Metformin reduces urate nephropathy in experimental nephrolithiasis


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The objective of the article is to evaluate the effectiveness of metformin in the prevention and treatment of experimental urate nephropathy. About 33% of the populations of developed countries suffer from metabolic syndrome. The relationship between metabolic syndrome, especially insulin resistance, with gout and urate nephrolithiasis, is now proven. The search for a common pathophysiological link in the development of these conditions allows us to identify insulin-dependent excessive urinary acidification due to impaired education and renal ammonium transport. We suggested the use of drugs that can increase the sensitivity of tissues to insulin, and induce a decrease in the manifestations of urate nephropyathy. The study was performed on 30 male Wistar rats weighing 250–300 g. For the induction of urate nephropyathy in rats, we used the classical model of inhibition of uricase by oxononium acid. Metformin was administered at a dose of 150 mg/kg in the treatment and prophylactic regimens. It was found that the use of metformin both in prophylactic and therapeutic regimen leads to a reliable decrease the level of uric acid in blood plasma and urine in rats with experimental urate nephrolithiasis. We found that the preventive use of metformin brought significant relief after experimental urate nephropathy, as evidenced by a shift in urine pH to the alkaline side, a decreased lactate dehydrogenase activity in urine, as well as a decrease in the processes of free radical oxidation in the blood and in the kidneys of the animals.

Keywords: oral hypoglycemic agents; uric acid; urate deposits; free radical oxidation

Introduction

According to modern statistical data, on average 33% of the populations of developed countries suffer from metabolic syndrome, and in patients older than 60 years this indicator exceeds 40% (Kim et al., 2013; Roberts et al., 2013; Choo et al., 2016). As is known, metabolic syndrome includes abdominal obesity, dyslipidemia and hyperglycemia, impaired glucose tolerance, and hypertension (Choo et al., 2016; Srikanthan et al., 2016). Along with disorders in carbohydrate and lipid metabolism, metabolic syndrome shows signs of a disorder of the metabolism of purines. Today, the relationship between metabolic syndrome and gout and urate nephropathy has been proven (Srikanthan et al., 2016; Won et al., 2016; Yarovoi et al., 2017). Urinary nephro lithiasis is a pathological process that occurs as a result of a disorder of purine metabolism that leads to hyperuricemia and hyperuricosuria, and is accompanied by the deposition of uric acid crystals in the kidneys. It is known that the overall frequency of metabolic syndrome in patients with gout is on average 57.0%, and the frequency of urate nephrolithiasis is 21.9% (Akman et al., 2013). Thus, the aim of the study was to evaluate the effectiveness of metformin in the prevention and treatment of experimental urate nephropathy.

The search for a common pathophysiological link in the development of metabolic syndrome and urate nephrotisits revealed excessive urinary acidification due to impaired generation and renal transport of ammonium (Sakhaee and Maalouf, 2008; Bobulescu et al., 2013). The results of the studies show that the main reason for lowering the acidity of urine is insulin resistance (Abate et al., 2004; Cameron et al., 2006). Insulin, activating the insulin receptors in the renal tubules, stimulates the activity of the isofor 3Na+/H+ exchanger, which provides transfer through the membrane of hydrogen ions for subsequent interaction with NH3 and direct transport of NH4+ into the lumen of the tubule (Curthoys, 2013). It is also known that insulin activates the metabolism of glutamine through glutamate and α-ketoglutarate, resulting in the formation of ammonia in the cells of the proximal renal tubules (Nissim et al., 1995). The solubility of uric acid crystals is directly dependent on pH. In the blood (pH = 7.4), uric acid is in soluble form. However, the pH of the urine can vary considerably. At a pH of 6.5, the uric acid in the urine, as well as in the blood, is predominantly in a soluble form. But when urine pH drops to 5.5, the solubility of uric acid decreases by 20 times. Thus, even under conditions of normal excretion, the probability of crystallization of uric acid and the formation of urate microcrystals is significantly increased (Liebman et al., 2007; Curthoys, 2013; Liu et al., 2017).

