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Screening and identification of SUMO-proteins in sub-acute treatment with diazinon

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ABSTRACT

Objective(s): Small ubiquitin-like modifiers (SUMOs) are a family of ubiquitin-related, proteins that are involved in a wide variety of signaling pathways. SUMOylation, as a vital post translational modification, regulate protein function in manycellular processes. Diazinon (DZN), an organophosphate insecticide, causses oxidative stress and subsequently programmed cell death in different tissues. The aim of this study was to evaluate the role and pattern of SUMO modificationas a defense mechanism against stress oxidative, in the heart tissuesof the DZN treated rats.

Materials and Methods: Diazinon (15 mg/kg/day), corn oil (control) were administered via gavageto male Wistar rats for four weeks. SUMO1 antibody was covalently crosslinked to protein A/G agarose. heart tissue lysate were added to agarosebeads,After isolation of target proteins(SUMO1- protein)SDS-PAGE gel electrophoresis was performed. Protein bands were identified using MALDI-TOF/TOF and MASCOT). Fold change of (DZN/Ctrl) separated proteins was evaluated using UVband software (UVITEC, UK).

Results: Our result showed that subacute exposure to DZN increased SUMOylationoffour key proteins involved in the metabolic process including; Acyl-CoA dehydrogenase, creatine kinase, glyceraldehyde-3-phosphate dehydrogenase and ATP synthase, in the heart tissue of animals .A probability value of less than 0.05 was considered significant (P<0.05).

Conclusion: It seems that protein SUMOylation provides a safeguard mechanism against DZN Toxicity.

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Introduction

Small ubiquitin-relatedModifier (SUMO) are a family of proteins express in mammals that conjugate to a broad spectrum of proteins. SUMOylation, as an vital post-translational modification, modulates diverse cellular functionsofproteins including transcriptional regulation, apoptosis, protein stability, response to stress, and the cell cycle (1, 2).

SUMOylation and ubiquitination (Ub) compete for the same lysine residue in target proteins and Sumoylatedlysinesleading to protect proteins from Ub labeling and degradation by Proteasome pathway (3).

Change in SUMO homeostasis has been in relation with development and progression of cancer (4), disregulation of mitosis (5), serious placental pathology (1) and neurodegenerative disorders (6-8). SUMOylation of target proteins affects their activity, intracellular localization, stability and interaction with other proteins (9).

Previous studies have consistently demonstrated that SUMOylation regulates intracellular stress and protect cells against oxidative stress (10).

Diazinon, is one of the commonly used organo-phosphorous compounds that reduces the activities of antioxidant enzymes and induces oxidative stress in different tissues (11). Recent studies have demonstrated that DZN may damage the heart in sub -acute exposure in rats through oxidative stress (12, 13).

It was reported that DZN causes histological and biochemical damages in different organs (14).

And also has been proved DZN induced cardio-toxicity in rats (12, 13, 15) major purpose of this study

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was to identify differentially expressedSUMO1-modified heart proteome in the subacute administration of DZN.

Materials and Methods

Animals

Adult male Wistar rats weighing 250-300 g were obtained from the animal house of School of Pharmacy, Mashhad University of Medical Sciences. Rats were kept on a 12-hr light/dark period and at 23±1 ˚C with a free access to food and water. Experiments were accomplished in accordance to Ethical Committee Acts of Mashhad University of Medical Sciences for care and use of laboratory animals.

Chemicals

Pierce Crosslink Immunoprecipitation Kit (Cat. N: 26147) was purchased from the Thermo Scientific Company. DZN was obtained from Bazodin (Syngenta, Singapore, purity 96%). SUMO1 Antibody (#4930) was purchased from Cell Signaling (USA). Other chemicals used in this work were described in the related section.

Treatment

12 Rats were divided into two groups (n=6), control group received corn oil and DZN group, administered 15 mg/kg/day DZN via gavage for four weeks. After four weeks of treatment, rats were sacrificed and the heart tissues were taken and washed using 0.9% normal saline solution, then immediately placed at -80 ˚C until the analysis.

Protein extraction

For performing Immunoprecipitation, 200 mg of the heart tissues were homogenized in 1 mlextraction buffer (IP lysis/wash buffer)containing 25 mM Tris pH 7.4, 1 mM EDTA, 1% NP-40, 150 mMNaCl, 5% glycerol and 2 µl complete protease inhibitor cocktail (Sigma P8340) and 1 mM phenylmethylsulfonyl fluoride (PMSF) using a Polytron Homogenizer (Kinematica, Switzerland) for 10 sec. After sonication for 40 sec (UP100H, Hielscher), homogenates were centrifuged (Hettich Universal 320R, Germany) at 14000 gfor 10 min at 4 ˚C.

Supernatants were removed and stored on ice. Total protein content in supernatants were measured by the PierceBCA protein assay kit (BioRad).

