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Original article

The impact of dapagliflozin on glucose excursions related to early proatherogenic derangement in the aortic wall☆

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ABSTRACT

Introduction: Cardiovascular risk in the course of diabetes depends greatly on glycemic variability which is even more significant than chronic hyperglycemia. Optimal management of diabetes involves a multidisciplinary approach focused in particular on decreasing the risk of atherosclerosis. Therefore, our purpose was to evaluate the impact of dapagliflozin on glucose excursions and related proatherogenic changes in the aortic wall.

Methods and materials: Animal model of type 2 diabetes rich-fat/STZ rats was used. Wistar rats were randomized into 3 groups: dapagliflozin-treated with glucose excursions, placebo-treated with glucose excursions and placebo-treated with stable diabetes. Dapagliflozin was administered once a day, 1 mg/kg, for 8 consecutive weeks. Glucose levels were measured twice a week at fasting and postprandially. The samples of aortas were taken for histopathological and immunochemistry examinations at the end of the experiment. The derangement in the aortic wall and the distribution of $CD68^+$ cells in the aorta were considered early signs of atherosclerosis.

Results: Dapagliflozin reduced glucose excursion to the level characteristic for stable, well-controlled diabetes. It was related to a significant decrease in histopathological changes which were observed in the placebo-treated rats with glucose variability. Dapagliflozin significantly reduced also the accumulation of $CD68^+$ macrophages in the aortic adventitia.

Conclusion: Dapagliflozin provides not only mere beneficial regulation of metabolic status with the depletion of glucose variability, but is also helpful in the prevention of early atherosclerosis related to the course of diabetes type 2.

1. Introduction

According to recent reports, exacerbation of glycemic variability in the course of diabetes has a deleterious effect on the cardiovascular risk (Di Flaviani et al., 2011; Tang et al., 2016) which is even more significant than the influence of chronic hyperglycemia (Ceriello et al., 2008). High mean amplitude of glycemic excursions is associated with an endothelial dysfunction (Suh and Kim, 2015) and autonomic neuropathy (Xu et al., 2016), which may lead to exercising intolerance, asymptomatic ischemia and myocardial infarction (Vinik et al., 2003). Moreover, high inpatient glycemic variability increases the number of cardiovascular events in patients with acute ischemic stroke (Yoon et al., 2017) and patient mortality (Timmons et al., 2016). Glucose variability is associated with both postprandial glucose peaks, blamed for atherogenic action, and increased incidence of hypoglycemia, that leads to a release of inflammatory cytokines, neutrophil and platelet activation, as well as increased adrenaline secretion with consequent arrhythmia and cardiac workload (Suh and Kim, 2015). Interestingly, it was found that dapagliflozin reduces mean amplitude of glycemic excursion in type 2 diabetic patients and this effect is related to the attenuation of the oxidative stress (Li et al., 2016).

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The ability to improve glycemic control up to the point of achieving individual therapeutic goals is essential for the therapy of diabetes, however, the modern treatment should also reduce diabetic complications, among which cardiovascular disease plays a very significant role (Rydén et al., 2013).

Dapagliflozin is an oral anti-hyperglycemic drug, classified as a selective sodium-glucose cotransporter 2 inhibitor (SGLT2-inhibitor). Its mode of action – inhibition of glucose reabsorption in the renal proximal tubule – leads to intensified glucose and sodium excretion, loss of caloric value and, ultimately, loss of body weight and lowering of blood pressure (Vivian, 2014). It is very possible that dapagliflozin also decreases cardiovascular risk, as it has been suggested by recent studies (Persson et al., 2017).

However, there are only few data confirming preventive effects of dapagliflozin on the cardiovascular system and explaining the potential mechanisms of this effect. Mostly, there are available some indirect data which support the idea that dapagliflozin reduces cardiovascular risk e.g. it was documented that empagliflozin – another SGLT2-inhibitor – reduces the risk of death due to cardiovascular reasons by about one-third (Fitchett et al., 2016).

Moreover, dapagliflozin affects several risk factors for atherosclerosis, but the studies investigating the effects of dapagliflozin on the visceral fat, insulin sensitivity, serum lipids, inflammation and adipocytokines are very limited (Yanai et al., 2016).

Taking into consideration the data mentioned above, it seems to be very possible that the attenuation of daily glucose fluctuations might be a plausible mechanism by means of which dapagliflozin influences the development of atherosclerosis. The following study was designed to estimate the impact of dapagliflozin on glucose variability in the animal model of type 2 diabetes and its relationship to the early stages of atherosclerosis expressed by the accumulation of macrophages in the adventitia of the aorta (Kortelainen and Porvari, 2014).

