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MIF/CD74 axis is a target for metformin therapy in diabetic podocytopathy — real world evidence

Oś MIF/CD74 jako cel terapii metforminą w podocytopatii cukrzycowej — rzeczywista praktyka kliniczna

Yan Xing[1, 2], Shandong Ye[2], Yan Chen[2], Aihong Fan[2], Zhuohua Xu[2], Wen Jiang[2]

[1]*Shandong University, Jinan, China*
[2]*Department of Endocrinology , Anhui Provincial Hospital, Hefei, 230001, Anhui Province of China*

Abstract

Introduction: To observe the effects of metformin on urinary excretion of MIF, CD74, and podocalyxin in type 2 diabetics, and to explore its possible renoprotective mechanisms.

Material and methods: A total of 202 uncontrolled type 2 diabetics, who were previously prescribed sulfonylurea monotherapy (n = 100) or sulfonylurea in combination with metformin (n = 102), were enrolled in the study. The amount of macrophage migration inhibitory factor (MIF) and CD74 in serum, urinary MIF-to-creatine ratio (UMCR), urinary CD74-to-creatine ratio (UCCR), urinary albumin-to-creatine ratio (UACR), and urinary podocalyxin-to-creatine ratio (UPCR) were determined.

Results: Metabolic parameters including fasting blood glucose, postprandial two hours blood glucose, haemoglobin A1c, MIF, and CD74 in serum were comparable between the two groups. Moreover, metformin add-on therapy showed significantly better efficacy in reducing UMCR, UCCR, UPCR, and UACR in comparison with those in the sulfonylurea monotherapy group, respectively. UPCR had a positive correlation with UACR, UMCR, and UCCR ($r = 0.73$, $r = 0.69$, $r = 0.62$, $P < 0.01$), respectively.

Conclusions: Metformin could present its podocyte-protective capacity in type 2 diabetics, and the underlying mechanisms may be partly attributed to its effects in suppressing MIF-CD74 axis-mediated inflammatory cascade response. **(Endokrynol Pol 2018; 69 (3): 264–268)**

Key words: *metformin, type 2 diabetes, macrophage migration inhibitory factor, CD74, podocyte*

Streszczenie

Wstęp: Celem pracy było zaobserwowanie efektów działania metforminy na wydalanie MIF, CD74 i podokaliksyny w moczu pacjentów z cukrzycą typu 2 oraz zbadanie jej możliwych mechanizmów nefroprotekcyjnych.

Materiał i metody: W badaniu wzięło udział 202 pacjentów z niewyrównaną cukrzycą typu 2, którym zalecono wcześniej monoterapię sulfonylomocznikiem (n = 100) lub sulfonylomocznikiem skojarzonym z metforminą (n = 102). Podczas badania określono ilość czynnika hamującego migrację makrofagów (MIF) i CD74 w surowicy krwi, wskaźnik MIF/kreatynina (*urinary MIF to creatinine ratio*; UMCR), wskaźnik CD74/kreatynina (*urinary CD74 to creatinine ratio*; UCCR), wskaźnik albumina/kreatynina (*urinary albumin to creatinine ratio*; UACR) oraz wskaźnik podokaliksyna/kreatynina (*urinary podocalyxin to creatinine ratio*; UPCR).

Wyniki: Parametry metaboliczne, w tym glikemia na czczo, stężenie glukozy we krwi dwie godziny po posiłku, hemoglobina A1c, MIF i CD74 w surowicy krwi były porównywalne w obu grupach. Ponadto, terapia z dodatkiem metforminy wykazała znacznie lepszą skuteczność w redukowaniu wskaźników UMCR, UCCR, UPCR i UACR w porównaniu z grupą, w której zastosowano monoterapię sulfonylomocznikiem. Wskaźnik UPRC wykazywał dodatnią korelację ze wskaźnikami UACR, UMCR i UCCR (odpowiednio: $r = 0{,}73$, $r = 0{,}69$, $r = 0{,}62$, $p < 0{,}01$).

