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Molecular Mechanisms Underlying the Nephrotoxicity of Cisplatin, Lead Acetate and Cyclosporine: Key Roles of *Myc* and *Smad4*

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Abstract

It is well documented that use of Cisplatin, Lead acetate and Cyclosporine in the chemotherapy and medical interventions is highly associated with nephrotoxicity and interrelated comorbidities. Here, we proposed the possible molecular mechanisms responsible for nephrotoxicity of these compounds. We utilized the microarray dataset GSE59913 consisting of approximately 600 different compounds profiled in up to 8 different tissues. After analysis with GEO2R, gene expression profiles of three aforementioned compounds were integrated with protein-protein interactions (PPI) networks and topological properties of the networks were measured using Cytoscape software. We found several key genes and signaling pathways that seem to be involved in nephrotoxicity of the examined compounds. Myc and Smad4 were identified as principal players of three compounds' nephrotoxicity through various pathways. Our results revealed the critical functions of Il2, Jak-Stat, Mapk-Pi3k, TGFβ and Ca^{2+} signaling pathways as well as novel biomarkers that may mediate the nephrotoxicity of Cisplatin, Lead acetate and Cyclosporine. The significantly altered genes in the compound-treated samples were substantially correlated with regulation of cell proliferation, apoptosis, inflammatory responses and homeostatic processes. This study reveals the important hub genes, biological networks and key pathways as well as novel biomarkers involved in nephrotoxicity of Cisplatin, Lead acetate and Cyclosporine.

Keywords: Nephrotoxicity; Cisplatin; Lead acetate; Cyclosporine; PPI Networks

Introduction

The kidney is an important organ prerequisite by the body to perform several essential regulatory roles including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs [1]. Therefore, the kidney can be considered as a vital target tissue for exogenous toxicants. Nephrotoxicity is a kidney-specific characteristic in which excretion does not go slowly owing to toxic chemicals or drugs [2,3]. Approximately 20% of nephrotoxicity in community and hospital acquired episodes is induced by drugs, among older adults, the incidence of drug-induced nephrotoxicity may rise as 66% as the average life span increases. Chemotherapy or anticancer medicine has been of limited use due to nephrotoxicity [4-7]. Cellular toxicity probably has a multifactorial etiology which is related to alterations in renal vascular cells, modifying renal hemodynamics and the relative ischemia induced by vasoconstriction potentiating sub lethal changes in renal tubular epithelial cells. Histopathological evidence of cell damage will be apparent only if the toxic injury exceeds the capacity of the cellular mechanisms to respond to the toxic insult [8]. It is widely acknowledged that patients treated with the Cyclosporine and Cisplatin are at a high risk of developing nephrotoxicity [9,10]. Studies have demonstrated that Cyclosporine causes vasoconstriction of the afferent and efferent glomerular arterioles and reductions in renal blood flow and glomerular filtration rate [11,12]. Cisplatin is an important antineoplastic drug used for the treatment of cancers. Its major dose limiting adverse effect is nephrotoxicity; 20% of patients receiving high-dose Cisplatin have intensive renal failure [10,13]. Furthermore, rat's exposure to environmental pollutants such as Lead acetate induced nephrotoxicity [14]. However, the mechanism behind these Compounds remains a matter of debate.

Since nephrotoxicity largely affects human health and has a poorly understood pathogenesis, several studies have examined this condition. Moreover, the spectrum of temporal pathway deregulation has not been studied using integrative framework. Bearing this fact in mind, understanding the toxic mechanisms for nephrotoxicity renders advantageous information on the development of drugs with both potential therapeutic benefits and reduced adverse effects. To this end, analysis of PPI and gene regulatory networks (GRNs) has emerged as a promising tool and can help to decipher in-depth biological aspects of

various disorders [15,16]. However, there are no high-throughput investigation between nephrotoxicity compounds and kidney that modulate host gene expression. Therefore, our network-based study has been carried out to investigate the specific pathways and regulatory genes that are critical for renal failure and nephrotoxicity in the rats treated with Cisplatin, Lead acetate and Cyclosporine.

Materials and Methods

Preparation of microarray data

A complete drug matrix dataset for kidney (Accession number: GSE59913) consisting of approximately 600 different compounds profiled in up to 8 different tissues was obtained from Gene Expression Omnibus database (GEO; http://www.ncbi.nlm.nih.gov/geo/). The authors of this dataset collected 2862 samples, in biological triplicates, from test compound-treated and vehicle control-treated subjects for gene expression analysis in response to studied compounds. Owing to their important role in nephrotoxicity, we chose Cisplatin-, Lead acetate- and Cyclosporine-treated samples for further analyses. The differentially expressed genes (DEGs) were determined by using GEO2R tool [17]. Subsequently, DEGs were further restricted to a log 2 fold change larger than 1 and smaller than -1 (p-value <0.05).

Functional annotation of DEGs

Functional annotation of selected DEGs was conducted utilizing Gene Ontology (GO) database [18]. In parallel, the network-based Biological Networks Gene Ontology (BiNGO) tool, a popular plugin of Cytoscape software [19], was used as an alternative tool for validating the GO results. This plugin is a flexible and extendable tool which is widely used to analyze GO terms overrepresented in a given biological network [20].