2. Material and methods

2.1. Animals and induction of type 2 diabetes

The experiment on animals was performed in accordance with the Directive 2010/63/EU of the European Parliament and of the European Council and with the Polish governmental regulations (2005.01.21), and was approved by the Local Ethics Committee on the Use of Laboratory Animals in Poznan, Poland (No. 54-55/2013). All animals received humane care and study protocols complied with the institution's guidelines.

The experiment was performed on 8-week-old male Wistar rats weighting initially 312 ± 21 g. The animals were housed in the Department of Pharmacology's Animal House in an environmentally controlled room at 22 °C with reversed 12:12 h light:dark cycle. All animals received water *ad libitum*.

After 1 week of acclimatization, the animals were randomized into 3 groups: a dapagliflozin-treated type 2 diabetes model group with glucose excursions (Dapa-T2D-GE), n = 15, a placebo-treated type 2 diabetes model group without glucose excursions (T2D-noGE), n = 15 and a placebo-treated type 2 diabetes model group with glucose excursions (T2D-GE), n = 15.

The type 2 diabetes model was induced by 4 weeks of feeding with a high fat diet (Labofeed B, 61% of fat) coupled with an intraperitoneal injection of 20 mg/kg streptozotocin. The intragastric glucose tolerance test was performed to confirm diabetes. After 12 h of overnight food deprivation the rats were given 1 g/kg of glucose as a solution via oral gavage. Glucose was measured at 0 and 1 h in samples obtained from superficial capillary vessels on the tip of rats' tail. The animals showing fasting glucose $\geq$ 126 mg/dl (7.0 mmo/l) and/or glucose concentration measured 1 h after glucose solution administration $\geq$ 140 mg/dl (7.8 mmol/l) were designated to the study. The rats with glucose levels > 200 mg/dl (11.1 mmol/l) and/or animals with clinical symptoms of hyperglycemia were excluded from the study.

Glucose excursions were stimulated by limiting the access to the standard chow except for 2 h in a day: between 11.00 and 12.00 am and between 5.00 and 6.00 pm. The animals from the T2D-noGE group were receiving standard chow *ad libitum*. The rats from the Dapa-T2D-GE group were receiving 1 mg/kg dapagliflozin suspended in 1% methylcellulose solution via oral gavage for 8 weeks. The T2D-noGE and T2D-GE groups were receiving the same volume of 1% methylcellulose at the same time as placebo. Blood glucose was measured 4 times a day: at 10.00 am as fasting glycemia (FG); at 1.00 pm as postprandial glycemia no. 1 (postG1); at 5.00 pm as preprandial glycemia (preG) and at 7.00 pm as postprandial glycemia no. 2 (postG2).

After 8 weeks, the animals were decapitated and the samples of blood and tissue were collected for further analyses.

2.2. Analyses

2.2.1. Glycemia, insulin and C-peptide serum levels

Glucose concentration in the blood samples was measured using a glucometer (Diagnostic GOLD Strip/System; Diagnosis S. A.; Poland).

Insulin and C-peptide in the serum were measured with rat specific ELISA (Insulin rat Elisa, Demeditec Diagnostics; C-peptide rat Elisa, R&D Systems).

2.2.2. Histopathology

On ventral position the chest of the animal was opened with incision, aorta was traced and a distance of 2 cm was dissected away. Isolated tissues were fixed in 10% buffered formalin. The sections were made and stained with Haematoxylin and Eosin (H&E stain) and were observed for early atherogenic/atherosclerotic changes in the aorta.

2.2.3. Immunochemistry

Staining and immunohistochemical analysis were performed on the paraffin-embedded tissue samples from the aortic wall to determine morphological changes and localize the distribution of the accumulation of macrophages. Macrophages were identified using mouse anti rat–CD68 antibody (dilution 1/50, Serotec MCA341GA). Endogenous peroxidase activity was quenched and the antigen was retrieved by treating the paraffin-embedded sections with sodium citrate 10 mmol/L, pH 6 (Sigma-Aldrich). Immunostaining employed appropriate biotinylated secondary antibodies (1/200, Vector laboratories, Burlingam, CA), streptavidin–horseradish peroxidase (Vectastain ABC kit, Vector laboratories), and the 3-amino-9-ethylcarbazole substrate–chromogen system (Sigma-Aldrich) for visualization. Finally, the slides were mounted with Glycergel (DAKO Corporation) and analyzed under a light microscope. Negative controls were tested both by omitting the primary antibody, as well as replacing it with an unrelated primary antibody. Both sets of controls yielded negative results, as expected.