Wnioski: Metformina może wykazywać zdolność do ochrony podocytów u pacjentów z cukrzycą typu 2, a mechanizmy leżące u podstaw tego procesu mogą być częściowo przypisane jej właściwościom hamowania kaskady reakcji zapalnych zależnych od osi MIF-CD74. **(Endokrynol Pol 2018; 69 (3): 264–268)**

Słowa kluczowe: *metformina, cukrzyca typu 2, czynnik hamujący migrację makrofagów, CD74, podocyt*

Introduction

Apart from metabolic and haemodynamic abnormalities, both enhanced inflammation reaction in local renal tissue and podocyte injury participate in the progression of diabetic nephropathy (DN) [1, 2]. For decades, physicians have attempted to present microalbuminuria as a clinical marker for DN. However, by the time renal dysfunction becomes apparent, podocyte injury has already occurred [3]. Emerging evidence has shown that podocyte-related proteins, such as podocalyxin [4], nephrin, shed in the urine may facilitate earlier detection of disease and allow closer monitoring of response to therapy [5, 6].

Macrophage migration inhibitory factor (MIF), a pleiotropic inflammatory mediator, is quasi-constitutively

Shandong Ye, Ph.D, Department of Endocrinology, Anhui Provincial Hospital, Hefei, 230001, Anhui Province of China; tel.: +86 551 228 3524; fax: +86 551 228 3524, e-mail: ysd196406@163.com

expressed by a variety of different cells and tissues, including immune and nonimmune cells like podocyte, mesangial cells in kidney [7]. It is the only cytokine that is stored in the secretary cells and is thus secreted rapidly on stimulation of CD74, and it was the first membrane receptor for MIF that was discovered; meanwhile, MIF and its homolog D-dopachrome tautomerase are the only ligands of CD74 [8]. Current research in vitro demonstrates that MIF exerted proinflammatory effects in podocyte via the CD74 receptor under stimulation [9, 10]. Metformin, a biguanide drug, is widely prescribed to treat high blood glucose in individuals with type 2 diabetes mellitus [11].

Despite the long clinical experience with metformin and active investigation in this area, its exact mechanisms of action remain unclear. Hence, by virtue of analysis the changes of a series of cytokines and podocalyxin in urine, the current study aimed to test the hypothesis that podocyte injury was associated with the inflammatory processes, and the podocyte-protection of metformin was related to its inhibiting role of MIF--CD74 axis in diabetes mellitus.

Material and methods

Study design and participants

A total of 202 uncontrolled type 2 diabetics, who were previously prescribed sulfonylurea monotherapy (group I, n = 100) and sulfonylurea in combination with metformin (group II, n = 102) were enrolled in the research. According to physician's prescription, the participants received sulfonylureas such as glimepiride (dosage range, 2 to 6 mg daily) or gliclazide (dosage range, 80 to 240 mg daily), or add-on extended-release metformin (500 mg/day, up-titrated weekly to a maximum 2000 mg/ /day) for 24 weeks. Moreover, all the type 2 diabetics were required to maintain their fasting blood glucose range from 4.4 to 8.3 mmol/L and to maintain the two-hour postprandial blood glucose level at less than 10 mmol/L. Consequently, the dosage of each agent was titrated on the basis of each study subject's blood glucose levels.

Exclusion criteria, including patients who: 1. had acute illness or chronic systemic diseases such as hypertension; 2. had primary or secondary renal diseases except DN, abnormal serum creatinine; 3. had inflammatory diseases (including urinary system infection), tumour, or immunity diseases; 4. had acute complications or serious chronic complications of diabetes mellitus such as ketoacidosis, hyperosmolar coma, and lactic acidosis recently; and 5. had statins, aldosterone receptor antagonists, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists in the past two weeks.

Another 100 healthy volunteers were chosen as a normal control group. Informed consent was obtained from all the participants, meanwhile the study protocol was reviewed and accepted by the Ethics Committee of Anhui Provincial Hospital, China. The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Data collection

Clinical data including the age, gender, course of diabetes, body mass index (BMI), and blood pressure were checked for all subjects, and peripheral venous blood samples were obtained. Meanwhile, first morning urine (15 ml) was obtained with a sterile cup and preserved at –40°C to test the concentrations of albumin, MIF, CD74, and podocalyxin.