Signaling pathways data

Pathway enrichment was carried out using SPEED web tool to identify the signaling pathways underlying nephrotoxicity of Cisplatin, Lead acetate and Cyclosporine [21]. This server is an intuitive approach for discovering signaling pathways responsible for regulating various biological processes. Additionally, the signaling pathways corresponding to the DEGs of each aforementioned compounds were collected from the Kyoto Encyclopedia of Genes and Genomes (KEGG) [22]. The daily updated KEGG databases consist of information about genomic, cellular pathways and chemical compounds.

Determination of regulatory relationships between the degs

A protein-protein interactions (PPIs) network was constructed for each studied compound utilizing BisoGenet, a plugin of Cytoscape [23]. BisoGenet is a multi-tier tool which constructs the PPI networks based on the regulatory relationships data accumulated from several PPI databases including Database of Interacting Proteins (DIP; http://dip.doe-mbi.ucla.edu), BioGRID, Human protein reference database (HPRD), Biomolecular Interaction Network Database (BIND), Molecular interaction database (MINT) and IntAct [24-28]. In addition, some other PPIs between the DEGs were retrieved from the most recent studies.

Topological analysis of the PPIs networks

Topological properties of each PPIs network were measured using Network Analyzer, a network analysis plug-in of Cytoscape, to identify the most important functional hub genes within the networks and simplify interpretation of the results. We employed eight common measures including, Degree, Betweenness Centrality, Clustering Coefficient, Closeness Centrality, Eccentricity, Neighborhood Connectivity, Topological Coefficient and Average Shortest Path Length for appraisal topological properties of the PPIs networks. The nodes that were repeatedly identified as hub gene in aforementioned measures were adjudged as functionally important hub genes.

Functional motifs within the PPI networks

MCODE plugin of Cytoscape was applied to discover the functional regulatory motifs embedded in constructed PPI networks. This plugin can discern the highly interconnected complexes in a given network by finding regions of significant local density. These complexes are often associated with a specific cellular process.

Overlap nephrotoxicity mechanisms between the compounds

In order to identify the common and signature genes implicated in nephrotoxicity of Cisplatin, Lead acetate and Cyclosporine, a clustering of DEGs was conducted using Venny 2.1.0, an interactive web tool for comparing lists with venn diagrams [29]. Prior to clustering, the up- and down-regulated DEGs of each compound were separated. Gene overlaps were determined with a three-way Venn diagram and were further analyzed.

Results

PPI networks of cisplatin, lead acetate and cyclosporine

By integrating the regulatory relationships obtained from BisoGenet plugin as well as published data, a PPI network was constructed for each DEG list resulted from processing Cisplatin, Lead acetat and Cyclosporine gene expression profiles. The nodes and edges number of Cisplatin, Lead acetate and Cyclosporine were 367 and 292, 467 and 408 and 502 and 436, respectively. After determination of each depicted PPI sub-network, the most integrated sub-networks were extracted and illustrated in Figure 1. Furthermore, node degree distribution of each PPI network was significantly right-skewed implying that three PPI networks have a biological scale-free pattern, as majority of nodes had low numbers of edges and only a few numbers of nodes were highly connected.

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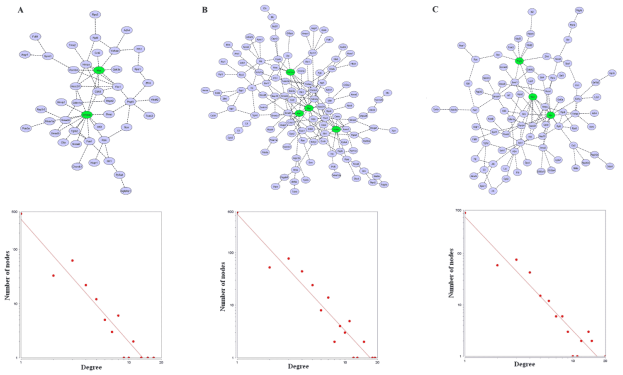

Figure 1: The main sub-networks extracted from PPI networks of (A) Cisplatin, (B) Lead acetate and (C) Cyclosporine along with their respective degree distribution charts (below each sub-network). The nodes with higher betweeness centrality are shown in green color.

The most significant hub genes of PPI networks

Using various algorithms, we found a set of hub genes for each PPI network constructed for Cisplatin, Lead acetate and Cyclosporine DEGs (Table 1). Expectedly, several hub genes were identified with more than one algorithm, and therefore were propounded as important hub genes. The hub genes in Cisplatin PPI network including *Ascc3l1, Bcr, Cdk9, Cdkn1a, Hnrpu, Lep, Myc, Pak1, Pcgf4, Ptpn6, Ran, Slc25a13, Slc8a1, Smad2, Smad4, Smad5, Stat5a, Tgm2, Tpm1, Ttn, Vapa, Ybx1* and *Ywhae* were distinguished as hub genes in more than one topological measure. Whereas, *A2m, Alb, Ewsr1, Hnrpu, Iqcb1, Myc, Ncoa6, Pcgf4, Ptpn2, Smad4, Ybx1* and *Ywhae* were singled out as the highest-scored hub genes of Cyclosporine PPI network. The important hub genes of Lead acetate PPI network were included *Adrm1, Alb, Apoa2, Apoc1, Cdk9, Cdkn1a, Hnrpu, Icam1, Myc, Pak1, Pdlim5, Prph1, Psma3, Ptpn2, Ptprr, Smad1, Smad4, Ybx1* and *Ywhae*. We also found several novel biomarkers that are likely involved in nephrotoxicity of each studied compound (bold genes in Table 1).