2.3. Calculations

2.3.1. Glucose excursions

Glucose excursions were evaluated by measuring mean daily differences between pre-prandial and post-prandial glucose levels (exactly: between FG and postG1 and between preG and postG2).

Glucose excursions were then expressed then in the following ways:

(1) as a mean calculated from the sum of differences between preprandial FG and postprandial postG1 glycemia, as below

$$\text{mean} = \frac{\sum_{i=1}^{n}(\text{postG1}_n - \text{FG}_n)}{n}$$

(2) as a mean calculated from the sum of differences between preprandial preG and postprandial postG2 glycemia, as below

$$\text{mean} = \frac{\sum_{i=1}^{n}(\text{postG2}_n - \text{preG}_n)}{n}$$

(3) as a total mean calculated from the sum of all differences between preprandial and postprandial glycemia, according to the formula below

$$\text{total mean} = \left[\frac{\sum_{i=1}^{n}(\text{postG1}_n - \text{FG}_n) + (\text{postG2}_n - \text{preG}_n)}{2}\right] : n$$

where:
FG – fasting glycaemia (measured at 10.00 am)
postG1 – postprandial glycaemia no. 1 (measured at 1 pm)
preG – preprandial glycaemia (measured at 5.00 pm)
postG2 – postprandial glycaemia no. 2 (measured at 7 pm)

(4) as mean pre-prandial (FG, preG) and post-prandial (postG1, postG2) glucose levels observed in each group at the onset (at the 1st day) and at the end (at the 56th day) of the experiment
(5) as the sum of daily differences between pre-prandial and post-prandial glycemia according to the formula:
daily differences = (postG1-FG) + (postG2-preG)

2.3.2. $HOMA_{IR}$ (Homeostatic Model Assessment of Insulin Resistance)

$HOMA_{IR}$ (Homeostatic Model Assessment of Insulin Resistance) index was used to measure insulin resistance, according to the formula proposed by Matthews et al. (Matthews et al. 1985).

2.3.3. Statistical calculations

Statistical calculations were performed with STATISTICA v. 12 on the license owned by Poznan University of Medical Sciences. All data is shown as mean ± standard deviation and (median). ANOVA or ANOVA Kruskall-Wallis tests were used to compare three or more groups of independent values with normal or non-normal distribution, respectively. Friedmann ANOVA analysis was performed to compare dependent values with non-normal distribution, while t-Student test for the comparison of two dependent values with normal distribution. Differences were considered significant, if p-value was ≤ 0.05.

3. Results

3.1. Characteristics of studied groups

There were no differences in the final body weight and C-peptide serum levels among all groups, however $HOMA_{IR}$ was lower for Dapa-T2D-GE group in comparison to both T2D-GE and T2D-noGE (Table 1).

3.2. Glucose excursions

Fasting glycemia (FG) values at the onset of the experiment (day 1st) in the Dapa-T2D-GE group were significantly higher in comparison to the T2D-noGE and to T2D-GE groups, while at the end of the experiment (day 56th), the FG values were comparable in all groups. Postprandial glycemias at 1.00 pm (postG1) and at 7.00 pm (postG2), both at the onset and at the end of the experiment, were similar in the Dapa-T2D-GE and in the T2D-noGE groups and were significantly higher in the T2D-GE group. Preprandial glycemia (preG) at the beginning of the experiment was higher in the Dapa-T2D-GE group that in other groups, while at the end of the experiment preprandial glycemia was comparable in all groups (Table 2 and 3).

The comparison of FG, preG, postG1, post G2 values between the onset and the end of the experiment within examined groups revealed the significant decrease in Dapa-T2D-GE group for all these parameters, while in T2D-GE and T2D-noGE groups the sig-

Table 1
The general characteristics of the examined groups.

Dapa-T2D n = 15 mean ± SD	T2D-noGE n = 15 mean ± SD	T2D-GE n = 15 mean ± SD
Final body weight (g)		
555.5 ± 26.5	550.9 ± 20.5	558.5 ± 22.1
C- peptide (ng/ml)		
0.8 ± 0.4	1.0 ± 0.2	1.1 ± 0.3
$HOMA_{IR}$		
4.8 ± 1.6[a]	7.1 ± 2.2	8.6 ± 1.5

[a] Statistically significant against other groups; $p \leq 0.05$ (ANOVA Kruskall-Wallis test).

Table 2
The comparison of the glucose serum concentration at the onset (at the 1st day) of the experiment between the examined groups.