Laboratory assays

The concentration of MIF and CD74 in serum and urine, as well as urinary podocalyxin were measured by enzyme-linked immunosorbent assay (ELISA) method. Urinary albumin was assayed using immune turbidimetry kits purchased from Northern Biotechnology Research Institute (Beijing, China). Creatine, both in urine and serum, was detected by a Hitachi automatic biochemical analyser, blood glucose measured by glucose oxidase method, and HbA1c determined by high-performance liquid chromatography. The urine samples from each patient were assayed in triplicate on the same plate. To eliminate the impact of urine volume, the levels of urinary albumin, MIF, CD74, and podocalyxin were expressed relative to the urinary creatinine concentration and referred to as UACR (Urinary albumin/UCr: mg/gCr), UMCR (Urinary MIF/ /UCr: ng/mgCr), UCCR (Urinary CD74/UCr: ng/mgCr), and UPCR (urinary podocalyxin/UCr, ng/mgCr), respectively.

Statistical analysis

Data were expressed as the means ± SD and were analysed by Statistical Package for the Social Sciences 13.0. Some non-normally distributed variables have to be compared with parametric tests after log-transformation. All the parameters between two groups were assessed by independent-sample T test. Correlations between UPCR and clinical variables were examined by spearman correlation. P-values < 0.05 were considered to be statistically significant.

Results

Comparison of biochemical parameters between diabetic subjects and control subjects

As shown in Table I, anthropologic and laboratory characteristics including age, gender, BMI, blood pressure, and serum creatinine are comparable among the

Table I. *Clinical indexes between sulfonylurea monotherapy group and metformin-add on therapy group*

Tabela I. *Wskaźniki kliniczne między grupą pacjentów, u której zastosowano monoterapię sulfonylomocznikiem a grupą, u której zastosowano terapię sulfonylomocznikiem skojarzonym z metforminą*

Variable	Normal control	Study groups	
		Sulfonylurea monotherapy group	Metformin add-on therapy group
Age (y)	51.77 ± 9.26	56.31 ± 2.80	57.16 ± 3.87
Number (Male/Female)	100 (49/51)	100 (49/51)	102 (52/50)
Diabetes duration (year)	—	3.34 ± 0.69	3.45 ± 0.51
BMI [kg/m^2]	23.74 ± 4.29	24.77 ± 3.36	23.84 ± 4.01
SBP [mmHg]	124.55 ± 12.28	126.11 ± 10.23	124.89 ± 9.46
DBP [mmHg]	74.27 ± 6.84	72.11 ± 7.26	73.18 ± 8.94
SCr [μmol/L]	62.27 ± 9.32	66.12 ± 7.16	64.33 ± 6.29
FBG [mmol/L]	4.89 ± 0.57	6.87 ± 0.69*	6.71 ± 0.73*
PBG [mmol/L]	5.78 ± 0.64	9.71 ± 0.67*	9.46 ± 0.92*
HbA1c (%)	5.21 ± 0.54	7.16 ± 0.73*	7.09 ± 0.66*
Serum MIF-1 [ng/ml]	2.16 ± 0.30	3.39 ± 0.30*	3.21 ± 0.27*
Serum CD74 [ng/ml]	11.53 ± 2.36	16.58 ± 4.11*	15.98 ± 4.28*
UACR [mg/gCr]	13.27 ± 2.41	31.14 ± 8.12*	24.33 ± 6.94*
UMCR [ng/mgCr]	0.51 ± 0.17	2.54 ± 0.29*	2.08 ± 0.23*
UCCR [ng/mgCr]	0.29 ± 0.03	3.44 ± 0.41*	2.92 ± 0.44*
UPCR [ng/mgCr]	0.78 ± 0.23	66.82 ± 12.25*	59.76 ± 11.95*