Hub Genes of Cisplatin PPI network					
Average Shortest Path Length	**Closeness Centrality**	**Degree**	**Betweenness Centrality**	**Topological Coefficient**	**Clustering Coefficient**
Oprm1	*Tpm1*	*Smad2*	*Tpm1*	*Stat5a*	*Stat5a*
Vapa	*Lep*	*Myc*	*Lep*	*Smad5*	*Smad5*
Ryr1	*Lcn2*	*Ywhae*	*Myc`*	*Tgm2*	*Tgm2*
Agtpbp1	*PVR*	*Pak1*	*Smad2*	*Strap*	*Cdk9*
Arts1	*Otc*	*Ybx1*	*Ywhae*	*Prkar1a*	*Ascc3l1*
Phgdhl1	*Mmp9*	*Pcgf4*	*Bcr*	*Nr5a2*	*Pdgfrb*
Npy5r	*Ryk*	*Ascc3l1*	*Pak1*	*Ngfr*	*Ptpn2*
Il2	*Nrp2*	*Cdk9*	*Slc8a1*	*Tnfrsf1a*	*Psmb4*
Fdft1	*Tomm20*	*Bcr*	*Ptpn6*	*Ttn*	*Gtf3c1*
Asgr1	*Tnfrsf12a*	*Hnrpu*	*Ybx1*	*Vapa*	*Hnrpu*
Vgcnl1	*Tnnt2*	*Cdk5*	*Pcgf4*	*Cct2*	*Ybx1*
Ihpk2	*Ghrl*	*Smad4*	*Slc25a13*	*Slc25a13*	*Smad4*
Adra1b	*Srxn1*	*Cdkn1a*	*Ran*	*Cdc25b*	*Myc*
Ttn	*Ucn*	*Ran*	*Alb*	*Slc8a1*	*Bcr*
Pc	*Myc*	*Ptpn6*	*Cdkn1a*	*Blk*	*Pak1*
Hub Genes of Lead acetate PPI network					
Average Shortest Path Length	**Closeness Centrality**	**Degree**	**Betweenness Centrality**	**Topological Coefficient**	**Clustering Coefficient**
Gdf15	*Mc4r*	*Myc*	*Reep6*	*Apoc1*	*Apoc1*
Tpm1	*Ppara*	*Hnrpu*	*Pdlim5*	*Apoa2*	*Apoa2*
Mapk14	*Sln*	*Psma3*	*Hnrpu*	*Adrm1*	*Adrm1*
Clta	*Npy*	*Ybx1*	*Myc*	*Rpl13a*	*Ptpn2*

Srxn1	*Wrnip1*	*Cdkn1a*	*Psma3*	*Ptpn2*	*Sfrs3*
Etv6	*Gclm*	*Smad4*	*Pak1*	*Prph1*	*Ascc3l1*
Ptprr	*Vps26*	*Smad1*	*Cdkn1a*	*Cd3z*	*Syncrip*
Cpb2	*Nup107*	*Ywhae*	*Ybx1*	*Paics*	*Ghr*
Otc	*Ryk*	*Pak1*	*Vtn*	*Cks2*	*Csda*
Efs	*Prim1*	*Icam1*	*Smad4*	*Mme*	*Mrps28*
Vapa	*Prim2*	*Alb*	*Lnx1*	*C3*	*S100a9*
Tacc3	*Pln*	*Cdk9*	*Prph1*	*Ptprr*	*Ccnh*
Txlnb	*Pdlim5*	*Ewsr1*	*Ywhae*	*Bcl2l1*	*Cct2*
Hmmr	*Fabp1*	*H2afx*	*Smad1*	*Ifit1*	*Cdk9*
Mmp9	*Txnl2*	*Plk1*	*Alb*	*Abcc2*	*Icam1*
Hub Genes of Cyclosporine PPI network					
Average Shortest Path Length	**Closeness Centrality**	**Degree**	**Betweenness Centrality**	**Topological Coefficient**	**Clustering Coefficient**
Pdgfrb	*Sstr3*	*Myc*	*Myc*	*Rps5*	*Ste2*
Tceb1	*Sstr2*	*Iqcb1*	*Ybx1*	*Ncoa6*	*Znf386*
Il3ra	*Cxcl9*	*Ybx1*	*Iqcb1*	*Slc8a1*	*Zfx*
C1s	*Stx7*	*Ywhae*	*Ewsr1*	*Ngfr*	*Zfp592*
Dusp7	*Onecut1*	*Smad4*	*Pcgf4*	*C1qb*	*Zfp536*
Ptpn2	*Foxa1*	*Pcgf4*	*Hnrpu*	*Hp*	*Zfp496*
Cish	*Efs*	*Ewsr1*	*Ywhae*	*Elavl3*	*Zfp422*
Il2ra	*Hadh2*	*A2m*	*Smad4*	*Zfml*	*Ypel4*
Npy5r	*Sftpc*	*Hnrpu*	*Rara*	*Id3*	*Xpnpep1*
Atf3	*Grsf1*	*Rbpms*	*Anxa2*	*Ttn*	*Wrnip1*
Nrcam	*Mapk10*	*Map3k1*	*A2m*	*Il2*	*Vsnl1*
Oprm1	*Gfra1*	*Alb*	*Vamp2*	*Nupr1*	*Vnn1*
Oprk1	*Tomm20*	*Vapa*	*Ncoa6*	*Arpc1b*	*Vgcnl1*
Cln8	*Gdnf*	*Ascc3l1*	*Alb*	*Ppp2r2c*	*Vash2*
Apod	*Gch*	*Cdk9*	*Ghr*	*Ptpn2*	*Usp18*