Dapa-T2D n = 15 mean ± SD (median) mmol/l	T2D-noGE n = 15 mean ± SD (median) mmol/l	T2D-GE n = 15 mean ± SD (median) mmol/l
FG - 1st day	FG -1st day	FG - 1st day
7.8 ± 1.2 (7.8)[a]	6.8 ± 0.5 (6.8)	6.7 ± 0.5 (6.7)
preG - 1st day	preG - 1st day	preG - 1st day
7.8 ± 1.2 (7.8)[a]	6.9 ± 1.0 (6.9)	6.5 ± 0.5 (6.5)
postG1- 1st day	postG1- 1st day	postG1- 1st day
9.1 ± 1.0 (9.1)	8.3 ± 0.6 (8.3)	10.7 ± 1.2 (10.7)[a]
postG2- 1st day	postG2- 1st day	postG2- 1st day
9.0 ± 1.5 (9.0)	8.7 ± 0.5 (8.6)	11.3 ± 1.6 (11.3)[a]

FG – fasting glycemia measured at 10.00 am.
postG1 – postprandial glycemia measured at 1.00 pm.
preG – preprandial glycemia measured at 5.00 pm.
postG2 – postprandial glycemia measured at 7.00 pm.
[a] Statistically significant against other groups; $p \leq 0.05$ (ANOVA test).

Table 3
The comparison of the glucose serum concentration at the end (at the 56th day) of the experiment between the examined groups.

Dapa-T2D n = 15 mean ± SD (median) mmol/l	T2D-noGE n = 15 mean ± SD (median) mmol/l	T2D-GE n = 15 mean ± SD (median) mmol/l
FG – 56th day	FG -56th day	FG - 56th day
6.9 ± 0.9 (6.9)	6.8 ± 0.7 (7.3)	6.8 ± 0.3 (6.8)
preG - 56th day	preG - 56th day	preG - 56th day
6.8 ± 3.4 (6.8)	6.6 ± 1.0 (7.2)	6.7 ± 0.4 (6.7)
postG1- 56th day	postG1- 56th day	postG1- 56th day
7.5 ± 1.4 (7.5)	7.3 ± 0.7 (7.4)	13.3 ± 1.0 (13.2)[a]
postG2- 56th day	postG2- 56th day	postG2- 56th day
7.7 ± 3.4 (7.7)	6.6 ± 0.5 (7.0)	11.3 ± 0.8 (11.4)[a]

FG – fasting glycemia measured at 10.00 am.
postG1 – postprandial glycemia measured at 1.00 pm.
preG – preprandial glycemia measured at 5.00 pm.
postG2 – postprandial glycemia measured at 7.00 pm.
[a] Statistically significant against other groups; $p \leq 0.05$ (Kruskall-Wallis ANOVA or ANOVA tests).

nificant decrease considered only postG1 and postG2 ($p \leq 0.05$; Wilcoxon or t-Student tests).

The excursions in the serum glucose expressed as a mean calculated separately from the sum of differences between postprandial (postG1, at 1.00 pm) and fasting conditions (FG, at 10.00 am) or from the sum of differences between postprandial (postG2, at 7.00 pm) and preprandial conditions (preG, at 5.00 pm), as well as calculated as a total mean of the sum of all differences between preprandial and postprandial glycemia in the T2D-GE group, were significantly higher than in the T2D-noGE and Dapa-T2D-GE groups (Table 4).

Fig. 1 presents mean daily differences in glucose concentrations on each measurement day in each examined group.

Table 4
Glucose excursions expressed as a mean calculated from the sum of all differences between preprandial and postprandial glycemia.

Dapa-T2D n = 15 mean ± SD	T2D-noGE n = 15 mean ± SD	T2D-GE n = 15 mean ± SD
a mean calculated from the sum of differences between preprandial FG and postprandial postG1 glycemia (mmol/l)		
1.0 ± 0.5	0.2 ± 0.3	4.1 ± 0.9[a]
a mean calculated from the sum of differences between preprandial preG and postprandial postG2 glycemia (mmol/l)		
0.6 ± 0.5	0.9 ± 0.2	4.7 ± 1.2[a]
a total mean calculated from the sum of all differences between preprandial and postprandial glycemia (mmol/l)		
0.8 ± 0.4	0.5 ± 0.2	4.4 ± 0.8[a]

[a] Statistically significant against other groups; $p \leq 0.05$ (ANOVA test).