Values are expressed as $\overline{\chi} \pm S$.
*P < 0.01 versus normal control subjects; P < 0.01 versus sulfonylurea monotherapy group
BMI — body mass index; SCr — serum creatinine; FBG — fasting blood glucose; PBG — postprandial 2 hours blood glucose; HbA1c — glycated haemoglobin A1c; SBP — systolic blood pressure; DBP — diastolic blood pressure; UACR — urinary albumin to creatine ratio; UMCR — urinary MIF to creatinine ratio; UCCR — urinary CD74 to creatinine ratio; UPCR — urinary podocalyxin to creatine ratio

three groups. We found that podocalyxin was barely detectable in the urine specimens from normal control subjects. Moreover, the levels of FBG, PBG, HbA1c, serum MIF, serum CD74, UACR, UMCR, UCCR, and UPCR were significantly higher in diabetic patients (P < 0.01).

Comparison of clinical indexes between the sulfonylurea monotherapy group and the metformin add-on therapy group, respectively

As shown in Table I, compared with the sulfonylurea monotherapy group, metformin add-on treatment significantly decreased UACR ,UMCR, UCCR, and UPCR (P < 0.01, respectively), while there was no significant difference in FBG, postprandial two hours blood glucose, HbA1c, MIF, and CD74 in serum between the two subgroups (P > 0.05).

Table II. *Correlations between urinary podocalyxin(UPCR) and other diabetic characteristics*

Tabela II. *Współzależności między podokaliksyną w moczu (UPCR) i innymi charakterystycznymi cechami dla cukrzycy*

	UPCR	
	r	P
UMCR	0.692*	< 0.01
UCCR	0.736*	< 0.01
UACR	0.727*	< 0.01

UPCR — urinary podocalyxin to creatine ratio; UACR — urinary albumin to creatine ratio; UMCR — urinary MIF to creatinine ratio; UCCR — urinary CD74 to creatinine ratio

Correlations between levels of urinary PCX and serum MIF and CD74, urinary MIF, CD74, and albumin excretion

As shown in Table II, UPCR has a positive correlation with UACR (r = 0.73), UMCR (r = 0.69), and UCCR (r = 0.62), respectively. There is an inferior correlation coefficient (r = 0.31) between urinary MIF concentration and serum MIF content. No correlations were found, however, between UPCR and other clinical variables, such as plasma glucose level or blood pressure.

Discussion

Podocyte injury and albuminuria are hallmarks of DN, which is the major cause of end-stage renal failure globally. However, to date, targeted therapies to halt or prevent these complications are currently unavailable [12]. Consequently, to identify better strategies for the detection of early stages of DN, numerous biomarkers present in urine have been identified that reflect kidney injury at specific sites along the nephron. Podocalyxin (PCX), initially identified in the glomeruli and known as PCX-like protein 1 (PODXL or PCLP1), is the most abundant heavily charged sialomucin prominently expressed by podocyte, whose expression and distribution has been correlated with podocyte development [13]. Alternatively, urinary podocalyxin has been used for reflecting the degree of podocyte injury in multiple reports [14, 15]. Our previous research confirmed that podocalyxin was barely detectable in the urine specimens from normal control subjects, whereas podocalyxin shedding occurs in DN [16]. Furthermore, urinary podocalyxin excretion was positively correlated with UACR (which is one of the earliest clinical manifestation of DN). These findings provide a basis for the involvement of podocalyxin in the occurrence and

development of albuminuria, corroborating previous research [17].

Macrophage migration inhibitory factor (MIF) is a widely expressed pleiotropic cytokine, exhibiting a broad range of immune and inflammatory activities. It can exert the proinflammatory effects in podocyte via the CD74 receptor [9]. In particular, MIF was secreted de novo from injured podocyte both in vitro and in vivo [18]. The contribution of MIF to renal injury could involve promoting inflammatory mediator expression and modulation of apoptosis and cell proliferation [19]. In the present study, we examined the expression of MIF as well as CD74 in type 2 diabetics to clarify the interplay between MIF and CD74 concerning DN progression. We found that both MIF and CD74 in serum and urine specimens were dramatically elevated compared with normal control subjects. Moreover, urinary MIF and CD74 excretion were positively correlated with the grade of podocyte injury. Alternatively, we examined whether elevated urinary MIF excretion reflects MIF expression within the injured kidney, or from the systemic effects of DN. The results showed an inferior correlation coefficient between urinary MIF concentration and serum MIF content ($r = 0.31$), indicating that increased urinary MIF excretion is not simply a reflection of increased clearance of serum MIF. These findings suggest the possibility that the MIF- CD74 axis might be involved in the mechanisms of local inflammation in diabetic podocytopathy, consistent with a proinflammatory state in diabetes [20].

