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Analysis of Hepatic Protein Expression in Fisher-344 Rats Fed 2-Aminoanthracene

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Abstract :*The effect of 2-aminothracene (2AA) ingestion in Fisher-344 rats was examined. Protein expression was examined via bulk proteins isolated from the liver and analyzed on a mass spectrometry. 2AA is an aromatic amine that can be classified as a polycyclic aromatic hydrocarbon (PAH). 2AA is used in the manufacture of chemicals, dyes, inks, and it is also a curing agent in epoxy resins and polyurethanes. 2AA has also been found in tobacco smoke and cooked foods.Total protein was extracted from liver tissues, separated via gel electrophoresis, trypsin-digested and analyzed using mass spectrometry. Mass spectrometric analysis of bulk hepatic proteins yielded a massive amount of proteins that seem to play varied roles in the maintenance of cellular homeostasis and integrity. The application of statistical algorithm to the dataset revealed the over expression of proteins that mediate energy related cellular and oxidative stress processes. Applying statistical algorithm to the bulk dataset reduced peptides to 28 common proteins that could be potential biomarkers of 2-aminoanthracene toxicity. Some of these peptides were Fetuin-B, Hemopexin, Pyrethroid hydrolase Ces2a and 2-hydroxyacyl-CoA lyase 1. Fetuin-b and hemopexin, which could be possible biomarkers of 2AA toxicity in the liver.Further analysis of these proteins via DAVID bioinformatics tool showed functional annotation terms related to oxidative stress responsive mechanisms, apoptosis related responses and various binding related processes such as monocarboxylic acid binding, cofactor binding and glutathione binding. Amount of quantified hepatic catalase concentration was found to be consistent to the bulk protein levels in the liver.*

Keywords :*2-Aminoanthracene, proteomics, Fisher-344 rats, mass spectrometry, catalase, hemopexin*

I. Introduction

Polycyclic aromatic carbons (PAHs) are a group of over 100 different chemicals that are formed during the incomplete burning of coal, oil and gas, garbage, or other organic substances like tobacco or charbroiled meat (ATSDR, 1995). PAHs are used to make plastic, dyes, pesticides, and some are even used to make medicine. Exposure to PAHs have caused tumors, birth defects, decrease in body weight, liver and kidney problems. Furthermore, problems with immune system in the animals that are highly exposed to certain PAHs have also been observed in experimental models. Humans can be exposed to PAHs in everyday living via tobacco smoke, fumes from vehicle exhaust, eating foods grown in contaminated soil, or through grilling meat (Rudel 2014). Coal tar, asphalt and aluminum, or that burns trash are additional sources of PAH contamination.Exposure to PAHs seem to correlate with cancer in humans (Rudel 2014).

2- Aminoanthracene (2AA) is an aromatic amine that can be classified as a PAH (Gato et al., 2014). 2AA can be found in tobacco smoke, burning of coal tar, and found in meat that charred or grilled with coal. A general examination of the compound structure on the material and datasheet described it as a yellow to brownish solid that gives off toxic fumes when heated. Its chemical formula contain 14 carbons atoms, 11 atoms of hydrogen, and one nitrogen atom which forms part of the amino group.

Biomarkers of toxicity or disease are measurable substances in an organism whose presence is indicative of some phenomenon such as disease, infection, or environmental exposure (Gupta, 2014; Quintaneiro et al., 2006). Biomarkers can be anything that a regular organism had but are then changed in a predicted infected organism. These biomarkers serve an important role in the drug approval process. Mass spectrometry is used as means of elucidating protein biomarkers. Proteins serve as good biomarkers because they are relatively easy to obtain and assess. Since proteins play essential rolein an organisms' cellular activity, minor changes in the protein expression may be caused by major dysfunctions in the body (Madasamy et al., 2014; Lin et al., 2014).

The goal for this work is to evaluate the protein expression in the liver of Fisher-344 (F344) rats exposed to 2AA. Protein expression was examined via bulk proteins isolated from the liver and analyzed on a mass spectrometry. We had the opportunity to directly quantify proteins that might provide insight into the toxicity associated with 2AA ingestion.

