www.nephropathol.com DOI: 10.34172/jnp.2021.31 J Nephropathol. 2021;10(3):e31

Journal of Nephropathology

Molecular patterns of oxidative stress in drug-induced nephropathy

Valentina Vitalyevna Lee[1*], Larissa Evgenievna Muravlyova[2], Ryszhan Yemelevna Bakirova[1], Ahmet Kiziltunc[3], Zhanara Zholtaevna Turkhanova[1], Bolatkyl Dguzbaevna Ashirbekova[1]

[1]Department of Internal Diseases, Karaganda Medical University, Karaganda-City, Kazakhstan
[2]Department of Fundamental Medicine, Karaganda Medical University, Karaganda-City, Kazakhstan
[3]Biochemistry Department, School of Medicine, Ataturk University, Erzurum, Turkey

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 12 February 2020
Accepted: 4 October 2020
Published online: 2 November 2020

Keywords:
Drug-induced nephropathy
Oxidative strees
Serum NGAL
Advanced oxidation protein products
Protein reactive carbonyl derivatives
CKD

ABSTRACT

Introduction: Drug induced kidney disorder is a frequent adverse event which contributes to morbidity and even incapacitation. The discovery and development of novel biomarkers and local (renal) response mechanisms, are needed for effective prevention of drug-induced nephrotoxicity.
Objectives: The main purpose of our study was to investigate the oxidative modifications of proteins in blood plasma and erythrocytes of patients with drug-induced nephropathies.
Patients and Methods: Around105 patients were divided into two groups: first group was represented by patients with psychotropic drug-induced nephropathy; the second one consisted of patients received nonsteroidal anti-inflammatory drugs (NSAIDs). Advanced oxidation protein products (AOPPs) were measured by Witko-Sarsat method. Protein reactive carbonyl derivatives (PRCD) were assayed in blood plasma and erythrocytes by the Levine method. Neutrophil gelatinase-associated lipocalin (NGAL) was determined with the use of a commercially available ELISA kit.
Results: Carbonyl derivatives are significantly higher in red blood cells of the 1st and 2nd group patients compared to the control subjects. AOPP statistically increased both in patients with various types of drug nephropathy and in patients with chronic kidney disease (CKD) compared with the control group. The NGAL was significantly higher in all groups compared to the control subjects.
Conclusion: The patients with drug-induced nephropathy have increased level of oxidative stress products and response NGAL reaction. The mechanisms that lead to the development of oxidative stress and the production of modified proteins are different in patients treated with different drugs. Establishing patterns of cell-molecular interaction permit the drug-induced nephropathy to be timely diagnosed and therapeutic programs to be optimized.

Implication for health policy/practice/research/medical education:
Establishment of predictors of nephropathy progression in the context of their relationship with the dynamics of molecular-cell patterns will make it possible to formulate recommendations for the timely diagnosis of drug-induced nephropathies.
Please cite this paper as: Lee VV, Muravlyova LE, Bakirova RY, Kiziltunc A, Turkhanova ZZ, Ashirbekova BD. Molecular patterns of oxidative stress in drug-induced nephropathy. J Nephropathol. 2021;10(3):e31. DOI: 10.34172/jnp.2021.31.

Original Article

Introduction

In recent years, the number of medicinal lesions of the kidneys has become much more frequent in the practice of doctors of all specialties (1). The reason is the continuous expansion of the medicines arsenal. Any drug may have potential nephrotoxicity. For example, the role of analgesic injury has significantly increased in the structure of causes of the end-stage renal failure, in the last 20 years (2). Moreover, a recent large database analysis concerned of potential acute kidney injury risk associated with antipsychotics (3,4) and cytotoxic effect of tricyclic antidepressants (5-8).