Metformin is the most widely prescribed drug to treat hyperglycaemia in type 2 diabetics and is recommended in conjunction with lifestyle modification (i.e. diet, weight control, and physical activity) as a first-line oral therapy in the recent guidelines of the ADA (American Diabetes Association) and EASD (European Association of the Study of Diabetes) [21]. The potential benefits of metformin therapy in patients with type 2 diabetes would extend beyond its anti-hyperglycaemic effects through improving glucolipid metabolism, decreasing advanced glycation end products (AGE), oxidative stress, and endoplasmic reticulum (ER) stress [22], inhibiting AMPK/mTOR pathway [23], downregulation of phosphorylated p38 mitogen-activated protein kinase (p-p38MAPK) [24], and so on. Zhai et al. [25], from our research group, have demonstrated that metformin could ameliorate podocyte damage by restoring renal tissue podocalyxin expression in type 2 diabetic rats. The ameliorative effect of metformin on diabetes-induced podocyte loss in experimental diabetic models were elaborated. In the present study, following metformin add-on therapy, urinary podocalyxin and albumin excretion were significantly decreased compared to those in the sulfonylurea monotherapy group, which is in agreement with our previous investigation from experimental diabetic models [25]. On the other hand, we found that metformin add-on treatment resulted in a significant fall in urinary MIF and CD74 excretion, which suggests that anti-inflammation mechanisms may be implicated in metformin effects on reducing podocyte injury and albuminuria. Glomerular podocytes are capable of synthesising MIF de novo, the first cytokine discovered more than 45 years ago [26], that could eventually trigger a cell-mediated immune reaction, thus promoting a perpetual cycle of injury. CD74 is a protein trafficking regulator and a cell membrane receptor for MIF [20]. Current studies have confirmed the increased CD74 expression in clinical and experimental DN and localised glomerular CD74 to podocyte surface was activated by MIF, leading to phosphorylation of extracellular signal–regulated kinase 1/2 and p38MAPK [9]. Yao et al. [27] confirmed that metformin could alleviate high glucose-induced oxidative stress and p-p38MAPK protein expression in rat glomerular mesangial cells. According to the previous study [9], we may speculate that metformin therapy was associated with a substantial reduction in urinary MIF and CD74 excretion, which was related with suppression of phosphorylation of extracellular signal–regulated kinase 1/2 and p38 MAPK pathways, thereby downregulating inflammatory cytokines such as TNF-related apoptosis-inducing ligand and monocyte chemoattractant protein-1 expression, and upregulation of podocyte apoptosis. It is worth noting that the results of our study show no difference in glycaemic control between the two groups, implying that metformin was cable of ameliorating podocyte injury and suppression of the local renal inflammation, independently of the hypoglycaemic manner. These findings may recommend the clinical use of metformin in the prevention of DN and improvement of clinical outcomes in patients with kidney injury.

However, our data do not identify the perplexity of whether MIF participate in the initialisation of diabetic podocytopathy or in the progression of DN. The exact interplay between MIF, CD74, and other factors concerning podocytopathy remains to be further elaborated in detail. Our findings raise the possibility of metformin therapy for targeting MIF-CD74 axis-mediated injury in DN. Most patients suffering from diabetes might not only benefit from therapeutic strategies targeting glycaemic control, but also MIF-CD74 axis as well.

Conclusions

In summary, our primary findings demonstrated that metformin could present its podocyte-protective capacity

in type 2 diabetics, and the underlying mechanisms may be partly attributed to its effects in inhibiting MIF-CD74 axis-mediated inflammatory cascade response. More clinical proof concerning the relationship between MIF-CD74 axis and podocytopathy needs to be elaborated in detail.

