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Serum metabolic fingerprinting after exposure of rats to quinolinic acid

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ABSTRACT

Quinolinic acid (QUIN), one of the end metabolites in the kynurenine pathway, plays an important role in the pathogenesis of several diseases. Serum QUIN concentration rises in patients with renal dysfunction, liver cirrhosis, and many other inflammatory diseases. In the present study, osmotic minipumps containing QUIN (0.3 and 1 mg/day) were implanted intraperitoneally into rats for 28 days. Then, the physiological and toxicological variables were evaluated and LC-QTOF-MS serum metabolic fingerprinting was performed. QUIN significantly decreased the serum concentrations of several amino acids (phenylalanine, valine, tyrosine, and tryptophan), pantothenic acid, branched chain C4 acylcarnitine, total cholesterol, and glucose; increased the serum concentrations of amides (pentadecanoic amide, palmitic amide, oleamide, and stearamide), polyamines (spermine and spermidine), sphingosine, and deoxy-prostaglandin; caused alterations in phospholipids. This is the first report of comprehensive metabolites analysis after chronic intraperitoneal administration of QUIN. Further studies could develop new therapeutics for patients with disorders accompanied by increased serum level of QUIN.

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1. Introduction

Quinolinic acid (QUIN) is one of the end metabolites in the kynurenine pathway of tryptophan metabolism. The two structurally distinct enzymes, tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygnease (IDO), are involved in the depletion of tryptophan and production of kynurenine pathway metabolites. Under physiological conditions, tryptophan metabolism takes place mainly through TDO in the liver. In the case of inflammation, proinflammatory cytokines activate IDO in the tissues other than the liver and the tryptophan degradation *via* kynurenine pathway occurs mainly in the blood and lymphoid tissues [1]. Under pathological conditions, tryptophan degradation becomes much higher than in physiological conditions. Proinflammatory cytokines also activate kynurenine-3-monooxygenase, the other enzyme in the kynurenine pathway [2]. These circumstances lead to increased serum production of QUIN in patients with several inflammatory diseases [3–5].

Chronic inflammation is a well-known feature in kidney disease. The causes of chronic inflammation are multiple, including decreased renal function, volume overload, factors associated with the dialysis procedure and genetic factors [6]. Serum level of QUIN increases in patients with the chronic renal disease, and correlates with disease severity. Moreover, hemodialysis incompletely removes QUIN [3]. Increased serum QUIN concentrations in renal insufficiency may be due to impaired renal elimination and decreased aminocarboxymuconate-semialdehyde decarboxylase activities [7]. Serum QUIN concentrations are also chronically elevated in patients with liver cirrhosis and may reflect the degree

Abbreviations: ALT, alanine transaminase; AMYL, amylase; AspAT, serum aspartate aminotransferase; BBB, blood-brain barrier; CID, collision-induced dissociation; CREA, creatinine; GLU, glucose; HGB, haemoglobin; HCT, hematocrit; IDO, indoleamine 2,3-dioxygnease; lyso PCs, lysophosphatidylcholines; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MFE, molecular feature extraction; NMDAr, N-methyl-D-aspartate receptors; PCA, principal component analysis; PCs, phosphatidylcholines; PLS-DA, partial least squares discriminant analysis; PLT, platelets; QC, quality control; QUIN, quinolinic acid; RBC, erythrocytes; TDO, tryptophan 2,3-dioxygenase; T-BIL, total bilirubin; T-CHOL, total cholesterol; WBC, white blood cells.

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of liver dysfunction. High serum levels of QUIN may serve as a sensitive indicator of hepatic encephalopathy [5].

The knowledge about the effect of chronically elevated serum QUIN levels on general toxicity is limited. Comprehensive evaluation of the effects evoked by QUIN can be performed by use of metabolomics approach, what may help to understand the biochemical alterations caused by diseases in which the serum levels of QUIN are increased and to develop new therapeutic strategies for patients with renal dysfunction, liver cirrhosis, and many other inflammatory diseases. This is the first attempt to evaluate the general physiological and toxicological variables after chronic systemic administration of QUIN in rats by use of serum metabolic fingerprinting with LC-QTOF-MS.

2. Materials and methods

2.1. Animals

Twenty-two male Wistar rats were used in the experiment. Rats were purchased from the Centre of Experimental Medicine of the Medical University of Bialystok. They were housed in specific pathogen-free conditions according to Good Laboratory Practice rules with a 12 h light/dark cycle in temperature and humidity controlled room. Rats were grouped cages as appropriate and allowed to have ad libitum access to a sterilized standard chow and tap water. A health surveillance programme monitored the animals' health status according to Federation of European Laboratory Animal Science Associations (FELASA) guidelines. Rats were free of all bacterial, viral, and parasitic pathogens listed in the FELASA recommendations. All the procedures involving animals were approved by Local Ethical Committee on Animal Testing at the Medical University of Bialystok (Permit Number 19/2013) and conducted by ARRIVE guidelines [8], Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes. Exsanguination euthanized all animals at the end of the experiment.