Nephrotoxicity can be defined as any kidneys damage caused by drugs. Nephrotoxicity is associated most commonly with injury in the tubulointerstitial compartment manifested as either acute tubular injury or acute interstitial nephritis. A growing number of reports have also highlighted the potential for drug-induced

*Corresponding author: Valentina Vitalyevna Lee, Email: liv@qmu.kz

glomerular disease, including direct cellular injury and immune-mediated injury up to the development of nephrotic syndrome (9). Nephropathy can be diagnosed by simple blood tests. Nephrotoxicity is usually estimated by hydroelectric disturbance, serum creatinine concentration, estimated glomerular filtration rate (eGFR) and creatinine clearance. However, these assessments of nephrotoxicity are only possible when most of kidney functions are damaged (10). Therefore, other biomarkers have been developed permitting earlier intervention that may improve patient's prognosis. Although qualitative studies do not confirm their advantages, here are some promising candidates: beta-2-microglobulin, kidney injury molecule-1 (KIM), neutrophil gelatinase-associated lipocalin (NGAL) (11).

The mechanisms of the drug nephropathy development are still poorly understood. It has recently been shown that oxidative stress in the kidneys underlies acute nephrotoxicity caused by drugs. Proteins could be directly modified by reactive oxygen species which leads to amino acid oxidation and cross-linking. The formation of oxidized modified proteins is accompanied by a loss of the protein functional activity, an alteration of conformation, the formation of aggregates, or fragmentation (12). Carbonyl metabolic products include advanced oxidation protein products (AOPPs) (13), protein reactive carbonyl derivatives (PRCD) (14). AOPPs have fairly pronounced biological properties similar to AGEs (advanced glycation end products), and can bind to the receptor for advanced glycation end products (RAGE), that leads to chronic kidney disease (CKD) development (15).

Thus, the problem of drug-induced nephropathies is of increasing importance due to the multifactorial nature of damaging agents that tend to grow continuously, as well as the lack of knowledge of systemic and local (renal) response mechanisms. The main purpose of our study was to investigate the oxidative modifications of proteins in blood plasma and red blood cells of patients with drug-induced nephropathies.

Objectives

The main purpose of our study was to investigate the oxidative modifications of proteins in blood plasma and red blood cells of patients with drug-induced nephropathies.

Patients and Methods

Study design

The present study was cross-sectional. Around 105 patients from 22 to 50 years old (64 females, 35 males) were divided into two groups depending on the type of the drug. The first group (n=30) was represented by patients with psychotropic drug-induced nephropathy; the second one (n=25) consisted of patients with nephropathy caused by nonsteroidal anti-inflammatory drugs (NSAIDs). Patients were recruited in the toxicological department of the hospital in Karaganda city. All the patients had acute intoxication with remedies.

Patients with the age less than 18 and more than 50 years, patients with acute pyelonephritis, glomerulonephritis, acute surgical pathology, concomitant diseases in the acute stage including; heart failure, diabetes, decompensated thyroid disease, diffuse connective tissue diseases, acquired immunodeficiency syndrome and gestational nephropathy were excluded.

Drug-induced nephropathy was diagnosed by nephrologist considering the history of drug intake, physical examination, urine parameters (proteinuria, macro/microhematuria, cylindruria, and decreased urine relative density) and biochemical blood tests consisting of elevated serum creatinine, reduced eGFR, electrolyte abnormalities (hyperkalemia, hyper/hyponatremia or hypocalcemia) according to clinical protocol (16).

As a comparison group (third group) were recruited 25 patients with 1 and 2 stage of the CKD. The control group consisted of 25 matched persons.

Blood sampling and biochemical analysis

Blood samples were collected from the cubital vein and placed into vacutainer tubes containing heparin. Erythrocytes were separated from plasma by centrifugation and washed for three times with physiological saline. Analysis of blood plasma and red blood cells were performed within 1-2 hours after their collection. Biochemical measurements were carried out on a PD-303UV A PEL spectrophotometer (Japan).

AOPP were measured spectrophotometrically as described by Witko-Sarsat et al (17). Briefly, 0.35 mL of blood plasma and 1.75 mL of sodium/potassium buffer solution were placed into spectrophotometric cuvette and gently mixed, and then 0.2 mL of glacial acetic acid and 0.1 mL of potassium iodide were added and measured at 340 nm. A mixture of buffer solution, glacial acetic acid and potassium iodide served as a control. The concentration of oxidative products of proteins containing bityrosine cross-links (AOPP) was expressed in nmol/L.