Insulin resistance leads to a decrease in the formation and secretion of ammonium, which contributes to the acidification of urine. We assumed that the use of a drug that increases the sensitivity of tissues to insulin will lead to a decrease in urate nephropathy. We have chosen metfor min, a synthetic anti-diabetic agent, a biguanide derivative (Fig. 1). Metformin increases the affinity of insulin receptors to insulin, changes their conformation and stimulates receptor and postreceptor pathways (Rena et al., 2013). Thus, the aim of the study was to evaluate the effectiveness of metformin in the prevention and treatment of experimental urate nephropathy.
Material and methods

A study was performed on 30 male Wistar rats weighing 250–300 g. The animals were in individual cages adapted for collection of urine. Keeping animals met the requirements of the European Convention for the Protection of Vertebrates Used for Experimental and other Scientific Purposes (Strasbourg, 1986). To induce urate nephropathy we used a classic model of uricase inhibition (Stavric et al., 1969). Animals were divided into 3 groups: control group (n = 14), prevention group (n = 10), treatment group (n = 10). Rats of the control group in the diet for 3 weeks received 500 mg/kg of oxonic acid and 1000 mg/kg of uric acid daily. Rats of the control group received 500 mg/kg of oxonic acid and 1000 mg/kg of uric acid daily by oral administration for 3 weeks. Rats of the prevention group additionally received metformin at a dose of 150 mg/kg daily. Rats of the prevention group additionally received metformin at a dose of 150 mg/kg daily, rats of the prevention group – from 11 to 21 days of the experiment. At 21 days in the daily urine, the concentration of uric acid, total protein, creatinine, and activity of enzymes markers of renal dysfunction (lactate dehydrogenase, gamma-glutamyltransferase and N-acetylβ-D-glucosaminidase) was measured. In the blood, the concentration of uric acid, creatinine was measured. The parameters of free radical oxidation were also measured: activity of catalase, glutathione peroxidase, superoxide dismutase, concentration of reduced glutathione in erythrocytes; concentration of thiobarbiturate-reactive products in the blood plasma, total antioxidant activity and total antioxidative activity of blood plasma. These free radical oxidation parameters were also measured in the kidneys. The rat kidneys also underwent histological examination. Sections of tissue 4–6 μm thick were obtained, which were stained with hematoxylin and eosin according to a standard procedure. Quantitative counting of urate stones in the lumen of the kidney tubules was carried out using the AxioVision 4.7 software package (Carl Zeiss, Germany).

For calculations and statistical processing the software SigmaStat 3.5 (Systat Software Inc., USA) was used. To compare the three groups, a rank analysis of the Kruskal-Wallis variations was used among themselves. For a posteriori pairwise comparisons, a nonparametric Mann-Whitney test was used. Data were presented as a median and interquartile range – Me (25%; 75%). Differences were considered statistically significant at P < 0.05.

Results

It was found that prolonged administration of metformin in the preventive regimen caused a marked decrease in plasma uric acid concentration – 1.1 (0.8; 1.2) mg/dL in the prevention group vs. 1.4 (1.2; 1.8) mg/dL in the control group (P = 0.009). This was accompanied by a significant decrease in the excretion of uric acid in the urine – from 19.5 (11.0; 22.9) mg/day in the prevention group vs. 31.5 (24.5; 33.6) mg/day in the control group (P = 0.002). When the drug was administered in the treatment regimen, there was also a significant decrease in the plasma concentration of uric acid – 1.0 (0.7; 1.2) mg/dL in the treatment group vs. 1.4 (1.2; 1.8) mg/dL in the control group (P = 0.013). This resulted in a significant decrease in the excretion of uric acid in the urine – from 15.4 (14.5; 18.7) mg/day in the treatment group against 31.5 (24.5; 33.6) mg in the control group (P = 0.001).

When studying the effect of metformin on renal function in rats with experimental urate nephrolithiasis (Table 1), there was a tendency to increase the glomerular filtration rate in animals receiving metformin in the preventive regimen compared to the control group. In addition, it was found that prolonged administration of metformin leads to a significant increase in the pH of the urine compared with the control.