2.2. Design of experiment

Wistar rats weighing 151.5 ± 8.9 g were randomly divided into 3 groups and anesthetized intraperitoneally with ketamine (100 mg/kg) and xylazine (10 mg/kg). Osmotic minipumps (Alzet 2006, Palo Alto, CA, USA) containing vehicle (sterile 10% DMSO) or QUIN (0.3 and 1 mg/day) were implanted intraperitoneally in the lower abdomen under the rib cage. Osmotic pumps are miniature infusion pumps for the continuous dosing of laboratory animals and can be used for the systemic administration when implanted intraperitoneally. After 28 days of continuous administration (~2.5 human years, considering 11.8 rat days equals 1 human year) [9], rats were weighted and again anesthetized with the mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). The blood samples were taken from the cardiac puncture and centrifuged for 10 min at $4000 \times g$ to obtain serum for the biochemical and metabolomics analysis. After centrifugation, serum was stored and frozen −80 °C until further assays could be performed. The blood samples were also collected on dipotassium ethylenediaminetetraacetic acid (K_2-EDTA) as an anticoagulant only for the evaluation of haematological parameters. The results of QUIN administration on general physiological and toxicological variables are summarized in Table 1.

2.3. Biochemical and haematological parameters

Serum aspartate aminotransferase (AspAT), alanine transaminase (ALT), total cholesterol (T-CHOL), glucose (GLU), amylase (AMYL), total bilirubin (T-BIL), and creatinine (CREA) were measured using biochemical analyser BS-120 (Mindray, Nanshan, Shenzhen, China) with the appropriate kit (Cormay, Lublin, Poland). White blood cells (WBC), erythrocytes (RBC), haemoglobin (HGB), hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and platelets (PLT) were assessed with an Animal Blood Counter (ABC, Horiba, Viernheim, Germany) according to the manufacturer's instructions.

2.4. Metabolic fingerprinting with LC–MS

Serum samples for metabolic fingerprinting were prepared as described previously [10]. Samples were analyzed by the HPLC system consisting of a degasser, two binary pumps, and thermostatted autosampler (1200 series, Agilent Technologies, Waldbronn, Germany) connected to an Agilent QTOF (6530) mass spectrometer. Samples (10 μL) were applied to a reversed-phase column (Discovery HS C18 15 cm × 2.1 mm, 3 μm; Supelco) with a guard column (Discovery HS C18 2 cm × 2.1 mm, 3 μm; Supelco). Chromatographic conditions were the same as described previously [10]. Data were collected in positive and negative ESI ion modes

Table 1
Characteristics of study male Wistar rats.

	Vehicle	QUIN (0.3 mg/day)	QUIN (1 mg/day)
N	6	6	10
Weight gain during the experiment (g)	141.0 (129.7–154.8)	147.6 (125.0–171.0)	148.0 (125.0–173.0)
WBC ($10^3/mm^3$)	2.9 (2.0–4.2)	2.5 (1.6–3.3)	2.7 (1.7–4.4)
RBC ($10^6/mm^3$)	7.73 (7.28–8.85)	7.53 (6.90–8.20)	7.83 (7.51–8.57)
HGB (g/dl)	16.2 (14.6–17.2)	15.5 (13.9–16.5)	16.1 (14.7–16.5)
HCT (%)	42.9 (41.6–48.8)	42.9 (37.3–46.0)	43.2 (41.3–45.7)
MCH (pg)	20.1 (19.2–24.8)	20.2 (20.0–21.4)	20.8 (18.8–21.4)
MCV (μm^3)	55.5 (50.0–57.0)	56.0 (54.0–57.0)	56.0 (53.0–57.0)
MCHC (g/dl)	36.0 (35.1–38.7)	36.3 (35.8–37.5)	37.1 (35.3–37.9)
PLT ($10^3/mm^3$)	703.5 (538.0–742.0)	648.0 (609.0–742.0)	633.5 (580.0–722.0)
AspAT (U/l)	52.3 (47.0–63.0)	68.5 (48.0–86.0)	62.0 (42.0–93.0)
ALT (U/l)	25.5 (24.0–31.0)	30.7 (24.0–31.0)	27.8 (24.0–31.0)
GLU (mg/dl)	156.5 (140.0–213.0)	107.0 (95.5–137.0)*	113.0 (88.0–129.0)*
T-CHOL (mg/dl)	54.5 (48.0–69.0)	50.0 (36.0–73.0)	43.0 (34.0–48.0)*
T-BIL (mg/dl)	0.25 (0.23–0.26)	0.25 (0.24–0.27)	0.26 (0.23–0.28)
AMYL (U/l)	501.5 (401.0–605.0)	525.0 (426.0–630.0)	449.0 (405.0–559.0)
CREA (mg/dl)	0.47 (0.44–0.54)	0.49 (0.45–0.56)	0.49 (0.44–0.54)

*$p < 0.05$ *vs.* vehicle; Mann-Whitney test. WBC, white blood cell count; RBC, erythrocytes; HGB, hemoglobin; HCT, hematocrit; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet count; AspAT, aspartate aminotransferase; ALT, alanine transaminase; GLU, glucose; T-CHOL, total cholesterol; T-BIL, total bilirubin; AMYL, amylase; CREA, creatinine. Results are shown as a median with lower and upper limits.

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