PRCD were assayed in blood plasma and erythrocytes by the method of Stadtman and Levine (18). 0.1 mL of blood plasma, 0.5 mL of 20% trichloroacetic acid and 0.5 mL of dinitrophenylhydrazine were added to the centrifuge tube. The reaction mixture is incubated at room temperature for 1 hour and centrifuged at 3000 rpm for 5-7 minutes. Supernatant discarded. The obtained precipitate is resuspended in 0.5 mL of ethyl acetate and centrifuged at 3000 rpm for 3 minutes. This procedure is repeated 2 times. The resulting precipitate is incubated at 37°C for 18 hours. After incubation, 2 mL of urea is added

to the precipitate and measured on a spectrophotometer at 370 nm. Distilled water serves as control.

Serum NGAL was determined with the use of a commercially available ELISA kit (Affymetrix eBioscience, Vienna, Austria) and presented in pg/ml.

Ethical considerations

This paper was extracted from the general physician thesis of Li Valentina at the Karaganda Medical University. The study was conducted in accordance with the Helsinki Declaration and was approved by the ethics committee affiliated with the Karaganda Medical University (Refrence #47 from 16/05/2019; Protocol #18). Informed consent was obtained from all the patients before their inclusion in the study. During the presence of patients in the toxicological department all of them received standard therapy, corresponding to the poisoning severity and developed complications.

Statistical analysis

Statistical analysis of the obtained data was done using the software package SPSS Version 22. All the data were expressed as median (interquartile range) ± standard deviation.The difference between case and control groups was assessed using Kruskal-Wallis test. For all outcomes a nominal P value <0.05 was considered significant.

Results

Table 1 shows that carbonyl derivatives are significantly higher in red blood cells of the 1st and 2nd group patients compared to the control subjects ($P<0.001$). In patients with CKD of the 1-2 stage the opposite situation is noted: the PRCD level in the erythrocytes was in three times lower than in the control group. In the plasma, a pronounced increase in carbonyl derivatives was detected only in the 3rd group ($P<0.003$).

AOPP statistically increased both in patients with various types of drug nephropathy and in patients with CKD when compared with the control group.

As to the serum NGAL, it is significantly higher in all three groups compared to the control subjects ($P<0.01$). No correlations of NGAL with other indicators were found.

Discussion

Our data confirm the serum NGAL elevation even at the stage of nephropathy formation due to the development of acute oxidative stress. The high level of the serum NGAL was showed in patients with normal GFR. As NGAL expression increases in proximal tubule cells due to drug-induced nephrotoxicity or ischemia, it may be considered as a sensitive biomarker for early diagnosis of acute kidney damage. A pronounced increase of carbonyl derivatives, advanced oxidative protein products in patients with psychotropic drug-induced nephropathy and in patients with nephropathy caused by NSAIDs, may underlie specific mechanisms.

NSAIDs-induced nephrotoxicity is realized through several mechanisms of cell death. It has been established that drugs with the properties of acids (nitrosalicylic acid, acetylsalicylic acid, diclofenac, naproxen, ibuprofen, indomethacin and piroxicam) can inhibit mitochondrial oxidative phosphorylation (2). In renal tubules, favorable conditions are created for the inhibitory effect of NSAIDs on oxidative phosphorylation: a normal, slightly acidic urine reaction and the high content of NSAIDs due to the concentration and reabsorption of urine.The uncoupling of oxidative phosphorylation and the loss of ATP lead to the disturbance of cellular ion homeostasis, namely, to the decrease of intracellular K^+ content and to the increase of Na^+ content that results in membrane depolarization and cell death. The disturbance of energy-dependent calcium homeostasis is also associated with the increased content of free Ca^{2+} in cytosol, that plays a critical role in cell death (2).