Table 1: The 15 top hub genes for each constructed PPI network. The putative novel biomarkers for nephrotoxicity of each compound are shown in bold.

Myc and *Il2* were found as key nodes of most significant functional motifs

In order to identify the functional motifs within constructed PPI networks, we analyzed the networks via MCODE algorithm. Prior to network analysis, the up- and down-regulated DEGs of each compound were dissected and a corresponding network was reconstructed for each DEG set. The results illustrated that a four-node complex consisting of *Myc, Cdk9, Hnrpu* and *Ascc3l1* genes is present in the most significant up-regulated motif of three aforementioned compounds. Furthermore, *Il2* and *Ngfr* were observed in the most significant down-regulated motif of Cisplatin and Cyclosporine PPI networks (Figure 2).

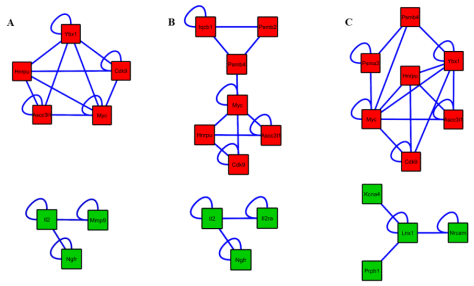

Figure 2: The most significant regulatory motif of (A) Cisplatin, (B) Cyclosporine and (C) Lead acetate PPI networks along with their respective most significant down-regulated motifs. Up- and down-regulated motifs are shown in red and green colors, respectively.

Functional annotation of DEGs

GO analysis unveiled that the dominant biological processes of Cisplatin, Lead acetate and Cyclosporine DEGs are obviously associated with the cell proliferation, apoptosis, inflammatory responses, homeostatic processes, response various stresses and modulation synaptic transmission responses. The overrepresented functional categories are listed in Table 2.

SPEED and KEGG results demonstrate Mapk-Pi3k as the crucial upstream signaling pathway upregulated in Cisplatin and Lead acetate nephrotoxicity. Moreover, results show that Jak-Stat signaling pathway is remarkably modulated after treatment of cells with Cisplatin and Lead acetate postulating the possible protective effect of this signaling pathway in nephrotoxicity. In contrast, Jak-Stat signaling pathway was found as the highest-scored signaling pathway upregulated in Cyclosporine-treated cells. The enriched signaling pathways are sorted by FDR score and graphical outputs aid in interpretation of the results (Figure 3).

Cisplatin					
Up-regulated DEGs			**Down-regulated DEGs**		
GO Term	**Count**	**P-value**	**GO Term**	**Count**	**P-value**
Regulation of muscle system process	11	0.0298	Chemical synaptic transmission	15	5.67E-05
Response to toxic substance	11	0.0475	Regulation of ion transmembrane transport	12	0.00794
Cellular homeostasis	23	0.00404	Regulation of homeostatic process	12	0.0372
Response to hormone	23	0.0246	Negative regulation of developmental process	15	0.0452
Chemical homeostasis	26	0.00544	Positive regulation of protein phosphorylation	17	0.0338
Response to organic cyclic compound	25	0.0165	Regulation of multicellular organismal process	19	0.00894
Regulation of cell proliferation	34	0.0304	Chemical homeostasis	17	0.0433
Cellular response to organic substance	40	0.0111	Generation of neurons	23	0.00431
Organonitrogen compound metabolic process	38	0.0314	Regulation of cell proliferation	25	0.00248
Response to stress	68	1.36E-06	Response to endogenous stimulus	22	0.0293
Regulation of cellular protein metabolic process	48	0.00191	Cell surface receptor signaling pathway	31	0.00111
Positive regulation of cellular metabolic process	51	0.0218	Regulation of intracellular signal transduction	24	0.049
Negative regulation of cellular process	74	0.000116	Animal organ development	37	0.000415
Cellular metabolic process	114	0.0267	Response to organic substance	34	0.00184
Biological process	189	0.000119	Regulation of molecular function	34	0.0169
Lead acetate					
Up-regulated DEGs			**Down-regulated DEGs**		
GO Term	**Count**	**P-value**	**GO Term**	**Count**	**P-value**
Cardiac muscle tissue development	12	0.0148	Cellular divalent inorganic cation homeostasis	11	0.0322
Regulation of blood pressure	14	0.00212	Blood circulation	12	0.0116
Response to hypoxia	20	0.000117	Cellular metal ion homeostasis	12	0.0393
Leukocyte migration	17	0.0031	Regulation of ion transport	14	0.0345