II. Materials and Methods

Experimental Design

Rats were purchased from Harlan Laboratories. The animals were 3-4 weeks old at the time of treatment. Animals were randomly assigned into dose regimens of 0mg/kg-diet (control), 50mg/kg-diet (low dose), 75mg/kg-diet (medium dose), and 100mg/kg-diet (high dose) 2-aminoanthracine (2AA) for either 14 or 28 days. There were three animals in each treatment group. Animals were housed in individual cages at Southern Illinois University Animal Facility with a 12h/12h light/dark cycle. Rats had access to distilled water *ad libitum*. The animals were handled according to the guidelines from the National Institute of Health (NIH) and Southern Illinois University Guide for Care and Use of Laboratory Animals [IACUC prototcol# 09-039]. At the expiration of treatment, the rats were euthanized and blood was collected by cardiac puncture. Excised livers were immediately frozen in liquid nitrogen.

Diet Preparation

2AA (CAS# 613-13-8) [98+% pure] was obtained from Sigma-Aldrich (St Louis, MO USA). Diet preparation involved the immersion of 1kg diet obtained from PMI (Nutrition International, LLC Brentwood MO USA) in 1-L molecular grade ethyl alcohol containing the appropriate amount of 2AA. The alcohol was then evaporated under the hood with periodic mixing. This was followed by storage in freezer protected from light.

Total Protein Extraction

Total protein was extracted from liver tissues using Qproteome Mammalian Protein Prep Kit (Qiagen, Valencia 2006). Approximately 40 mg of tissues were lysed in 1 mL mammalian cell lysis buffer including 10 µl protease inhibitor and 1 U benzonase nuclease. This was followed by tissues disruption for 30 s at medium speed in 15 mL propylene centrifuge tube. Samples were then transferred into 2 mL precooled microcentrifuge tubes and centrifuged at 7800 rpm for 10 minutes. The supernatant was filtered through Whatman 0.2 µm PVDF Filter Media and concentrated via 3 K Amicon centrifugal filter device for 20 minutes. Samples were then aliquoted and stored at -20 ^{o}C.

1D Gel Electrophoresis

Protein samples were separated using one dimensional gel electrophoresis. Total hepatic protein concentration was determined using the modified Lowry Assay method according to the manufacturer's recommendations (Pierce Biotechnology, Rockford IL). Approximately 20 mg of protein samples were diluted with Laemmli sample buffer in a ratio of 1:1. The Laemmli buffer was prepared by adding 25 µl β-mercaptoethanol to 475 µl Laemmli sample buffer. The protein-Laemmli mixture was vortexed briefly and heated for 5 minutes at 95 ^{o}C. Thirty microliters of the mixture along with a molecular weight marker were loaded onto mini-protean TGX precast gel (Bio-Rad, Hercules, CA). The gel was run at constant 200 V and 50 mA using 1X Tris/glycine/SDS gel running buffer for 35 minutes. Then, gel was pulled of the cassette and rinsed three times for 5 minutes each with high purity MilliQ water. After this, the gel was stained with 50 ml of Bio-Safe Coomassie G-250 stain (Bio-RAD, Hercules, CA) for 1hr with gentle shaking. Finally, the gels were washed again with high purity MilliQ for 30 minutes and imaged.

Digestion of 1D Gels

Gel bands were excised from control,low dose and high dose treatment groups and mailed to Proteomic and Mass Spectrometry Core Facility, University of Georgia, Athens GA for analysis. The gel bands were processed for in-gel trypsin digestion and peptide extraction using the following protocol: destaining, reduction and alkylation, in-gel digestion and extraction. The supernatant was analyzed directly without further processing.

Mass Spectrometry Protocol

The tryptic peptides from in-gel digestion were analyzed by ProxeonnanoLC HPLC system is coupled to a Thermo-Fisher LTQ Orbitrap Elite. Peptides were loaded load the sample direct to the analytical column that is self-packed with C18, similar to Jupiter Proteo resin (Phenomenex). The preferred method is to measure both MS and MS/MS in the orbitrap at 120,000 and at 3,000, respectively.LC/MS runs were searched against Rat of SwissProt database using Proteome Discoverer with Mascot (Matrix Sciences) to identify unique peptide signatures.