Another nephrotoxicity mechanism of NSAIDs is associated with the formation of active metabolites. With the participation of cytochrome P-450 (mainly its isoform CYP2E1), the electrophilic intermediates are formed which, covalently bind to cell macromolecules, disrupting mitochondrial and nuclear functions, thereby triggering reactive oxygen species (ROS) production, protein

Table 1. AOPP, PRCD and NGAL level in patients with drug-induced nephropathy compared to CKD patients (Mean ± SD)

Groups	PRCD		AOPP	NGAL
	Blood plasma (nmol/L)	Erythrocytes (nmol/L)	Blood plasma (nmol/L)	Blood plasma (pg/mL)
(1st group) Patients with psycho-tropic drug-induced nephropathy	1.13 ± 0.71	14.8 ± 2.66*	0.312 ±0.086*	332.2± 112*
(2nd group) Patients with NSAID drug-induced nephropathy	0.79 ± 1.62	15.73 ± 1.01*	0.301 ± 0.09*	324.17 ± 96*
(3d group) Patients with CKD 1-2	2.27 ±0.86*	3.74 ± 1.91*	0.382 ±0.13*	351.27± 107*
Control subjects	0.72 ± 0.16	10.76 ± 1.26	0.192 ± 0.052	80.49 ± 85.13

*In comparison to control subjects ($P \leq 0.05$).

oxidation and PRCD formation in erythrocytes. In patients with CKD of the 1-2 stage, the carbonyl derivatives level in erythrocytes was in three times lower than in the control group. It can be explained by conformation of the oxidized proteins and exposure of hydrophobic parts to the outside during the excessive PRCD formation, which creates conditions for the formation of protein-protein conglomerates and insoluble protein aggregates and causes the progression of nephropathy (12).

In addition, when taking NSAIDs, an immuno-allergic reaction can be developed which is characterized by macrophage interstitial infiltration - the main producers of reactive oxygen species and nitric oxide (2), whose targets are tyrosine residues in proteins, cytochromes and myeloperoxidase that is confirmed by the high level of AOPP in these patients. Each of the above mediators has a direct cytotoxic effect or is able to enhance the inflammatory response of tissues to other damaging factors.

The safety of psychotropic drugs is nowadays one of the most ambiguous research problems. Concerns regarding the possible detrimental effect of psychotropic medications on kidney function are supported by a recent meta-analysis (3,4). Recently, several independent researchers have reported that psychotropic remedies exhibit cytotoxic effect in human cell cultures, increasing oxidative stress.

Several studies showed that tricyclic antidepressant specifically inhibited mitochondrial complex III activity due to low mitochondrial membrane potential (5-7). The decrease of cytochrome C, citrate synthase and catalase activities lead to ROS overproduction. This in turn leads to cascade process of protein oxidation and glycoxidation resulting in excessive production of carbonyl derivatives, AOPP and AGE products. The end glycation products are one of the factors of post-translational modification of proteins. They can also have effect on the cell at the level of gene expression binding to specific cell surface receptors that leads to phosphorylation of mitogen-activated protein kinases, activation of NF-κB, secretion of pro-inflammatory cytokines (15). Macrophage-mediated cell infiltration initiates AOPP production, exacerbating the course of nephropathy.

Conclusion

Thus, the results of the present study permit us to draw the following conclusions. The patients with psychotropic and NSAIDs drug-induced nephropathy have increased level of oxidative stress products and response NGAL reaction. The mechanisms that lead to the development of oxidative stress and the production of modified proteins are different in patients treated with different drugs. Establishing patterns of cell-molecular interaction permit the drug-induced nephropathy to be timely diagnosed and therapeutic programs to be optimized.

Limitations of the study

Several limitations must be noticed in this study. First, there was considered only acute intoxication with remedies, i.e. chronic drug intoxication (during month or years) has not been reviewed here. Second, in this study, only two drug groups were included; psychotropic and non-steroidal anti-inflammatory drugs. This limitation is due to the prevalence of suicidal drugs. Third, there was an age limitation to avoid influence of age-related changes.

Authors' contribution

LEM and RYB participated in the design and in describing the methodology of the study, amending the manuscript draft of the article. AK participated in the statistical analysis of the data and amending the manuscript draft of the article. LVV prepared the draft of the proposal and participated in conduct of the study (data collection, blood sampling, enter data to software), participated in the statistical analysis, amending the manuscript draft of the article. ZZT and BDA participated in conduct of the study. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest

Ethical considerations

Ethical issues including plagiarism, double publication, and redundancy have been completely observed by the authors.

Funding /Support

This study was supported by an interior grant from the Karaganda Medical University 001/KFNI3 "Vesiculogenesis and neutrophil reactivity in acute kidney injury".