Response to oxidative stress	19	0.0135	Regulation of multicellular organismal process	26	0.000753
Positive regulation of MAPK cascade	25	0.000298	Response to organic substance	34	0.0139
Cellular response to cytokine stimulus	29	0.000262	Single-organism cellular process	87	0.0237
Inflammatory response	21	0.0423			
Negative regulation of apoptotic process	32	0.00268			
Response to organonitrogen compound	30	0.00858			
Regulation of proteolysis	27	0.0329			
Chemical homeostasis	33	0.00806			
Regulation of cell differentiation	47	0.00375			
Regulation of cell proliferation	47	0.00476			
Regulation of cellular component organization	67	6.03E-05			
Cyclosporine					
Up-regulated DEGs			**Down-regulated DEGs**		
GO Term	**Count**	**P-value**	**GO Term**	**Count**	**P-value**
Response to temperature stimulus	11	0.0494	Regulation of cytosolic Ca^{+2} ion concentration	10	0.0154
Regulation of muscle system process	13	0.017	Locomotory behavior	10	0.0205
Activation of immune response	19	0.0326	Modulation of synaptic transmission	15	6.76E-05
Cytokine-mediated signaling pathway	20	0.0245	Regulation of membrane potential	15	0.00077
Regulation of cytokine production	23	0.0137	Regulation of homeostatic process	16	0.00402
Negative regulation of cell proliferation	25	0.0109	Response to extracellular stimulus	15	0.0245
Immune response	38	0.000314	Positive regulation of Mapk cascade	15	0.0452
Negative regulation of cell death	30	0.0169	Regulation of hormone levels	15	0.0487
Chemical homeostasis	30	0.021	Cation transmembrane transport	17	0.015
Defense response	40	0.000663	Inorganic ion transmembrane transport	17	0.0294
Response to oxygen-containing compound	43	0.00122	Response to nitrogen compound	23	0.003
Regulation of phosphate metabolic process	44	0.0177	Positive regulation of cell differentiation	22	0.00555
Regulation of protein modification process	44	0.0361	Response to organic cyclic compound	23	0.00449
Organonitrogen compound metabolic process	47	0.0284	Response to oxygen-containing compound	36	1.69E-06
Animal organ development	66	0.0054	Response to endogenous stimulus	33	7.91E-05
			Generation of neurons	27	0.0491
			Regulation of multicellular organismal development	31	0.0463
			Cellular response to chemical stimulus	41	0.00221

Table 2: Significant overrepresented GO terms in DEGs of Cisplatin, Lead acetate and Cyclosporine.

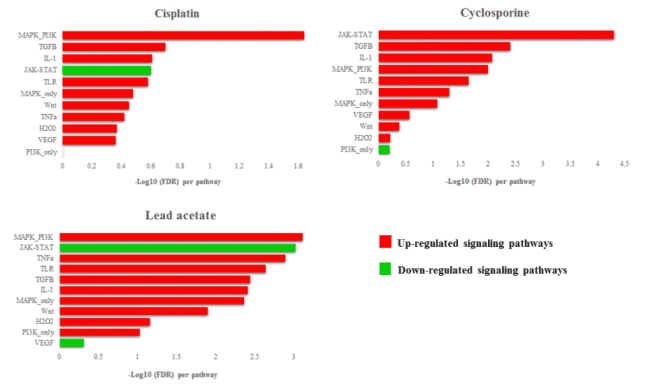

Figure 3: The most enriched signaling pathways for the DEGs resulted from gene expression profile of Cisplatin, Cyclosporine and Lead acetate. Up- and down-regulated signaling pathways are shown in red and green colors, respectively.

As illustrated in Figure 4, mechanism of nephrotoxicity caused by Cisplatin, Lead acetate and Cyclosporine is noticeably mediated by upregulation of TGFβ signaling pathway. Several genes of this signaling pathway including *Smad1, Smad2, Smad4, Myc* and *Id1* were found to be up-regulated after treatment with Cisplatin, Lead acetate and Cyclosporine. However, up-regulation of *Rock1* was only observed in Lead acetate-treated samples.

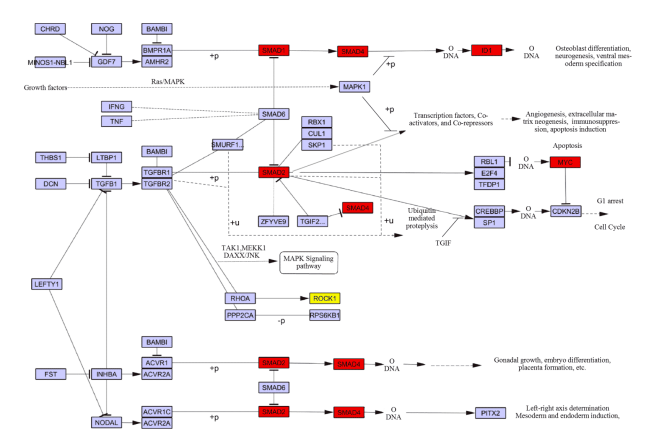

Figure 4: Representation of TGFβ signaling pathway extracted from KEGG. Common up-regulated genes in the samples treated with three studied compounds (Cisplatin, Lead acetate and Cyclosporine) are shown in red color. The node with yellow color was only up-regulated in the samples treated with Lead acetate.