In the group of animals receiving metformin in the treatment regimen, an increase in the glomerular filtration rate was observed by 20% compared to the control animals. Metformin in animals with experimental urate nephrolithiasis caused a shift in the pH of the urine to the alkaline side by 3% in comparison with the control values. However, these changes did not reach statistical significance (Table 1).

The positive effect of metformin on the kidney is confirmed by a marked decrease in the activity of lactate dehydrogenase in the urine of rats of the prevention group by more than 1.5 times in comparison with animals from the control group (Table 2). When analyzing the effect of metformin on the kidney, a marked decrease in the activity of lactate dehydrogenase (cytosolic enzyme of the tubular epithelium) in the urine of the rats of the treatment group is also noticeable by 47% compared with the control. The activity of gamma-glutamyltransferase and N-acetylβ-D-glucosaminidase in the urine of animals receiving metformin in both regimes did not change significantly.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Glomerular filtration rate, ml/min</th>
<th>Urine pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n = 14</td>
<td>18.3 (14.8; 21.5)</td>
<td>6.8 (6.5; 6.9)</td>
</tr>
<tr>
<td>Prevention, n = 10</td>
<td>23.6 (20.9; 26.4)</td>
<td>7.9 (7.5; 8.2)*</td>
</tr>
<tr>
<td>Treatment, n = 10</td>
<td>21.9 (17.0; 28.4)</td>
<td>7.0 (6.6; 7.4)</td>
</tr>
</tbody>
</table>

Note: * - significant difference in comparison with the control (Mann-Whitney test, P<0.05).

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Lactate dehydrogenase activity, U/l</th>
<th>Gamma glutamyltransferase activity, U/l</th>
<th>N-acetylβ-D-glucosaminidase activity, U/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n = 14</td>
<td>1.6 (1.1; 2.3)</td>
<td>32.2 (21.5; 45.7)</td>
<td>0.3 (0.2; 0.4)</td>
</tr>
<tr>
<td>Prevention, n = 10</td>
<td>1.0 (0.7; 1.1)*</td>
<td>49.1 (36.5; 55.3)</td>
<td>0.3 (0.2; 0.4)</td>
</tr>
<tr>
<td>Treatment, n = 10</td>
<td>0.9 (0.7; 1.0)*</td>
<td>39.7 (30.2; 56.2)</td>
<td>0.6 (0.4; 1.2)</td>
</tr>
</tbody>
</table>

Note: see Table 1.

Analysis of the effect of metformin on the processes of free radical oxidation in the blood of rats with experimental urate nephrolithiasis showed that in animals receiving the drug in the preventive regimen, a significant decrease in the total antioxidative activity was recorded at the end of the 3 weeks of the experiment (Table 3). This was not due to the activation of antioxidative enzymes: the total antioxidative activity of the blood plasma, the activity of catalase, superoxide dismutase and glutathione peroxidase did not differ from the control. Concentrations of reduced glutathione of erythrocytes and thiobarbiturate-reactive plasma products also did not change. The administration of metformin in the treatment regimen additionally caused an increase in the concentration of reduced glutathione.

Table 4 presents the markers of the processes of free radical oxidation in rat kidneys under the influence of metformin. There was a significant increase in the total antioxidative activity of the renal tissue and the concentration of reduced glutathione. As in the analysis of blood markers, changes in the total antioxidative activity, antioxidative enzyme activity, and the concentration of thiobarbiturate-reactive products are not observed. In the quantitative assessment of the number of urate microliths in the histological sections of the kidneys, the positive effect of the prophylactic administration of metformin on the course of experimental urate nephrolithiasis was confirmed. As can be seen in Figure 2, in the prevention group there was a significant decrease in the amount of uric acid in comparison with the control group. In the prevention group, the number of urate microliths was 3.5 (1.0; 6.0) per 100 tubules vs. 19.0 (11.8; 29.0) in the control group (P = 0.002).

hereditary diseases are also characterized by hyperuricemia and may be
the development of severe hyperuricemia (Davidson et al., 2004). Some
the cellular destruction of purines, and a change in its activity leads to
transferase enzyme (Lesch-Nyhan syndrome). This enzyme prevents
them, the genetic defect of the hypoxanthine-guanine phosphoribosyl-
nied by an increase in the excretion of uric acid in the urine. Among