Immunoprecipitation protocol:

SUMO1-proteins were enriched by immunoprecipitation using anti SUMO1 antibody and Pierce Crosslink IP Kit according to manufacturer's protocol (Cat. N: 26147). Briefly, about 20 µg SUMO1 antibody was covalently cross linked to protein A/G resin using disuccinimidylsuberate (DSS). Tissue lysates, containing total proteins (500 µg in 500 µl) were added to the control Agarose resin, to minimize unspecific interactions of SUMO1-proteins with stationary phase resin. After centrifugation (1000 × g for 1 min), the supernatant was added to the antibody linked resin.

Wash the resin two times with Coupling Buffer (provided in kit) and then proteins were eluted using elution buffer (pH 2.8). Elutes were pooled, and dialyzed at 4 ˚C in deionized water using a 2000 Da cutoff (Spectrapor, USA) dialysis membrane to remove electrolytes. Desalted sample proteins were freeze dried and stored at -80 ˚C until use.

Separation of target proteins using SDS-PAGE

Freeze dried samples from the heart were dissolved in 20 µL of 2XSDS buffer containing 100 mMTrisHCl pH 6.8,4% (w/v) SDS (sodium dodecyl sulfate), 0.2% (w/v) bromophenol blue,20% (v/v) glycerol, and 200 mM β-mercaptoethanol. Samples were incubated in boiling water for 5 min and then were loaded on to 12% SDS-PAGE. Electrophoresis was performed at 120 V per gel until the tracking dye reached to the end of the gel.

Sensitive, Mass compatible silver staining was performed to visualize the proteins. Fold change of (DZN/Ctrl) separated proteins was measured byusingUVband software (UVITEC, UK).Differently expressed protein species in DZN group were excised and then collected in microtube containing 1% acid acetic and sent to the Center for Genomic Sciences at the University of Hong Kong for in-gel digestion, identification and characterization by MALDI-TOF/TOF mass spectrometry (Matrix Assisted Laser Desorption Ionization-Time of Flight Analyzer).

MS data were analyzed using the MASCOT search engine (version 2.1.0, Matrix Science, London, UK) MASCOT software database search settings were as follows: +1 monoisotopic peaks were searched with a mass tolerance of 75 ppm for precursor Mass; ±0.2 Da for MS/MS. Trypsin was selected as the enzyme while allowing one missed cleavage, cysteine carbamidomethylation as fixed modification and methionine oxidation as a variable modification; Rattus taxonomy filter was applied when searching against SwissProt and NCBInr databases. Proteins with a score of > 50 and confidence interval (CI) > 95% were accepted.

Classification of proteins

All identified proteins were submitted to the PANTHER online database (http://www. pantherdb.-org) for the classification of proteins according to protein functions, biological process and biological pathways.

Results

To analyze the effect of DZN onexpression SUMO1-protein pattern, immunopercipitated SUMO1-proteins of heart tissue in DZN and control

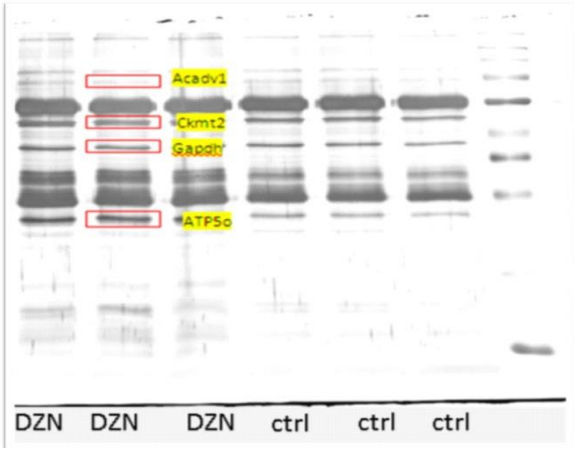

Figure 1. 1D gel image of Sumo1-protein isolated from the heart DZN treated rats

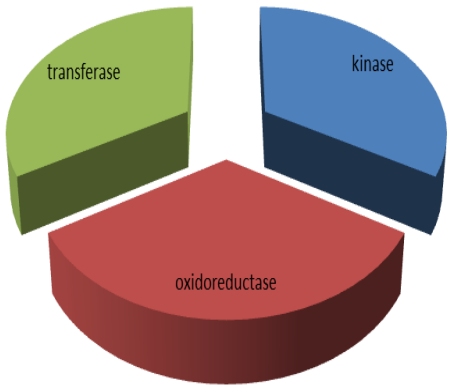

Figure 2. Protein classification of SUMO1- proteins in DZN treated group

groups were compared and four differently expressed proteins were identified by MS analysis; Acyl-CoAdehydrogenase, Creatin kinas s-type mitochondrial, Glyceraldehyde-3-phosphate dehyrogenase, ATP synthase subunit-mitochondrial (Figure 1, and Figure 2). The information of identified proteins, including protein names/gene names, size, protein score, sequence coverage (%), theoretical pI, fold change, sequence of identified peptide with the highest ion score were reported in Table 1.