Bulk Protein Data Analysis

Bulk protein data were combined to find the same accessions in four treatment groups. Then select the same accessions in four treatments to analyze.

Data were analyzed using SAS 9.3 statistical software package for Windows (SAS Institute Inc., Cary, NC, USA). Descriptive analysis was applied to variable 'score' by treatment group. The dependent variables were also compared descriptively across groups to illustrate temporal trends. The treatment effect is analyzed using general linear model. The treatment effect is statistically significant ($p<0.05$). And the scores for each accession is different ($p<0.05$).

Analysis of protein associations via DAVID

DAVID (database for annotation, visualization and integrated discovery) was employed to analyze protein relationships within the sample. The Gene Ontology (GO) comparison tool provides the opportunity to show the relationship between and the association of peptides with respect to each other in functional and biochemical pathways (Huang *et al*, 2009).This tool organizes transcripts into hierarchical categories via biological process, molecular function, and cellular components. Proteins from the 2HD-4HD group were imported into DAVIDbioinformatics tool for analysis.

III. Results

Proteins are direct target of toxicants. Proteins that might mediate the toxicity of 2AA in the liver were examined. Protein extracts from liver homogenates were separated via SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Mass spectrometry was then employed to identify specific protein signatures that might be essential in understanding the toxicity of 2AA. Samples analyzed included control (0 mg/kg-2AA), 2HD (100 mg/kg-2AA for two weeks), 4LD (50 mg/kg-2AA for four weeks) and 4HD (100 mg/kg-2AA for four weeks). The total number of spectral matching a peptide ranged from 888 to 1244 with a false discovery rate between 0 and 1%.

To investigate which proteins might be of primary importance, SAS statistical software was employed to analyze the data. Table 1 presents a summary of statistical details of bulk proteins analyzed. Shown in Figure 1 is the box plot of the treatments. Protein scores indicating the sum of the highest ion scores for each distinct sequence ranged from a minimum of 62.57 to 3511.5. Each box plot shows the mean (diamond), the quartile (lower bound bar) and 75^{th} percentile (upper bound bar). The interaction plot relates scores to peptides via comparing their scores across all treatment groups analyzed (Figure 2). Generally it appears the low dose group (red cross in Figure 2) show higher scores relative to controls while the high dose (plots with x and triangles in Figure 2)groups for 2 weeks seem to have lower scores. As expected, serum albumin had the highest score among all the groups.

Figure 3 provides a summary of the level of peptides within each treatment group. Comparison of all treatment groups revealed that the 2HD-4HD had the most peptides, followed by the 2C-4LD and 2C-2HD-4LD-4HD respectively. That is 28 unique proteins were common to all groups followed by 47 common to control and low dose while 65 peptides were similar in the high dose rats. Table 2 lists a summary of peptides common to all groups. Some of the peptides within this group include; 60 kDa heat shock protein, mitochondrial; Thioredoxinreductase 1, cytoplasmic; Pyruvate kinase PKLR; Catalase and Hemopexin.

To further examine proteins list, the 65 peptides common to the 2AA treated groups were loaded into the DAVID bioinformatics database. DAVID provides the opportunity to understand biological applications from the protein list. We used DAVID to glean additional information from this list. Findings from the DAVID analysis were presented as pathways affected (Table 3) and summary of select gene ontology categories (Table 4). Gene ontology categories were observed as biological process (BP), cellular component and molecular function (MF). There were more biological process categories than molecular function and then cellular component.Pathways identified from gene-association analysis indicate proteins generally involved in drug metabolism, antioxidant activity and the metabolism of carbohydrates, proteins and lipids.

Protein data was validated via determining the level of catalase (P24270) protein in liver samples. The level of mean catalase activity increased dose-dependently. That is the control group indicated the lowest catalase concentration. Though the low dose animals had higher mean catalase than the control, the difference is not significant. However rats fed 100 mg/kg of 2AA diet had significantly higher levels of catalase relative to control animals.