Conflicts of interest statement

There are no conflicts of interest.

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References

1. Cui S, Zhu Y, Du J, et al. CXCL8 Antagonist Improves Diabetic Nephropathy in Male Mice With Diabetes and Attenuates High Glucose-Induced Mesangial Injury. Endocrinology. 2017; 158(6): 1671–1684, doi: 10.1210/en.2016-1781, indexed in Pubmed: 28387853.
2. Li S, Liu X, Lei J, et al. Crocin Protects Podocytes Against Oxidative Stress and Inflammation Induced by High Glucose Through Inhibition of NF-κB. Cell Physiol Biochem. 2017; 42(4): 1481–1492, doi: 10.1159/000479212, indexed in Pubmed: 28719912.
3. Fiseha T, Tamir Z. Urinary Markers of Tubular Injury in Early Diabetic Nephropathy. Int J Nephrol. 2016; 2016: 4647685, doi: 10.1155/2016/4647685, indexed in Pubmed: 27293888.
4. Ye H, Bai X, Gao H, et al. Urinary podocalyxin positive-element occurs in the early stage of diabetic nephropathy and is correlated with a clinical diagnosis of diabetic nephropathy. J Diabetes Complications. 2014; 28(1): 96–100, doi: 10.1016/j.jdiacomp.2013.08.006, indexed in Pubmed: 24075693.
5. Petrica L, Vlad A, Gluhovschi G, et al. Proximal tubule dysfunction is associated with podocyte damage biomarkers nephrin and vascular endothelial growth factor in type 2 diabetes mellitus patients: a cross-sectional study. PLoS One. 2014; 9(11): e112538, doi: 10.1371/journal.pone.0112538, indexed in Pubmed: 25397960.
6. Pereira SV, Dos Santos M, Rodrigues PG, et al. Increased urine podocyte-associated messenger RNAs in severe obesity are evidence of podocyte injury. Obesity (Silver Spring). 2015; 23(8): 1643–1649, doi: 10.1002/oby.21156, indexed in Pubmed: 26147062.
7. Dandona P, Aljada A, Ghanim H, et al. Increased plasma concentration of macrophage migration inhibitory factor (MIF) and MIF mRNA in mononuclear cells in the obese and the suppressive action of metformin. J Clin Endocrinol Metab. 2004; 89(10): 5043–5047, doi: 10.1210/jc.2004-0436, indexed in Pubmed: 15472203.
8. Chen SJ, Liao DL, Shen TW, et al. Genetic signatures of heroin addiction. Medicine (Baltimore). 2016; 95(31): e4473, doi: 10.1097/MD.0000000000004473, indexed in Pubmed: 27495086.
9. Sanchez-Niño MD, Sanz AB, Ihalmo P, et al. The MIF receptor CD74 in diabetic podocyte injury. J Am Soc Nephrol. 2009; 20(2): 353–362, doi: 10.1681/ASN.2008020194, indexed in Pubmed: 18842989.
10. Hara S, Kobayashi N, Sakamoto K, et al. Podocyte injury-driven lipid peroxidation accelerates the infiltration of glomerular foam cells in focal segmental glomerulosclerosis. Am J Pathol. 2015; 185(8): 2118–2131, doi: 10.1016/j.ajpath.2015.04.007, indexed in Pubmed: 26072030.
11. Viollet B, Guigas B, Sanz Garcia N, et al. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond). 2012; 122(6): 253–270, doi: 10.1042/CS20110386, indexed in Pubmed: 22117616.
12. Fiorina P, Vergani A, Bassi R, et al. Role of podocyte B7-1 in diabetic nephropathy. J Am Soc Nephrol. 2014; 25(7): 1415–1429, doi: 10.1681/ASN.2013050518, indexed in Pubmed: 24676639.
13. Pavenstädt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. Physiol Rev. 2003; 83(1): 253–307, doi: 10.1152/physrev.00020.2002, indexed in Pubmed: 12506131.