Moreover, down-regulation of Ca^{+2} signaling pathway was significantly observed after treatment of rats with Cisplatin, Lead acetate and Cyclosporine. This suggests that modulation in Ca^{+2}-mediated gene expression is a common event upon nephrotoxicity of three studied compounds. *Slc8a2, Chrm1, Cysltr1* and *Mylk4* were found as overlap down-regulated genes of Ca^{+2} signaling pathway, whereas *Chrna7* and *Ppp3ca* were down-regulated after treatment with Cyclosporine and Cisplatin, respectively (Figure 5).

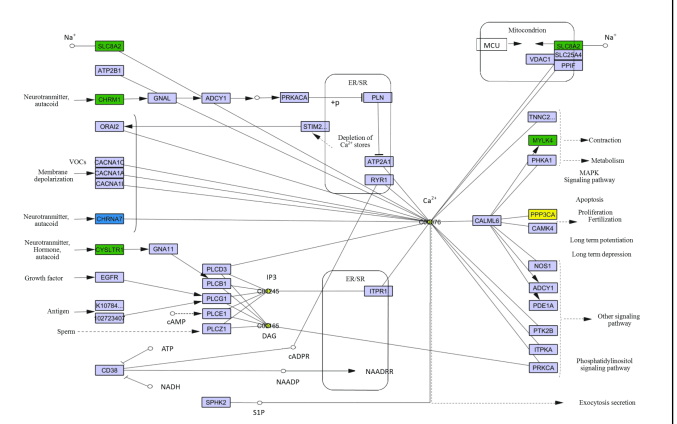

Figure 5: Representation of Ca^{+2} signaling pathway extracted from KEGG. Common down-regulated genes in the samples treated with three studied compounds (Cisplatin, Lead acetate and Cyclosporine) are shown in green color. The nodes with yellow and blue colors were only up-regulated in the samples treated with Cisplatin and Cyclosporine, respectively.

Cisplatin, cyclosporine and lead acetate share overlap nephrotoxicity mechanisms

In order to investigate the common nephrotoxicity pathways, a clustering was conducted according to each compound's DEG list. The results indicated that 142 up-regulated DEGs (28.3%) and 41 down-regulated DEGs (13.3%) are joint between three nephrotoxic compounds. The lowest number of overlap DEGs was found between Cisplatin and Lead acetate (Figure 6).

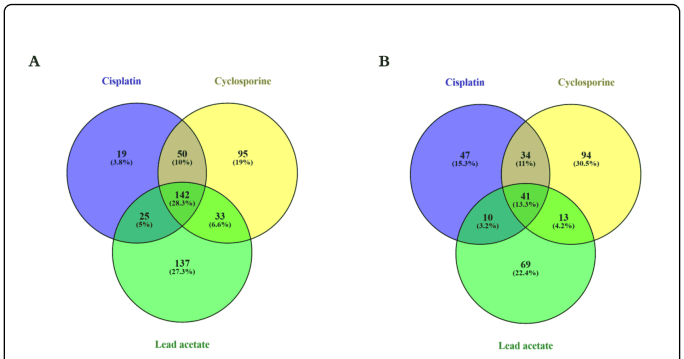

Figure 6: Clustering of (A) up-regulated and (B) down-regulated DEGs obtained from analysis of gene expression profiles of Cisplatin, Lead acetate and Cyclosporine. The numbers represent number of overlap DEGs.

DEG-GO networks revealed the most important overlap DEGs

A DEG-GO network was separately constructed for both common up-regulated and down-regulated DEGs. Common up-regulated DEGs and their respective biological processes were considered as source and target nodes, respectively. The results indicated that several overlap

DEGs up-regulated in all studied compounds including *Cdkn1a, Myc, Smad4, Pak1, Hmgb1, Kras* and *Pdgfrb* have significant roles in activation of nephrotoxicity pathways (Figure 7). On the other hand, among common down-regulated *DEGs, Il2, Pth* and *Hcrt* showed a pivotal role in regulation of nephrotoxicity pathways (Figure 8). These DEG-GO networks of the overlap DEGs uncovered that common up-regulated DEGs are highly enriched in regulation of cell metabolism, cell proliferation and cell death pathways, whereas the common down-regulated DEGs are notably involved in homeostatic processes.

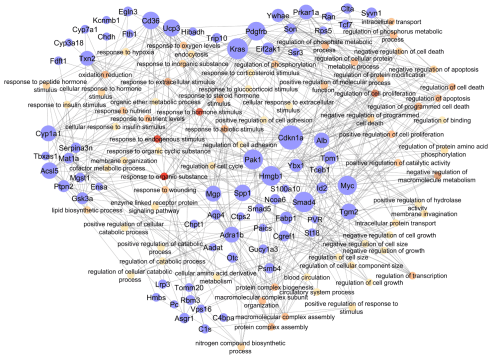

Figure 7: DEG-GO network for common up-regulated DEGs between Cisplatin, Lead acetate and Cyclosporine. Nodes (common up-regulated DEGs) with higher out-degree measure are shown in bigger circles, while nodes (biological processes) with higher in-degree measure tend to have red color.