Treatment	Mean	Median	Max	Min	Std.
Control group	672.05	313.25	3510.15	62.57	786.37
4LD group	802.50	557.99	3434.74	110.97	821.57
2HD group	602.83	201.36	3492.29	54.29	814.60
4HD group	701.77	386.07	3511.54	73.52	798.94

Table 1.Descriptive statistics for variable 'score'.

Obs	Accession Number	Score	Description
1	O89049	449.27	Thioredoxinreductase 1, cytoplasmic
2	P01946	839.53	Hemoglobin subunit alpha-1/2
3	P02091	1081.18	Hemoglobin subunit beta-1
4	P02770	3511.54	Serum albumin
5	P04762	2254.63	Catalase
6	P04785	322.86	Protein disulfide-isomerase
7	P05545	173.58	Serine protease inhibitor A3K
8	P07724	1712.33	Serum albumin
9	P11598	91.05	Protein disulfide-isomerase A3
10	P12346	182.4	Serotransferrin
11	P12928	753.14	Pyruvate kinase PKLR
12	P16303	767.35	Carboxylesterase 1D
13	P17475	126.55	Alpha-1-antiproteinase
14	P18418	530.38	Calreticulin
15	P20059	653	Hemopexin
16	P24090	228.6	Alpha-2-HS-glycoprotein
17	P24270	1519.82	Catalase
18	P26043	159.9	Radixin
19	P63038	1798.13	60 kDa heat shock protein, mitochondrial
20	Q02253	577.22	Methylmalonate-semialdehydedehydrogenase [acylating], mitochondrial
21	Q07116	503.44	Sulfite oxidase, mitochondrial
22	Q63010	180.1	Liver carboxylesterase B-1
23	Q63150	270.54	Dihydropyrimidinase
24	Q64573	287.56	Liver carboxylesterase 4
25	Q6P6R2	321.84	Dihydrolipoyl dehydrogenase, mitochondrial
26	Q8CHM7	73.52	2-hydroxyacyl-CoA lyase 1
27	Q8QZR3	108.97	Pyrethroid hydrolase Ces2a
28	Q9QX79	171.21	Fetuin-B

Table 2.Summary of proteins common to all treatment groups. Using algorithm written into SAS 9.3 statistical software package for Windows these set of peptides were found to be expressed in all treatment groups.

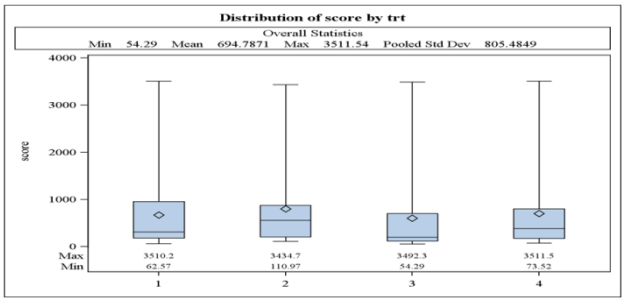

Figure 1 Boxplot of groups (diamond is mean), line in the box is the median based of score of proteins. Upper bound of each box is the 75th percentile. The lower bound is the first quartile (25th percentile). Overall statistics show minimum and maximum trt (treatment) scores.

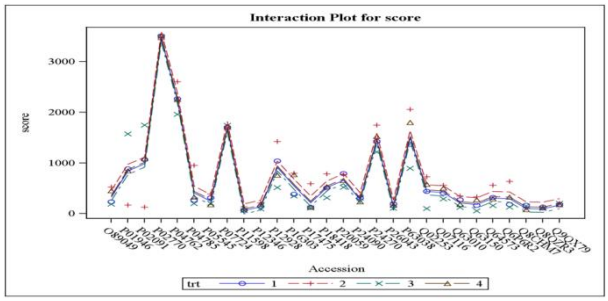

Figure 2.Interaction plot of peptide score versus accession number indicating the score distribution of proteins similar across the four groups examined. This plot involves twenty eight proteins represented by their accession numbers. Treatments (trt) include; (blue circle: trt 1– control); (red cross: trt 2 – low dose); (green x: trt 3 – high dose for two weeks) and (black triangle: trt 4 – high dose for four weeks).

