PHD THESIS

DIABETES-RELATED STRUCTURAL, MOLECULAR AND FUNCTIONAL ALTERATIONS IN CAPILLARIES SUPPLYING TO THE MYENTERIC PLEXUS IN THE GUT OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Introduction

The enteric nervous system is considered to be the most complex division of the peripheric nervous system. It regulates all the functions of the gastrointestinal tract. The enteric neurons and glial cells create ganglionic plexuses and direct the processes of absorption, secretion and peristalsis. The nitrergic myenteric neurons, which are responsible for the descendent inhibition of peristaltic reflex, are especially susceptible for developing diabetic neuropathy. Impairment of the nitrergic innervation has a key role in the development of different gastrointestinal motility disorders.

We have recently demonstrated that nitrergic myenteric neurons located in different intestinal segments display different susceptibilities to diabetic damage. They also exhibit different levels of responsiveness to insulin treatment, which indicates the importance of the molecular difference in the neuronal microenvironment in the pathogenesis of diabetic nitrergic neuropathy.

Therefore, we investigated the diabetes-related pathological changes in the capillary endothelium in the vicinity of the myenteric ganglia in different intestinal segments of experimentally induced chronic diabetic rats. Because of their dominant clinical incidence, the diabetes-induced alterations in the microvasculature of the retina, lung and renal glomerulus have been at the focus of a vast number of studies, however, the impact of diabetes on the capillaries within the intestinal wall appears to have been almost completely overlooked to

date. The loss of modulatory function of the endothelium in these capillaries has been proposed as a potential mechanism underlying enteric neuropathy.

Recent data have demonstrated that hyperglycaemia is a major risk factor in the development of the endothelial impairments in diabetes, however, endothelial cells from different vascular beds exhibit metabolic and structural differences and may be affected differentially by hyperglycaemia. In retina and renal glomerulus, the thickening of the endothelial basement membrane (BM) and increasing capillary permeability was observed in diabetics. Based on literature data, the hyperglycaemia is clearly culprit in the pathogenesis of diabetic complications, through increasing oxidative stress and hypoxia.

Aims

The main question of our study was whether the endothelial cells in capillaries adjacent to the myenteric plexus are direct targets of diabetic damage:

- Does the thickness of capillary basement membrane (BM) and the separation of it from the endothelium change in diabetic animals?
- Does diabetes have an effect on the integrity of endothelium and the condition of the tight junctions (TJs) between adjacent endothelial cells?
- What kind of effects has diabetes on the density of endothelial caveolae are important in transcytosis across the endothelium?
- How does hyperglycaemia alter the endogenous albumin transport?
- Does the hyperglycaemia have an effect on endothelial nitric oxide synthase (eNOS) and caveolin-1 (Cav-1) expression?
- Is there any gut region-specific pathological alteration in the capillary endothelium?
- Could the immediate insulin replacement prevent the diabetes-related endothelial damage?

Materials and methods

The diabetes-related structural, functional and molecular alterations of capillaries in the vicinity of the myenteric ganglia were investigated in different gut segments of control, streptozotocin-induced diabetic and insulin-treated diabetic rats.

The effects of diabetes on the capillary basement membrane, the caveolar compartments and the endothelial TJs were evaluated morphometrically. The quantitative features of the endogenous albumin transport were investigated by post-embedding immunohistochemistry. The diabetes-related changes in Cav-1 and eNOS expression were assessed by post-embedding immunohistochemistry, western blotting and RT-PCR techniques.

Results

In diabetic animals, the capillary BM was significantly thickened, the distance between the endothelium and the BM was widened and the enlargement of caveolar compartments was demonstrated in the microvessels of the colon and the ileum, but not in the capillary walls in the duodenum.

The number of opened TJs was higher in all intestinal segments of diabetic rats.

Insulin replacement prevented the development of the structural alterations in the capillary walls in the ileum, but not in the colon. In the capillaries of the colon the diabetes-related structural changes increased further as a response to immediate insulin replacement. However, in ileal capillaries all of these structural alterations could be reversed by insulin treatment.

A net increase in the number of gold particles labelling the endogenous albumin was revealed in all three intestinal segments in diabetic rats.

However, the distribution of the gold particles between the endothelial and interstitial compartments differed significantly in the different gut segments. While in the colon of diabetics more gold particles were counted in the capillary endothelium, in the ileal capillaries the increase in the number of gold particles was most pronounced in the interstitium.

The density of gold particles in the duodenum of the diabetic rats was double both in the endothelium and the interstitium.

While the imbalance of the distribution of albumin labelling gold particles in the colon capillaries lasted also after insulin replacement, it was restored completely in the capillaries of the ileum.

