic syndrome and type 2 diabetes: new insight on the inflammatory paradigm. *FASEB J* 2014; 28(5): 2038–2046.
14. Tarr JM, Kaul K, Chopra M, Kohner EM, Chibber R. (2013). Pathophysiology of diabetic retinopathy. *ISRN Ophthalmol* 2013; 343560.
15. Fujii E, Nomoto T. Changes in glycosylated hemoglobin in short- and semi long-term streptozotocin-diabetic mice and rats. *Jpn J Pharmacol* 1984; 34(1): 113–115.
16. Derelanko MJ. Determination of erythrocyte life span in F-344, Wistar, and Sprague-Dawley rats using a modification of the (3H)diisopropylfluorophosphate ((3H)DFP) method. *Fundam Appl Toxicol* 1987; 9(2): 271–276.
17. Kanai Y, Smith CP, Hediger MA. A new family of neurotransmitter transporters: the high-affinity glutamate transporters. *FASEB J* 1993; 7(15): 1450–1459.
18. Lau JC, Kroes RA, Moskal JR, Linsenmeier RA. Diabetes changes expression of genes related to glutamate neurotransmission and transport in the Long-Evans rat retina. *Mol Vis* 2013; 19: 1538–1553.
19. Kusari J, Zhou SX, Padillo E, Clarke KG, Gil DW. Inhibition of vitreoretinal VEGF elevation and blood-retinal barrier breakdown in streptozotocin-induced diabetic rats by brimonidine. *Invest Ophthalmol Vis Sci* 2010; 51(2): 1044–1051.
20. Szatkowski M, Barbour B, Attwell D. Non-vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake. *Nature* 1990; 348: 443–446.
21. Billups,B., and Attwell,D.(1996). Modulation of non-vesicular glutamate release by pH. Nature. 379, 171–174.
22. Cunha-Vaz JG. Blood–retinal barriers in health and disease. *Trans Ophthalmol Soc* U.K. 1980; 100: 337–340.
23. Hofman P, Blaauwgeers HG, Tolentino MJ, Adamis AP, Nunes Cardozo BJ, Vrensen GF, Schlingemann RO. VEGF-A induced hyperpermeability of blood–retinal barrier endothelium *in vivo* is predominantly associated with pinocytotic vesicular transport and not with formation of fenestrations. Vascular endothelial growth factor-A. *Curr Eye Res* 2000; 21: 637–645.
24. Reichel V, Burghard S, John I, Huber O. P-glycoprotein and breast cancer resistance protein expression and function at the blood-brain barrier and blood-cerebrospinal fluid barrier (choroid plexus) in streptozotocin-induced diabetes in rats. *Brain Res* 2011; 25: 1370: 238–245.