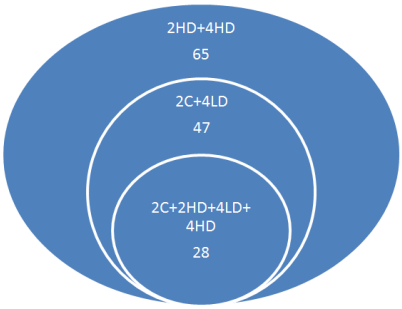

Figure 3. Distribution of analyzed peptides deduced from bulk hepatic protein homogenates. Animals were fed various levels of 2AA including 0 mg/kg (C – control), 50 mg/kg for four weeks (4LD), and 100 mg/kg diet for 2 weeks (2HD) and four weeks (4HD).

Terms	**Gene Count**	**%**	**P-Value**
Glutathione metabolism	5	8.1	6.2E-5
Pyruvate metabolism	4	6.5	6.5E-4
Metabolism of xenobiotics by cytochrome P450	4	6.5	2.3E-3
Drug metabolism	4	6.5	3.8E-3
Drug metabolism	3	4.8	1.6E-2
Amyotrophic lateral sclerosis (ALS)	3	4.8	2.8E-2
PPAR signaling pathway	3	4.8	4.0E-2
Pyrimidine metabolism	3	4.8	6.3E-2

Table3.Significant ($p < 0.05$) Pathways found through DAVID analysis to be essential in understanding the hepatic toxicity of 2AA. Proteins involved in each pathway ranges from 3 – 5 in number.

Category	Term	Count	P Value
BP	response to inorganic substance	14	4.70E-12
	cell redox homeostasis	9	4.30E-11
	hydrogen peroxide metabolic process	7	1.80E-10
	response to reactive oxygen species	8	3.00E-08
	oxygen and reactive oxygen species metabolic process	7	3.20E-08
	cellular homeostasis	13	3.50E-08
	hydrogen peroxide catabolic process	5	9.70E-08
	response to oxidative stress	9	2.20E-07
	response to hydrogen peroxide	7	2.40E-07
	cellular response to hydrogen peroxide	5	4.10E-07
	homeostatic process	14	6.70E-07
	oxidation reduction	13	9.20E-07
	response to metal ion	8	2.60E-06
	response to organic substance	14	1.20E-05
	negative regulation of apoptosis	9	1.90E-05
	negative regulation of programmed cell death	9	2.10E-05
	negative regulation of cell death	9	2.20E-05
	cellular response to reactive oxygen species	7	3.10E-10
	peroxiredoxin activity	5	8.60E-09
	cofactor metabolic process	7	7.30E-05
	regulation of apoptosis	11	1.00E-04
	regulation of programmed cell death	11	1.10E-04
	response to xenobiotic stimulus	4	1.20E-04
	regulation of cell death	11	1.20E-04
	glutathione metabolic process	4	1.40E-04
	protein complex assembly	8	5.90E-04
	protein complex biogenesis	8	5.90E-04
	sulfur metabolic process	5	6.10E-04
	response to wounding	8	6.70E-04
	peptide metabolic process	4	7.70E-04
	cellular response to stress	8	7.90E-04
	response to organic cyclic substance	6	8.70E-04
	regulation of DNA binding	4	6.40E-03
	energy derivation by oxidation of organic compounds	4	6.40E-03
CC	cytosol	23	2.10E-10
	mitochondrion	20	4.20E-07

	soluble fraction	9	3.80E-05
	peroxisome	6	4.00E-05
	microbody	6	4.00E-05
	endoplasmic reticulum lumen	5	9.90E-05
	cytosolic part	6	1.10E-04
MF	fatty acid binding	4	8.70E-04
	glutathione binding	3	8.80E-04
	transferase activity, transferring alkyl or aryl (other than methyl) groups	4	1.10E-03
	cofactor binding	7	1.40E-03
	regulation of binding	5	1.50E-03
	generation of precursor metabolites and energy	6	1.50E-03
	cellular respiration	4	1.50E-03
	regulation of protein kinase cascade	6	1.70E-03
	aging	5	1.90E-03
	positive regulation of binding	4	1.90E-03
	monocarboxylic acid binding	4	2.00E-03
	membrane-enclosed lumen	14	2.10E-03
	blood circulation	5	2.20E-03
	circulatory system process	5	2.20E-03
	coenzyme binding	6	2.20E-03
	macromolecular complex assembly	8	2.80E-03
	cellular amino acid derivative metabolic process	5	2.90E-03