3.3. Histopathology

The aortic sections were studied under the 200X Olympus photographic microscope and the following features in the aortic wall were considered: the status of the nuclei, the nuclei arrangement in the endothelium and in the smooth muscles, the arrangement of elastic fibers and smooth muscles in the media, the composition and components of perivascular adipose tissue and the localization of inflammatory cells. The gravest changes affecting the aortic wall were found in the T2D-GE group, much less advanced in the T2D-noGE and Dapa-T2D-GE groups – see details in Fig. 2.

3.4. CD68$^+$ cells (macrophages) localization

Immunostaining with anti-CD68, the marker of monocytes/-macrophages, revealed a lower amount of CD68$^+$ cells in the adventitia of the aorta from the Dapa-T2D-GE group, as compared to the T2D-noGE and to the T2D-GE groups (mean 1.1 ± 1.0, median 1.0 versus mean 2.7 ± 0.8, median 3.0 and mean 3.6 ± 1.5, median 4.0 cells/microscope field). These cells in the last two groups usually persist as single infiltrating cells and small clusters localized mainly around the *vasa vasorum*. Taking into consideration

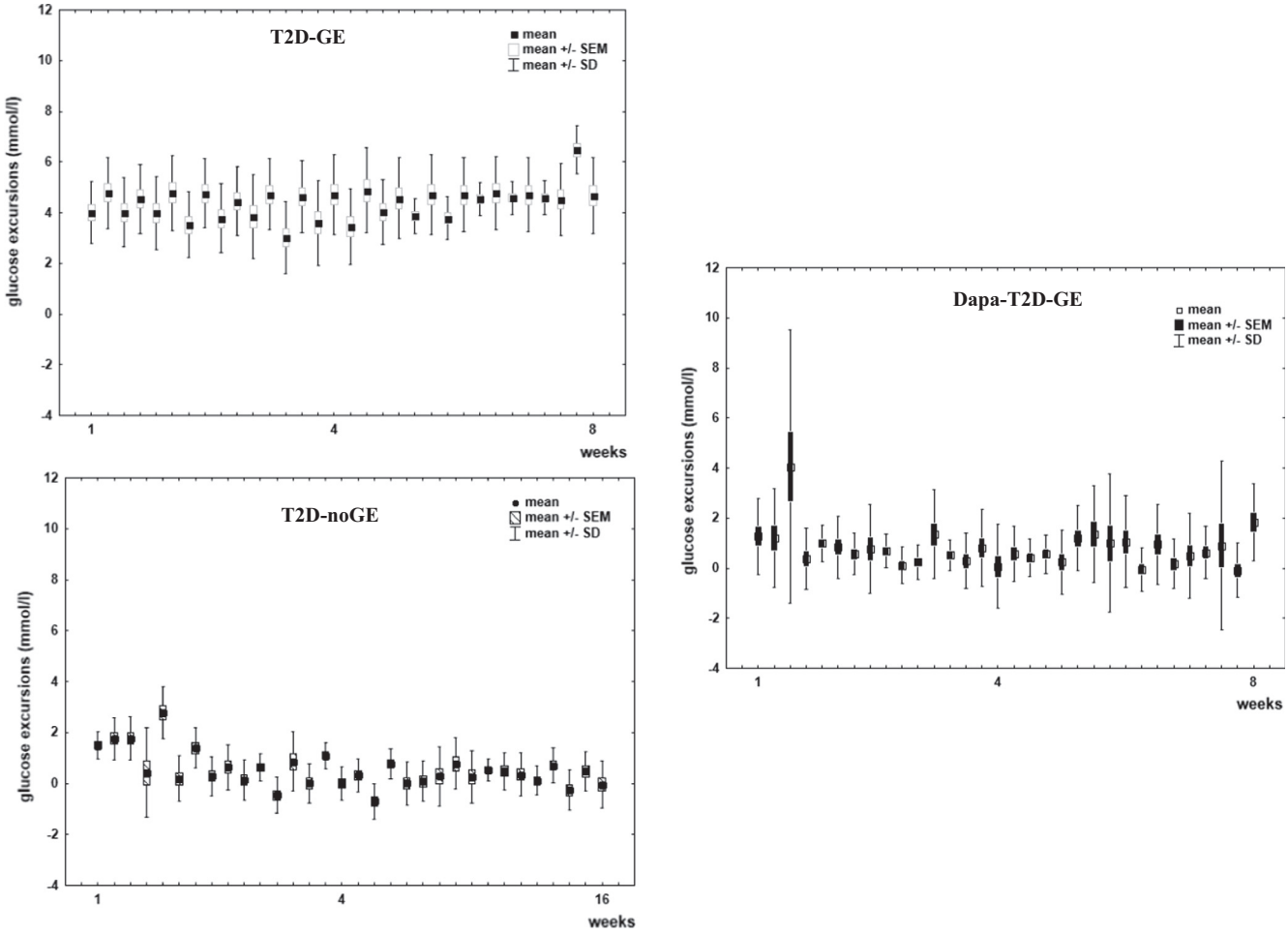

Fig. 1. Glucose excursions expressed as the sum of daily differences between preprandial and postprandial glycemia according to the formula: daily differences = (postG1-FG) + (postG2-preG). Glucose was measured 2 times a day, 2 days a week within 8 weeks. Each box expresses a daily differences found on each measure day.