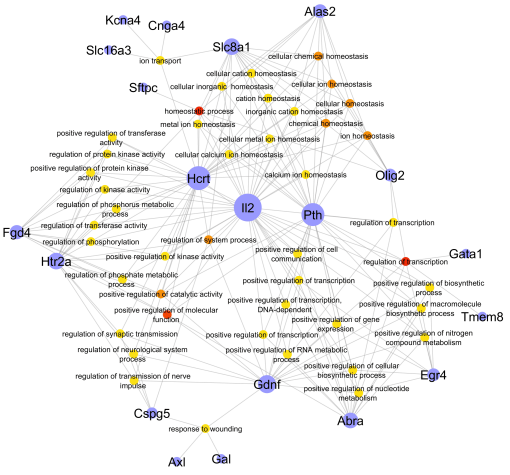

Figure 8: DEG-GO network for common up-regulated DEGs between Cisplatin, Lead acetate and Cyclosporine. Nodes (common up-regulated DEGs) with higher out-degree measure are shown in bigger circles, while nodes (biological processes) with higher in-degree measure tend to have red color.

Discussion

Nephrotoxicity is a harmful repercussion of some medications and toxic chemicals on renal function which its pathogenesis is not yet fully understood [30]. All available data on protein/gene expression in nephrotoxicity provide an interesting opportunity to identify markers for the diagnosis and treatment of the disease. It is thought that the incidence of nephrotoxicity is closely correlated with the abnormal expression of multitudinous genes. We used bioinformatics methods and a consolidated view to explore a new strategy for understanding toxicity-derived renal dysfunction mechanisms and find phenotype-related biomarkers.

GO annotation suggests that many of resulted DEGs in the samples treated with studied compounds are connected with multiple biological processes, including organonitrogen compound metabolic process, immune response, regulation of cell proliferation, regulation of apoptosis, response to oxidative stress, electrolyte abnormalities and regulation of blood pressure. *Myc, Pak1, Pcgf4, Ptpn6, Ran, Smad4, Smad2, Smad5, Stat5a, Ywhae, Slc25a13* and *Slc8a1* were identified as important hub genes in the PPI networks. Smad2 is actively participating in manifold cell processes such as cell differentiation, apoptosis, cell proliferation, cell-fate determination and morphogenesis. This gene is frequently upregulated in nephrotoxicity and activated by TGFβ receptor-type kinases [31]. *Slc25a13* belongs to super-family of SLC proteins which comprises 55 gene families, having at least 362 putatively functional gene targets. Members of this super-family functions in transportation of various molecules and ions across cellular and organelle membranes [32]. Recently, it has been established that cell toxicity can lead to dysregulation of these transporters [33]. Our study also provides further evidences that activation of *Pak1*, presumably an adaptive reaction against cell death, is a signature of Cisplatin-induced nephrotoxicity. Delving further into the issue, Pak1 is a serine/threonine kinase that is targeted by small GTP binding proteins. This protein is presented as a regulator of cytoskeletal remodeling, apoptosis and cell motility. It has been recently demonstrated that nephrotoxicity increases matrix remodeling and apoptosis properties [34,35]. We also report *Cdk9, Psma3, Icam1, Myc, Hnrpu, Pak1* and *Alb* as a consensus early signature of nephrotoxicity that induced by Lead acetate. During apoptosis Hnrpu is cleaved in a caspase-dependent way. Interestingly, Li et al. found that *Hnrpu* up-regulation is followed by cooper overload and vancomycin nephrotoxicity [36,37]. By analysis of PPI networks, we present *Hnrpu* as a promising biomarker for Lead acetate nephrotoxicity. Furthermore, the results indicated that Rock1 is important during Lead acetate nephrotoxicity. Interestingly, gentamicin-induced nephrotoxicity caused an increase in the activity of Rho-kinase enzyme. The activity of Rho-kinase enzyme in nephrotoxicity is highly associated with an increase in oxidative stress, leading ultimately to irreversible kidney dysfunction [38]. Moreover, a significant up-regulation of response to hypoxia, leukocyte migration and response to oxidative stresses was found in DEGs of studied compounds after GO analyses. Strikingly, these biological processes have been linked to the Lead acetate-induced nephrotoxicity [39,40]. Accordingly, we suggest the use of a specific inhibitor of Rock1 may represent a novel therapeutic approach in the prevention of nephrotoxic side effects during Lead acetate exposure. Psma3 gene was found as the most important hub gene obtained from topological analysis of Lead acetate nephrotoxicity network. The *Psma3* has a key role in determination of protein's fate via ubiquitin-independent proteasomal degradation [41]. In addition, DNA damage can induce phosphorylation of *Psma3* during the cell cycle transition and apoptosis [42]. Surprisingly, Lead acetate can accelerate proteasome activity and this activity is likely associated with Mapk pathway and inflammatory response [43-45]. Taken together, we suggest that