Table 3
Markers of free radical oxidation in the blood of rats with experimental urate nephrolithiasis
with the administration of metformin in the prevention and treatment regimen

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood plasma</th>
<th>Erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thio-barbiturate-reactive products, μM</td>
<td>total antioxidant activity, %</td>
</tr>
<tr>
<td>Control, n = 14</td>
<td>5.0 (4.8, 5.1)</td>
<td>67.2 (58.3, 76.8)</td>
</tr>
<tr>
<td>Prevention, n = 10</td>
<td>5.7 (4.5, 6.4)</td>
<td>45.2 (39.7, 48.1)*</td>
</tr>
<tr>
<td>Treatment, n = 10</td>
<td>4.5 (3.8, 5.1)</td>
<td>50.2 (49.7, 51.4)*</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test

P 0.111 0.004 0.432 0.028 0.930 0.877 0.252

Note: see Table 1.

Table 4
Markers of free radical oxidation in the kidneys of rats with experimental urate nephrolithiasis
with the administration of metformin in the prevention and treatment regimen (per g protein)

<table>
<thead>
<tr>
<th>Group</th>
<th>Thio-barbiturate-reactive products, μM</th>
<th>total antioxidant activity, %</th>
<th>total antioxidant activity, %</th>
<th>Reduced glutathione, mmol</th>
<th>Catalase activity, %</th>
<th>Superoxide dismutase activity, %</th>
<th>Glutathione peroxidase activity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n = 14</td>
<td>0.3 (0.2, 0.3)</td>
<td>4.6 (4.4, 5.9)</td>
<td>2.3 (1.6, 2.8)</td>
<td>1.8 (1.4, 2.3)</td>
<td>0.03 (0.02, 0.04)</td>
<td>2.5 (1.9, 3.1)</td>
<td>14.2 (13.2, 19.6)</td>
</tr>
<tr>
<td>Prevention, n = 10</td>
<td>0.3 (0.2, 0.4)</td>
<td>5.1 (4.6, 5.5)</td>
<td>4.2 (3.4, 4.6)*</td>
<td>2.5 (2.1, 3.2)*</td>
<td>0.04 (0.03, 0.05)</td>
<td>3.0 (2.6, 3.3)</td>
<td>16.5 (15.0, 19.6)</td>
</tr>
<tr>
<td>Treatment, n = 10</td>
<td>0.3 (0.2, 0.5)</td>
<td>5.0 (4.6, 5.4)</td>
<td>4.3 (3.6, 4.6)*</td>
<td>2.6 (2.2, 2.8)*</td>
<td>0.04 (0.02, 0.04)</td>
<td>2.7 (2.1, 3.2)</td>
<td>16.9 (14.6, 18.4)</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test

P 0.827 0.939 0.003 0.023 0.427 0.443 0.635

Note: see Table 1.

Metformin administration in the treatment regimen significantly re-
duced the amount of urate microoliths: 8.0 (5.5; 12.0) per 100 tubules in the
treatment group versus 19.0 (11.8; 29.0) in the control group (P = 0.044).

Discussion
There are several basic conditions that determine the development of
urate nephrolithiasis, which include an excessively acidic pH of
urine, hyperuricemia and hyperuricosuria, a decreased volume of urine.
A low volume of urine is a nonspecific and universal factor contributing
to the formation of all types of renal calculi. Due to the limited solubility
of uric acid, increasing its concentration with a reduced volume of urine
leads to supersaturation and the creation of conditions conducive to
precipitation of uric acid and its sodium salt (Robertson, 2003).