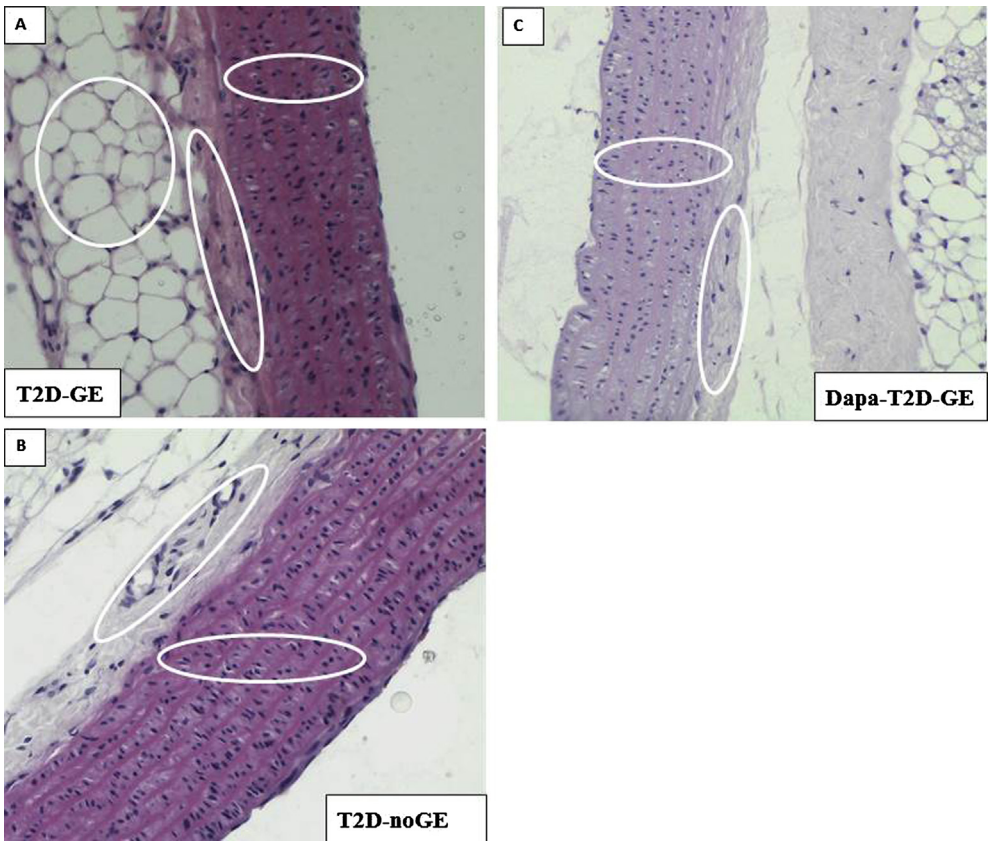

Fig. 2. Rat's aorta - H&E stain. A – T2D-GE group. The nuclei of endothelial cells are arranged in various directions, protruded into the lumen of the aorta. Endothelial nuclei are activated with a significant amount of euchromatin. The nuclei of smooth muscles are arranged in various directions, elastic fibers are wrinkled. The adipose tissue disseminates adventitia with dominant white adipose tissue. Many inflammatory cells are seen in the adipose tissue. B – T2D-noGE group. The nuclei of endothelial cells have mostly a regular shape and occur in a parallel arrangement; a limited focal activation of the nuclei is visible. The nuclei of smooth muscles are linearly arranged with no activation; the parallel arrangement of elastic fibers is found. The adipose tissue is localized not very close to the adventitia, single white adipose tissue cells with the dominating brown adipose tissue cells are seen. Single inflammatory cells are present in the adventitia. C – Dapa-T2D-GE group. The nuclei of endothelial cells have a regular shape and occur in a parallel arrangement; there is no activation of the nuclei. The nuclei of smooth muscles are linearly arranged with no activation; the parallel arrangement of elastic fibers is found. The adipose tissue is localized not very close to the adventitia, single white adipose tissue cells are found, with the dominating brown adipose tissue cells. No inflammatory cells are observed in the adventitia.

exclusively the T2D-noGE and T2D-GE groups, the increased count of the macrophages in the adventitia was observed in the T2D-GE group, as compared to the T2D-noGE group (Fig. 3).