Table 4.Functional annotation of protein list showing gene ontology (GO) categories of biological process (BP), cellular component (CC) and molecular function (MF).GO categories significantly ($p < 0.01$) noted to mediate 2AA toxicity in the liver.

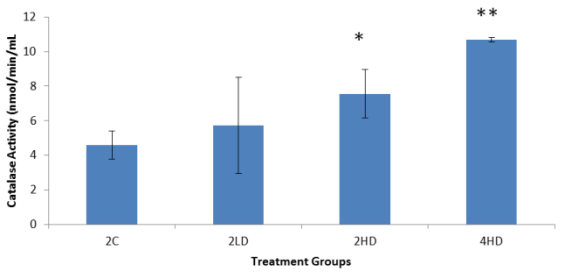

Figure 4.Hepatic catalase activity in Fisher-344 rats that ingested 2AA for two and our weeks. Animals were treated to 2AA adulterated diet for 14 days: control (2C) – 0 mg/kg; 50 mg/kg – low dose (2LD); 100 mg/kg – high dose (2HD) and twenty eight days: 100 mg/kg – high dose (4HD). Differences in catalase amount was significant at *$p < 0.05$ or *$p < 0.01$.

IV. Discussion

According to Dail*et al* (2008), the full potential of proteomics techniques in identifying and quantifying proteins is not yet fully realized. Proteomics (large scale study of proteins as active agents in the cell) also provide the opportunity to examine global protein associations in response to drugs and xenobiotic intoxication. In the present study, the responses of rats to 2AA diet were investigated via profiling protein levels in the liver. As is typical with this type of study, mass spectrometric analysis of bulk hepatic proteins yielded a massive amount of proteins. These bulk proteins seem to play varied roles in the maintenance of cellular homeostasisand integrity. Some of these process include: communication; cell differentiation; cell organization and biogenesis; cell proliferation; cellular component movement; defense response; development; metabolic process; regulation of biological process and response to stimulus.

To elucidate which proteins might be essential in understanding the effect of the xenobiotic 2AA on the animals, statistical algorithm was applied to the dataset. This resulted in a significant reduction of the number of proteins deemed to play a role in the toxic effects of 2AA. For instance, liver carboxylesterase 4mediates phase I metabolism of xenobiotics.Dihydrolipoyl dehydrogenase, mitochondrial is reported to contribute to significant generation of reactive oxygen species by the mitochondria in *Saccharomyces cerevisiae* (Tahara*et al*., 2007). On the other hand, 60 kDa heat shock protein, mitochondrial belong to a class of molecular chaperones that play vital roles in protein synthesis, transportation and degradation. This particular protein was reported to be a target of T cell and Ab responses in atherosclerosis and chronic inflammation (Chen et al., 1999). Similarly, pyruvate kinase PKLR plays a critical role in energy producing process such as glycolysis. Some isoforms of this enzyme were noted to mediate aerobic glycolysis and tumor growth (Sun *et al*., 2011).These examples seem to suggest the over expression of these proteins that mediate energy related cellular and oxidative stress processes.

Comparison of protein scores point to generally higher protein intensity within the low dose 2AA treated group in reference to the controls. On the contrary, the high dose 2AA animals indicated slightly lower protein scores. The results also suggest a stronger similarity in the high dose groups treated for two and four weeks. This observation conforms to other trends we have noted previously. We have previously observed the similarity in the toxicological response of animals exposed to 100 mg/kg 2AA for two and four weeks (Gato*et al*., 2012; Gato and Means, 2011). In these previous investigations, hepatic and pancreatic global gene expression alterations indicated similar responses in the high dose groups for 14 and 28 days.