14. Skoberne A, Konieczny A, Schiffer M. Glomerular epithelial cells in the urine: what has to be done to make them worthwhile? Am J Physiol Renal Physiol. 2009; 296(2): F230–F241, doi: 10.1152/ajprenal.90507.2008, indexed in Pubmed: 18842819.
15. Lin H, Ye S, Xu J, et al. The alpha-lipoic acid decreases urinary podocalyxin excretion in type 2 diabetics by inhibiting oxidative stress in vivo. J Diabetes Complications. 2015; 29(1): 64–67, doi: 10.1016/j.jdiacomp.2014.09.011, indexed in Pubmed: 25312599.
16. Xing Y, Ye S, Hu Y, et al. Podocyte as a potential target of inflammation: role of pioglitazone hydrochloride in patients with type 2 diabetes. Endocr Pract. 2012; 18(4): 493–498, doi: 10.4158/EP11378.OR., indexed in Pubmed: 22441004.
17. Hara M, Yamagata K, Tomino Y, et al. Urinary podocalyxin is an early marker for podocyte injury in patients with diabetes: establishment of a highly sensitive ELISA to detect urinary podocalyxin. Diabetologia. 2012; 55(11): 2913–2919, doi: 10.1007/s00125-012-2661-7, indexed in Pubmed: 22854890.
18. Djudjaj S, Lue H, Rong S, et al. Macrophage Migration Inhibitory Factor Mediates Proliferative GN via CD74. J Am Soc Nephrol. 2016; 27(6): 1650–1664, doi: 10.1681/ASN.2015020149, indexed in Pubmed: 26453615.
19. Morand EF, Leech M, Bernhagen J. MIF: a new cytokine link between rheumatoid arthritis and atherosclerosis. Nat Rev Drug Discov. 2006; 5(5): 399–410, doi: 10.1038/nrd2029, indexed in Pubmed: 16628200.
20. Sanchez-Niño MD, Sanz AB, Ruiz-Andres O, et al. MIF, CD74 and other partners in kidney disease: tales of a promiscuous couple. Cytokine Growth Factor Rev. 2013; 24(1): 23–40, doi: 10.1016/j.cytogfr.2012.08.001, indexed in Pubmed: 22959722.
21. Nathan DM, Buse JB, Davidson MB, et al. Medical Management of Hyperglycemia in Type 2 Diabetes: A Consensus Algorithm for the Initiation and Adjustment of Therapy: A consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2008; 32(1): 193–203, doi: 10.2337/dc08-9025.
22. Piwkowska A, Rogacka D, Jankowski M, et al. Metformin reduces NAD(P)H oxidase activity in mouse cultured podocytes through purinergic dependent mechanism by increasing extracellular ATP concentration. Acta Biochim Pol. 2013; 60(4): 607–612, indexed in Pubmed: 24432311.
23. Ravindran S, Kuruvilla V, Wilbur K, et al. Nephroprotective Effects of Metformin in Diabetic Nephropathy. J Cell Physiol. 2017; 232(4): 731–742, doi: 10.1002/jcp.25598, indexed in Pubmed: 27627216.
24. Li Cj, Lv L, Li H, et al. Cardiac fibrosis and dysfunction in experimental diabetic cardiomyopathy are ameliorated by alpha-lipoic acid. Cardiovasc Diabetol. 2012; 11: 73, doi: 10.1186/1475-2840-11-73, indexed in Pubmed: 22713251.
25. Zhai L, Gu J, Yang Di, et al. Metformin Ameliorates Podocyte Damage by Restoring Renal Tissue Podocalyxin Expression in Type 2 Diabetic Rats. J Diabetes Res. 2015; 2015: 231825, doi: 10.1155/2015/231825, indexed in Pubmed: 26075281.
26. David JR. Delayed hypersensitivity in vitro: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. Proc Natl Acad Sci U S A. 1966; 56(1): 72–77, indexed in Pubmed: 5229858.
27. Yao XM, Ye SD, Xiao CC, et al. Metformin alleviates high glucose-mediated oxidative stress in rat glomerular mesangial cells by modulation of p38 mitogen-activated protein kinase expression in vitro. Mol Med Rep. 2015; 12(1): 520–526, doi: 10.3892/mmr.2015.3446, indexed in Pubmed: 25760137.