4. Discussion

The pathophysiological evidence appears to be highly suggestive of glycemic excursions being an important key determinant of vascular damage (Standl et al., 2011).

The results of the present study showed that dapagliflozin effectively attenuated glucose excursions in animals with type 2 diabetes to the level characteristic of the well-controlled, stable diabetes with no glucose fluctuations. This effect is mostly dependent on the decrease in postprandial glycemia, but partially depends also on the decrease in preprandial glucose levels. A similar effect was found earlier by Li et al. (Li et al., 2016) in humans with type 2 diabetes. These authors indicated also that decreased glycemic variations related to dapagliflozin activity were closely associated to the limited production of free radicals.

This phenomenon might be the base for the beneficial influence of dapagliflozin on the aortic wall found by us in rats with induced glucose excursions. Since in the recent years the glycemic variability need for personalized treatment of hyperglycemia has been advocated (Raz et al., 2013), the confirmation that dapagliflozin may be helpful to achieve this goal is especially useful for the clinical practice.

On the other hand, it was observed that insulin resistance – one of two fundamental pathologies in the course of type 2 diabetes – was lower in dapagliflozin-treated group comparing to other groups. It allows to suppose that lower insulin resistance could be associated with the beneficial influence of dapagliflozin on early proatherogenic derangement in the aortic wall. While C-peptide serum level – an indicator of beta-cells function – did not differ within all examined groups, $HOMA_{IR}$ index was significantly lower in dapagliflozin-treated group. It is well known that insulin resistance can promote both atherogenesis and advanced plaque progression (DeFronzo (2010). Moreover, the oxidative stress, closely related to glucose variability, increases insulin resistance (Saisho, 2014).

Moreover, the present study proved also that dapagliflozin implies beneficial effects on glucose excursions that are closely related to the prevention of vascular dysfunction in the aortic wall, such as an irregular shape of nuclei in the endothelial and in the smooth muscles cells, irregular arrangement of both endothelial cells and smooth muscles cells, irregular arrangement of elastic fibers in the intima, disseminated adipose tissue in the adventitia with the dominant white adipose tissue, and numerous foci of inflammatory cells in the adventitia. The dapagliflozin administration prevented the development of all of the derangements con-