The consequence of an increase in the content of uric acid in the
blood is an increase in its excretion in the urine with the development of
hyperuricosuria. T. Yü and A. Gutman in their work showed a strict
linear relationship between the concentration of uric acid in the blood
plasma and urate nephrolithiasis. In patients with plasma uric acid levels
below 7 mg/dL, microoliths were only detected in 9.9% of cases, and
with an increase of uric acid concentration in plasma of up to 13 mg/dL
and higher, concerns were already detected in 53% of cases (Yü and
Gutman, 1967). Later, the researchers confirm these results and conclu-
ded that gout is a risk factor for the formation of kidney stones (Kramer
et al., 2003). Besides gout, there are other conditions that are accompa-
nied by an increase in the excretion of uric acid in the urine. Among
them, the genetic defect of the hypoxanthine-guanine phosphoribosyl-
transferase enzyme (Lesch-Nyan syndrome). This enzyme prevents
the cellular destruction of purines, and a change in its activity leads to
the development of severe hyperuricemia (Davidson et al., 2004). Some
hereditary diseases are also characterized by hyperuricemia and may be
accompanied by the development of urate nephrolithiasis. Among
them, Wilson-Konovalov’s disease, Hartup’s disease, Girk’s disease,
Kelly-Zygmilier syndrome, Fanconi syndrome (Talente et al., 1994).
Hyperuricemia can develop against the background of chemotherapy
in myelo- and lymphoproliferative diseases because of catabolism of
tumor cells, hemolytic and sickle-cell anemia, polychromatemia and
psoriasis as a result of enhanced cellular turnover (Davidson et al.,
2004). In addition, hyperuricemia may be the result of long-term use of
a number of drugs, including thiadizide and loop diuretics, carbonic an-
dehydraz inhibitors, a number of non-steroidal anti-inflammatory drugs,
some antibiotics, angiotensin converting enzyme inhibitors, angiotensin
receptor blockers, statins, modern antiviral agents (Burckhardt, 2012).
An equally important pathogenic factor contributing to the formation of
urate stones is hyperuricosuria. With an increase in the concentration of
uric acid in the primary urine, good conditions are created for its precipi-
tation in renal interstitium with subsequent crystallization. Both genetic
causes and the impact of acquired factors can lead to the development of
hyperuricosuria. Congenital hyperuricosuria occurs as a result of
 disorders of uric acid transport in the kidneys due to mutations of
kidney uric acid transporters. Acquired hyperuricosuria can be caused by
a purines-rich diet and the use of uricosuric medicines such as pyrazin-
amide, probenecid, benzthromoramone, sulfinpyrazone, phenylbutazone,
acetylsalicylic acid in high doses, losartan, fenofibrate, atorvastatin
(Liebman et al., 2007).

During the experiments, it was found that both preventive and the-
erapeutic use of metformin significantly facilitate the course of experi-
mental urate nephrolithiasis. First, there was a significant decrease in
plasma uric acid concentrations and a reliable decrease of it with urinary
excretion against the background of long-term administration of the
drug in the prevention and treatment groups compared to the control
values. The results obtained are in good agreement with the view that
the action of metformin is not limited to interfering with the metabolism
of carbohydrates, but it is more extensive, and affects other types of
metabolism, including purine metabolism (Najeed et al., 2002; Cur-
thoys, 2013; Rena et al., 2013). Metformin may also enable to influence
the transport of uric acid by increasing the number of its specific Glut9
transporters (SLC2A9) on the surface of the membranes of liver cells,
kidneys, intestines, chondrocytes, leukocytes. In the kidneys, this trans-
porter is located on the apical (isomorf “b”) and the basolateral (isomorf
“a”) membranes and is responsible for the reabsorption of uric acid
from the primary urine into the blood. In enterocytes, Glut9a provides
the transfer of uric acid from the blood to the lumen of the intestine,
which ensures its excretion with feces (So and Thorens, 2010). It pro-
vides reduction of the concentration of uric acid in the blood plasma and
excretion of uric acid with the urine.