References

1. Peraczella MA Drug-induced acute kidney injury: diverse mechanisms of tubular injury. Curr Opin Crit Care. 2019; 25(6):550-557. doi: 10.1097/MCC.0000000000000653.
2. Ungprasert P, Cheungpasitporn W, Crowson CS, Matteson EL. Individual non-steroidal anti-inflammatory drugs and risk of acute kidney injury: A systematic review and meta-analysis of observational studies. Eur J Intern Med. 2015;26(4):285-91. doi: 10.1016/j.ejim.2015.03.008.
3. Correll CU, Detraux J, De Lepeleire J, DeHert M. Effects of antipsychotics, antidepressants and mood stabilizers on risk for physical diseases in people with schizophrenia, depression and bipolar disorder. World Psychiatry. 2015;14(2):119-136. doi: 10.1002/wps.20204 .
4. Jiang Y, McCombs JS, Park SH. A retrospective cohort study

of acute kidney injury risk associated with antipsychotics. CNS Drugs. 2017;31(4):319-26. doi: 10.1007/s40263-017-0421-4.
5. Moreno-Fernandez AM, Cordero MD, de Miguel M, Delgado MD, Sanchez –Alcazar, Navas P. Cytotoxic effects of amitriptyline in human fibroblasts. Toxicology. 2008; 14;243(1-2):51-58. doi: 10.1016/j.tox.2007.09.021.
6. Cordero MD, Sánchez-Alcázar JA, Bautista-Ferrufino MR, Carmona-López MI, Illanes M, Ríos MJ, Garrido-Maraver J, Alcudia A, Navas P, de Miguel M. Acute oxidant damage promoted on cancer cells by amitriptyline in comparison with some common chemotherapeutic drugs. Anticancer Drugs. 2010;21(10):932-44. doi: 10.1097/CAD.0b013e32833ed5f7.
7. Hroudova J, Fisar Z In vitro inhibition of mitochondrial respiratory rate by antidepressants. Toxicol Lett. 2012; 18;213(3):345-52. doi: 10.1016/j.toxlet.2012.07.017.
8. Dokken K, Fairley P. Sodium channel blocker toxicity. StatPearls Publishing; 2020.
9. Sales GTM, Foresto RD. Drug-induced nephrotoxicity. Rev Assoc Med Bras. 2020;66:82-90. doi: 10.1590/1806-9282.66.S1.82.
10. Griffin BR, Faubel S, Edelstein CL. Biomarkers of drug-induced kidney toxicity. Ther Drug Monit. 2019;41(2):213-226. doi: 10.1097/FTD.0000000000000589.
11. Kim SY, Moon A. Drug-induced nephrotoxicity and its biomarkers. Biomol Ther (Seoul). 2012;20(3):268-272. doi: 10.4062/biomolther.2012.20.3.268.
12. Barnoux AS, Morena M, Badiou S, Dupuy AM, Canaud B, Cristol JP. Carbonyl stress and oxidatively modified proteins in chronic renal failure. Ann Biol Clin. 2009;67(2):153-8. doi: 10.1684/abc.2009.0295.
13. Piwowar A. Advanced oxidation protein products. Part I. Mechanism of the formation, characteristics and property. Pol MerkurLekarski. 2010;28(164):166-9. [Polish]
14. Husna AH, Ramadhani EA, Eva DT, Yulita AF, Suhartono E. The Role Formation of Methylglyoxal, Carbonyl Compound, Hydrogen Peroxide and Advance Oxidation Protein Product Induced Cadmium in Ovarian Rat. Int J Chem Eng Appl. 2014;5(4):319-23. doi: 10.7763/IJCEA.2014.V5.402.
15. Rabbani N, Thornalley PJ. Advanced glucation end products in the pathogenesis of chronic kidney disease. Kidney Injury. 2018;93(4):803-13. doi: 10.1016/j.kint.2017.11.034.
16. Clinical protocol №10 approved by the Joint Commission on the quality of medical services of the Ministry of Health and Social Development of the Republic of Kazakhstan. August 25, 2016. https://diseases.medelement.com/disease/14932.
17. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int. 1996;49:1304-13.
18. Stadtman ER, Levine RL. Protein oxidation. Ann N Y Acad Sci. 2000;899:191-208.