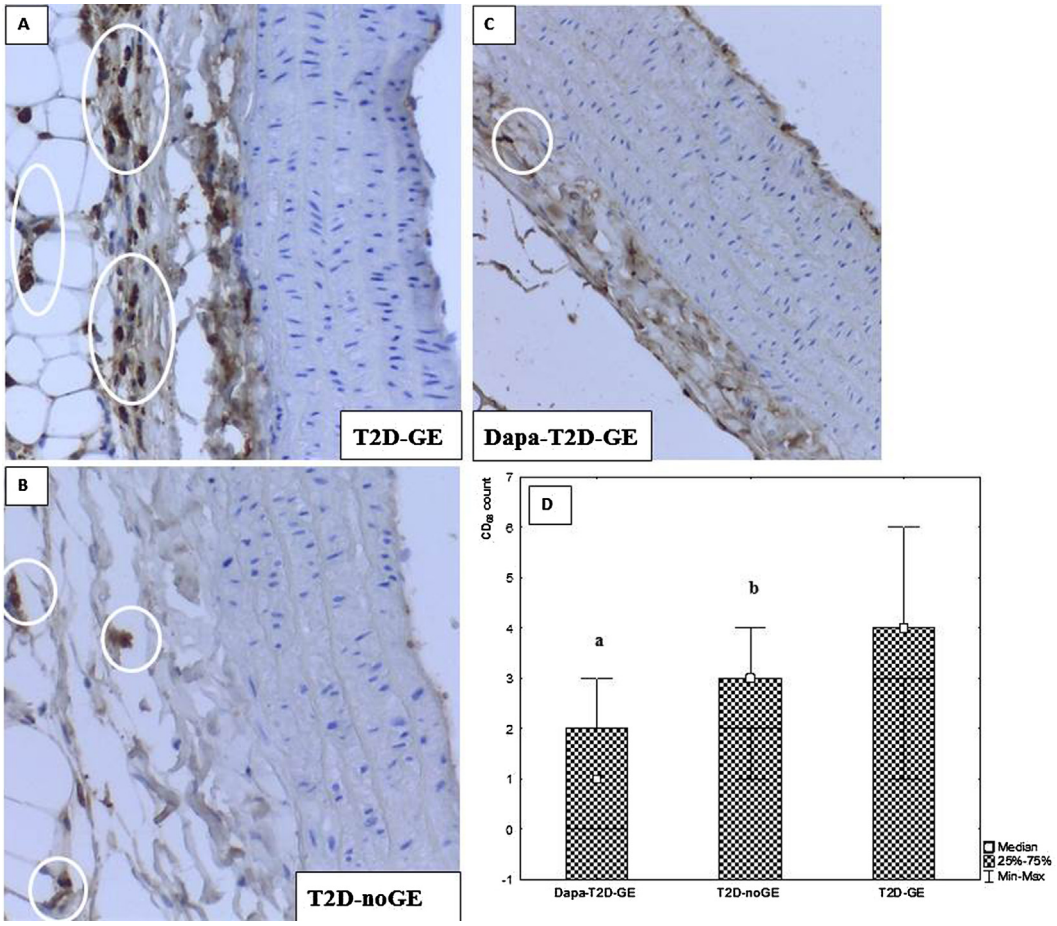

Fig. 3. Rat's aorta - CD68$^+$ cells distribution and count. A – Monocytes/macrophages CD68$^+$ in the adventitia in the T2D-GE group. B – Monocytes/macrophages CD68$^+$ in the adventitia in the T2D-noGE group. C – Monocytes/macrophages CD68$^+$ in the adventitia in the Dapa-T2D-GE group. Photomicrographs of the rat aortas were captured under 200X magnification, scale bar: 1 cm = 100 um. D – The CD68$^+$ cells count in the microscope field of view. a – statistically significant against other groups; $p \leq 0.05$. The comparison was carried out using the ANOVA Kruskall-Wallis test, with data expressed as median ± quartile 1 and quartile 3 values. b – statistically significant against the T2D-noGE; $p \leq 0.05$. The comparison between the T2D-noGE group and T2D-GE groups was carried out using the Mann-Whitney test, with data expressed as median ± quartile 1 and quartile 3 values.

cerning the aortic wall in rats with diabetes and induced glucose excursion.

The progression of atherosclerosis in our experiment occurred at a very early stage, because the only risk factor for its development was hyperglycemia (more precisely, glucose excursions) and the total time of the main study was no longer than 2 months. Nevertheless, we found the symptoms of pathologic activation of many cells and tissues in the aortic wall. The most interesting observation in this matter was the increased infiltration of the adventitia by the macrophages. While the distribution of CD68$^+$ macrophages into the intima was not very intensive in rats with glucose excursion, there was a significant accumulation of CD68$^+$ cells into the adventitia. The increased concentration of macrophages in the adventitia was revealed in a very advanced stage of atherosclerosis, however, this phenomenon was also correlated with its very early stages (Kortelainen and Porvari, 2014; Tavora et al., 2010).

The strength of our study is that we have proved first dapagliflozin-related beneficial effect on glucose fluctuation at very early stage of atherosclerosis. This observation suggests that dapagliflozin could protect from macroangiopathy development. This study has limitation to consider. However the model of type 2 diabetes used for the experiment mimics very well two main pathomechanisms of diabetes: beta-cells dysfunction and increased insulin resistance, the obtained results cannot be extrapolated directly to humans. The course of diabetes according to this model does not have the disease phase with hyperinsulinaemia. It does not influence the value of our results but does not give us the possibility to explain how dapagliflozin acts in terms of hyperinsulinaemia.

5. Conclusion

Dapagliflozin, by reducing glucose variability, decreased at the same time the accumulation of CD68$^+$ cells in the adventitia and this effect was even more evident than the one observed in a rat model of well-controlled stable diabetes without glucose excursions (T2D-noGE).

Considering the effects of glucose excursions on the aortic wall seen in the histopathologic examination and especially the decrease in macrophages concentration in the adventitia, it could be concluded that dapagliflozin not only provided mere beneficial regulation of the metabolic status with a depletion of glucose variability, but was helpful for the prevention of early atherosclerosis related to the course of diabetes type 2.

6. Statement on the welfare of animals

All procedures and protocols adhered to the Guide for the Care and Use of Laboratory Animals from the Institute for Laboratory

Animal Research, National Research Council, Washington, D.C., National Academy Press, 2011. Procedures and protocols were in accordance with the Polish governmental regulations (2005.01.21) and were approved by the Local Ethics Committee on the Use of Laboratory Animals in Poznan, Poland (permissions 25/2013 and 53/2013).

Conflicts of interest

None.

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