Swissprot accession number of target proteins were submitted to PANTHER database and categorized (Figure 2). The detected proteins belonged to 3 classes including kinases (33.3%), oxidoredoctases (33.3%) and transferases (33.3%).

Discussion

In humans, oxidative stress is related with many heart disorders, such as atherosclerosis, heart failure, myocardial infarction (16) some reports stated that DZN increases free radical production and induces oxidative stress in rat tissues by enhancing lipid peroxidation and decreasing the activities of antioxidant enzymes, and depletion of GSH (11, 17).

ProteinSUMOylation represents a very essential modification pathway duringoxidative stress and exposure to oxidative species is able to enhance theglobal protein SUMO conjugation profile. SUMOylationisbeneficial mechanism to protect cells from oxidative stress by activating or inhibiting key proteins in different signal pathways (18).

Utilizing immune pull down of SUMO1-proteins accompanied by a proteomics approach, we showed that there wasan increase in SUMOylationpattern of some proteins in DZN treated group in comparison with the control group. These four proteins were identified as differently expressed SUMO1-proteins in DZN treated group in rat heart tissues.

Level of SUMO1Glyceraldehyde-3-phosphate

Table1. Identified proteins by MALDI/TOF/TOFin DZN treated group

Protein name	Protein score	Matches	Fold change (dzn/ctr)	Mas (Da)	Coverage %	PI	Peptide sequence
Acyl-CoAdehydrogenase GN=Acadv1	67	2 (2)	1.87	71047	7%	9.01	K.ITAFVVER.S
Creatin kinas s-type mitochondrial GN=Ckmt2	128	3 (3)	1.45	47811	1%	8.76	R.GHSHPPACSR.A
Glyceraldehyde-3-phosphate dehyrogenase GN=Gapdh	164	3 (3)	1.992	36090	15%	8.14	M.VKVGVN
ATP synthase subunit0-mitochondrial GN=ATP50	82	2 (2)	1.46	23440	7%	10.03	R.LDQVEKELLR.V

dehydrogenase (GAPDH) was elevated by 92% after exposure to DZN. GAPDH is a key enzyme in glycolysis process. This enzyme is also involved in the transcription process, DNA replication, repair and apoptosis(19). It has been demonstrated that DZN can induce apoptosis and oxidative stress indifferent tissues such as theHeart (20). Previous studies showed that GAPDH protein overexpressed in apoptosis and stress conditions (20). According to another study GAPDH can be SUMOylatedunder oxidative stress (21). SUMOylationcouldbe a possible mechanism in adaptation of cells to stress condition during exposure to DZNbyenhancingtheflux through the glycolytic pathway for their survival (21, 22).

Mitochondrial Creatine kinase (CKm), a major protein responsible for cellular energy homeostasis, is used as a marker inthe evaluation of myocardial, muscular and cerebral damage(23). Creatine kinases are very sensitive to oxidative sress and is a target for ROS (reactive oxygen species) (24). In this projectweidentifiedCKm as another differentially expressed SUMO1-protein (45% increase in DZN group).

Some evidencesupported that CKm is over expressed in oxidative stress (25, 26) And its sumoylation form of CKm may a compensate mechanism to adjust the ATP level (26).

Thesumoylated form of ATP synthasewas previously detected when cells weresubjected to oxidative stress (27). Fold change ratio DZN/Ctrl of Sumoylation for ATP-synthase was 1.46. Also This protein up-regulated by some environmental stresses. These results show that sumoylation of ATP synthase has a role in stress response induced DZN in addition to its role in cell death regulation and producing ATP (28).

Acyl-CoA dehydrogenase is an enzyme that catalyzes the initial step in each cycle of fatty acid β-oxidation in the mitochondria. It seems protein sumoylationis essential for maintenance of protein performance in cells against oxidative stress and apoptosis (29). We showed that exposure to subacute DZN could increase SUMOyaltion by 87%.

Conclusion

In summary, we showed that protein SUMO1 modification could respond to DZN induced oxidative stress. In this study, levels of four SUMO1- proteins increased in the heart tissues of animals after subacute exposure to DZN; these proteins were Acyl-CoAdehydrogenase, mitochondrial Creatine kinase, Glyceraldehyde-3-phosphate dehyrogenase and ATP synthase.Increased protein SUMOylation may provide protection against oxidative stress (30).

To further elucidate the role of SUMOylation of proteins in exposure to DZN, more investigations have to be done.

Conflict of interest

The authors have declared no conflict of interest

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