Secondly, against the background of the use of metformin, the urine
pH shifted to the alkaline side in animals from both experimental groups
in comparison with the control values. As noted earlier, the excessively
cid acid pH is a potent factor contributing to urate lithogenesis (Liu
et al., 2017). Considering the significance of the acidity level of urine in
the formation of urate microoliths, it can be assumed that the pH shift to
the alkaline side under the influence of metformin had a beneficial ef-
fect. Increasing the sensitivity of the insulin receptors of the renal tubu-
les to insulin, metformin activates the function of NHE3 and, as a con-
sequence, the transport of NH4 to the lumen of the renal tubule, which
leads to an increase in the pH of the urine (Curthoys, 2013). It should
also be noted that a pronounced decrease in the level of lactate dehyd-
rogenase indirectly indicates a favorable effect of metformin on the state of tubule epitheliocytes. Of special interest is the study of the effect of metformin on the processes of free radical oxidation accompanying urate nephropathy, an important role of which in the development of this state was described by us earlier (Perfil’ev et al., 2017). Analyzing the dynamics of free radical oxidation processes in blood and kidneys of experimental animals in comparison with the control animals, it can be assumed that the total antioxidant activity of renal tissue increased under the influence of metformin led to a decrease in the blood total prooxidant activity of rats with experimental urate nephrolithiasis. Moreover, the noted absence of activation of antioxidant enzymes is possibly due to the ability of metformin to interfere with the processes of free radical oxidation. It is well known that metformin can inhibit oxidative reactions, including oxidative glycosylation of proteins (Najeed et al., 2002; Daskalopoulou et al., 2004). Probably, against the background of metformin, the activity of non-enzyme antioxidant protection increased at the expense of low-molecular compounds with antioxidant properties, including some vitamins, β-carotene etc. (Nissim et al., 1995). Thus, in the treatment of metformin in patients with type 2 diabetes mellitus, a tendency was registered to increase and normalize the content of endogenous α-tocopherol, which is known to be one of the main endogenous antioxidant compounds (Mah et al., 2015).

In addition, it is well known that uric acid is a powerful antioxidant, but under certain conditions it acquires prooxidant properties, caused by the formation of a urate radical during oxidation (Glantzounis et al., 2005; So and Thorens, 2010). It is possible that under the influence of metformin in plasma and kidneys, an optimal concentration of uric acid is created, which provides a greater manifestation of its antioxidant properties.

**Conclusion**

The use of metformin both in preventive and treatment regimens has a beneficial effect on the course of experimental urate nephrolithiasis. The use of the drug leads to a decrease in the number of renal stones, leads to a reliable decrease the level of uric acid in the plasma and urine of sick animals. Prophylactic use of metformin greatly facilitates the course of urate nephropathy, which is confirmed by a shift in the pH of the urine to the alkaline side, a decrease in the activity of lactate dehydrogenase, an enzyme that reflects the damage to the renal epithelium, and a decrease in free radical oxidation in the blood and in the kidneys.

**Fig. 2.** Morphological picture of the kidney of a rat on the 21st day of the experiment: arrows indicate urate microlites
syndrome risk: A retrospective database study. International Journal of Environ-
mental Research and Public Health, 13(7), 667.
Curthoys, N. P. (2013). Renal ammonium ion production and excretion. Seldin
and Giebisch’s the kidney (Fifth edition).
Davidson, M. B., Thakkar, S., Hix, J. K., Bhandarkar, N. D., Wong, A., & Schrei-
ber, M. J. (2004). Pathophysiology, clinical consequences, and treatment of
tumor lysis syndrome: The American Journal of Medicine, 116(6), 546–554.
4151.
Association of nephrolithiasis with metabolic syndrome and its components.
Metabolism, 62(6), 1070–1083.
The association between gout and nephrolithiasis in men: The health profes-
sis. Current Rheumatology Reports, 9(3), 251–257.
logic targeting ERK1/2 attenuates the development and progression of hyperuri-
cemic nephropathy in rats. Oncotarget, 8(20), 33807–33826.
Mah, E., Sapper, T. N., Chatchamrooncholchai, C., Failla, M. L., Schill, K. E.,
Clinton, S. K., & Bruno, R. S. (2015). α-Tocopherol bioavailability is lower
in adults with metabolic syndrome regardless of dairy fat co-ingestion: A ran-
donized, double-blind, crossover trial. The American Journal of Clinical
Nutrition, 102(5), 1070–1080.
of glyburide (glibenclamide) and rosiglitazone on QT dispersion: A potential
adenosine triphosphate sensitive K+ channel effect. The American Journal of
Cardiology, 90(10), 1103–1106.
An experimental model. Proceedings of the Society for Experimental Biology
and Medicine, 130(2), 512–516.
factors. Annals of Internal Medicine, 67(6), 1133–1148.