We have previously examined the role of PAHs in modulating diabetes related gene expression in rats (Gato et al, 2012; Gato and Means, 2011). Diabetes is known to affect many organs such as the liver (Sundsten and Ortsater, 2009).Diabetes mellitus is a heterogeneous disease as such it changes the expression of varied genes and proteins. The present investigation afforded the opportunity to directly analyze proteins that might mediate adverse conditions related to diabetes. GO analysis via DAVID showed pathways essential for studying the diabetic condition. Some of these include; PPAR signaling pathway, glutathione metabolism and pyruvate metabolism. Glutathione plays an important role in the regulation of the redox state (McLennan et al., 1991; Wu et al., 2004). The most common form of glutathione in mammalian cells is the reduced type seen as a low-molecular weight thiol (L-gamma-glutamyl-L-cysteinyl-glycine). Reduction in concentrations of this molecule has been implicated in various pathological conditions including diabetes (Carolo dos Santos *et al*., 2014; Franco *et al*, 2007).Pyruvate has also been implicated in the diabetes condition (Moon et al., 2012). Peroxisome proliferator-activated receptor (PPAR) on the other hand is reported to be a central regulator of glucose and lipid homeostasis (Grygiel-Gorniak, 2014). Recent studies have linked various isoforms of PPAR to diabetes. The genes associated with the PPAR signaling pathway have also been targets for therapeutic treatment for metabolic syndrome (Zheng*et al*., 2014).

Functional annotation also provides the opportunity to elucidate specific GO terms associated with diverse cellular conditions. GO terms appear to broadly mimic the pathways observed earlier. Some of the terms generally fall within oxidative stress responsive mechanisms, apoptosis related responses and various binding related processes such as monocarboxylic acid binding, cofactor binding and glutathione binding. Some of the oxidative stress related terms included; response to reactive oxygen species, cell redox homeostasis, glutathione metabolic process and oxygen and reactive oxygen species metabolic process.These GO terms noted in the present study are similar to others observed in a previous investigation involving global gene expression analysis(Gato*et al*., 2012). Some of the terms noted in the global gene expression study were; response to oxidative stress, apoptosis, regulation of programmed cell death and glutathione transferase activity. Though the correlation between proteins and quantified mRNA levels are typically weak (Nie*et al*., 2007), clearly there is a strong similarity in protein associations via DAVID.

Employing high throughput assay such as bulk protein analysis via mass spectrometry provides the opportunity to identify unique protein signatures (Matt *et al*., 2008).Applying statistical algorithm to the datasetreduced peptides to 28 common proteins that could be potential biomarkers of 2AA toxicity in the liver. Some of these peptides were Fetuin-B, Hemopexin, Pyrethroid hydrolase Ces2a and 2-hydroxyacyl-CoA lyase 1.Fetuin-b and hemopexin, which can be possible biomarkers of 2AA toxicity in the liver.

Bulk protein data was validated analyzing the hepatic concentration of catalase. Catalase is an enzyme found in all living organism. This protein is responsible for sequestering hydrogen peroxide in order to minimize cellular oxidative stress (Góth, 2008; Sofo et al., 2015). The activity of catalase in the livers of rats that ingested 2AA increased in a dose-dependent fashion. Level of hepatic catalase concentration is consistent with bulk protein catalase protein, confirming the accuracy of the mass spectrometry analytical methodology.

Summary

The toxicity associated with a polycyclic aromatic hydrocarbon, 2-aminoanthracene ingestion by rats was evaluated. Mass spectrometry was employed to identify proteins that might be mediate 2AA toxicity in the liver. Mass spectrometric analysis of bulk hepatic proteins yielded a massive amount of proteins that seem to play varied roles in the maintenance of cellular homeostasis and integrity. The application of statistical algorithm to the dataset revealed the over expression of proteins that mediate energy related cellular and oxidative stress processes. Further analysis of these proteins via DAVID showed functional annotation terms related to oxidative stress responsive mechanisms, apoptosis related responses and various binding related processes such as monocarboxylic acid binding, cofactor binding and glutathione binding. Amount of quantified hepatic catalase concentration was found to be consistent to the bulk protein levels in the liver.

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