In the duodenum of diabetics the number of gold particles labelled Cav-1 or eNOS increased significantly in the capillary endothelium. At the same time, the diabetes-related up-regulation of Cav-1 mRNA and the Cav-1 protein, the up-regulation of eNOS mRNA and the down-regulation of eNOS protein expression were demonstrated in the duodenal tissue homogenates.

Significant changes in Cav-1 or eNOS expression were not detected in either the ileum or the colon of the diabetic animals. Insulin treatment prevented the molecular alterations in the duodenum and significantly reduced the Cav-1 and eNOS expression in the colon.

Discussion

According to our results, the diabetes-related pathological changes in the endothelium of the mesenterial capillaries appear gradually along the colon-ileum-duodenum axis. When the quantitative features of BM and caveolar morphology changed irreversibly in colon-, but reversibly in ileal capillaries, structural alterations in duodenal microvessels were not seen.

At the same time the molecular mechanisms associated with the regulation of the amount of bioavailable NO were still active in the duodenal but not in ileal or colonic tissue samples. Therefore, we suggest that capillary endothelium in the duodenum but not in the ileum and colon were able to maintain the vascular homeostasis and accordingly the proper neuronal microenvironment even under the diabetic condition within the period of ten weeks of investigation in the present work.

Our data provide morphological, functional and molecular evidence indicating that the endothelial cells in capillaries adjacent to the myenteric plexus are direct targets of diabetic damage. These results are in accordance with our hypothesis that the microvessels in particular gut segments are affected differentially by the pathophysiological conditions, and provide the microenvironment for the enteric neurons in a strictly regional manner allowing neurons in one intestinal region to survive while causing them to die in another.

Thesis related publications

N. Bódi, P. Talapka, M. Poles, E. Hermesz, Zs. Jancsó, Z. Katarova, F. Izbéki, T. Wittmann, É. Fekete, M. Bagyánszki (2011) *The endothelium of capillaries supplying the myenteric plexus are direct targets of diabetic damage.* Microcirculation (under revision) IF: 2.533

M. Bagyánszki, **N. Bódi** (2011) *Diabetes-related alterations in the enteric nervous system and its microenvironment.* World Journal of Diabetes (submitted)

P. Talapka, **N. Bódi**, I. Battonyai, É. Fekete, M. Bagyánszki (2011) *Subcellular distribution of nitric oxide synthase isoforms in the rat duodenum.* World Journal of Gastroenterology 17(8):1026-1029. IF: 2.240

N. Bódi, I. Battonyai, P. Talapka, É. Fekete, M. Bagyánszki (2009) *Spatial pattern analysis of nitrergic neurons in the myenteric plexus of the duodenum of different mammalian species.* Acta Biologica Hungarica 60(4), pp. 347-358. IF: 0.793

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M. Bagyánszki, Z. Novák, **N. Bódi**, H. Orvos, A. Pál, É. Fekete (2009) *Structural differences in the umbilical vein wall after full-term and preterm delivery.* Anatomia, Histologia, Embryologia 38:387–391. IF: 0.646

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Abstracts published in conference proceedings

N. Bódi, P. Talapka, M. Poles, M. Bagyánszki, A. I. Rosztóczy, É. Fekete, F. Izbéki, T. Wittmann (2011) Diabetes-related structural, molecular and functional alterations in capillaries supplying to the myenteric plexus can be responsible for the region-specific nitrergic neuropathy in the gut of streptozotocin-induced diabetic rats. 19th United European Gastroenterology Week, Stockholm, Sweden.

Gut 60 (Suppl 3) A313 IF: 10,614

R. Á. Nagy, **N. Bódi**, P. Talapka, M. Bagyánszki, É. Fekete, T. Wittmann, F. Izbéki (2011) Effects of acute and chronic quinolinic acid treatment on gastrointestinal motility in mice. 19th United European Gastroenterology Week, Stockholm, Sweden.
Gut 60 (Suppl 3) A313 IF: 10,614

N. Bódi, P. Talapka, M. Bagyánszki, A. I. Rosztóczy, É. Fekete, F. Izbéki, T. Wittmann (2010) The role of structural capillary damage in the loss of nitrergic myenteric neurons in a rat model of diabetes mellitus. 18th United European Gastroenterology Week, Barcelona, Spain.
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N. Bódi, P. Talapka, M. Bagyánszki, A. Rosztóczy, É. Fekete, T. Wittmann, F. Izbéki (2010) Intestinal endothelial dysfunction may have a role in the development of myenteric nerve damage in a rat model of diabetes mellitus. Magyar Gasztroenterológiai Társaság 52. Nagygyűlése, Tihany, Magyarország.
Z Gastroenterol 48:598-599 IF: 1,131

Impact factor of full papers: ***11,783***
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