inhibition of *Psma3* should be recognized as a new anti-inflammatory strategy in Lead acetate nephrotoxicity. Our results revealed that expression of *Alb, Pcgf4, Ybx1* and *Myc* hub genes is significantly altered in Cyclosporine nephrotoxicity. Studies have shown that these genes have a role in cell toxicity of some cytotoxic compounds. Recently, it has been determined that reduction of *Pcgf4* can lead to apoptosis, senescence in tumor cells, and increased cell susceptibility to cytotoxic agents [46]. In addition, *Pcgf4* can prevent cell toxicity *in vitro* and *in vivo* by reducing oxidative stress [47]. The *Ybx1* is a key regulator of cell growth, apoptosis, drug resistance, DNA repair and transcription. It has been shown that overexpression of *Ybx1* via Erk/Akt pathway can monitor damage recognition and renal fibrosis of toxic agents such as Cyclosporine [48-50]. However, the role of *Pcgf4* and *Ybx1* in nephrotoxicity has not yet been fully evaluated. It has been shown that *Il-2* is down-regulated in Cisplatin and Cyclosporine nephrotoxicity [51,52]. Consistent with these studies, we found *Il-2* as a common hub gene in Cisplatin, Lead acetate and Cyclosporine nephrotoxicity. Our work further pinpointed two key genes including *Myc* and *Smad4* that may play a pivotal role in the development of nephrotoxicity. *Myc* is a transcription factor and multifunctional gene that plays a role in the control various cellular functions such as cell proliferation, apoptosis, cell migration, biogenesis of macromolecules and protein degradation pathways [53]. We figured out that the *Myc* and *Smad4* genes are the most important common hub genes procured from topological analysis of Cisplatin, Lead acetate and Cyclosporine PPI networks. Several studies have established that *Myc* has a crucial role in perturbation of gene expression of Mrp and SLC families which are efflux transporters and have important roles in the kidney function by regulating Ca^{2+} homeostasis and other organic, anionic conjugates [54-56]. In addition, the positive regulatory impact of Cisplatin and Cyclosporine on *Myc* expression in drug resistance and nephrotoxicity pathways has been previously reported [57,58]. Overall, our results suggest that *Myc* proto-oncogene not only regulates Cisplatin- and Cyclosporine-mediated nephrotoxicity, but also can be perceived as a key mediator of Lead acetate-induced nephrotoxicity.

Extracellular matrix proteins and tubulointerstitial fibrosis play pivotal role in nephrotoxicity [59]. Renal fibrosis is the final common result in loss of kidney function. TGFβ-*Smad* signaling has been determined to contribute in nephrotoxicity [60-62]. TGFβ signaling pathway plays multiple functions in cytokine-mediated signaling in many cell types, conditioning them for differentiation, survival, apoptosis and fibrosis [63]. Among the common hub genes of the present study, *Smad4* is an important protein in TGFβ signaling pathway. Although, it is widely accepted that TGFβ signaling pathway induces cell death through *Smad*-mediated pathways, the distinct role of this signaling pathway has insufficiently investigated [64]. However, the synergetic cooperation Cyclosporine with TGFβ signaling pathway in the modulation of renal paracellular permeability has been confirmed by Feldman and colleagues [65]. In another study, expression of TGFβ has been demonstrated to be enhanced in cisplatin-induced Acute Kidney Injury (AKI) [66]. In agreement with these studies, we propose that that Cisplatin, Lead acetate and Cyclosporine can induce nephrotoxicity, at least in part, by affecting TGFβ signaling pathway, particularly *Smad4* expression. Moreover, it is well established that distinct mechanisms such as inflammation, oxidative damages, and DNA damage are associated with development of nephrotoxicity [67]. Here, we found that the studied cytotoxic agents can exacerbate inflammation responses in rats. Activation of Hmgb1 in nephrotoxicity has been shown to increase inflammatory cytokine levels and tissue damages via immunological and non-immunological pathways [68,69]. Our data lending credence to the hypothesis that blockade of *Hmgb1*/TGFβ signaling cascade may constitute a therapeutic strategy for treatment of nephrotoxicity-induced tubulointerstitial fibrosis. The possible mechanisms of *Myc* and *Smad4* in Cisplatin, Lead acetate and Cyclosporine nephrotoxicity are illustrated in Figure 9.

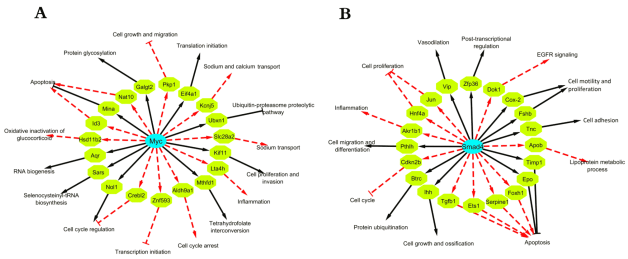

Figure 9: Schematic illustration of possible mechanisms of *Myc* and *Smad4* in Cisplatin, Lead acetate and Cyclosporine nephrotoxicity. Gene targets of *Myc* and *Smad4* transcription factors are illustrated in bright green octagonal.

In summary, this study indicated that characteristics of Cisplatin, Lead acetate and Cyclosporine nephrotoxicity are associated with differential expressions of several genes. We reported *Myc* and *Smad4* as a consensus early signature of in vivo toxicity in kidney. Inhibition of TGFβ signaling intermediates Smad4 and Myc could be an attractive new approach to treatment of Cisplatin, Lead acetate and Cyclosporine nephrotoxicity.

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Conflict of Interest

No conflict of interest to be declared by any of the authors.

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