Published in final edited form as:

Physiol Genomics. 2007 October 22; 31(2): 352–356. doi:10.1152/physiolgenomics.00276.2006.

Heat Map Visualization of High-Density Clinical Chemistry Data

J. Todd Auman, Gary A. Boorman, Ralph E. Wilson, Gregory S. Travlos, and Richard S. Paules*

National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

Abstract

Clinical chemistry data are routinely generated as part of preclinical animal toxicity studies and human clinical studies. With large-scale studies involving hundreds or even thousands of samples in multiple treatment groups, it is currently difficult to interpret the resulting complex, high-density clinical chemistry data. Clinical chemistry data were obtained from male rats each treated with one of eight different acute hepatotoxicants from a large-scale toxicogenomics study. The raw data underwent a Z-score transformation comparing each individual animal's clinical chemistry values to that of reference controls from all eight studies and then visualized in a single graphic using a heat map. The utility of using a heat map to visualize high density clinical chemistry data was explored by clustering the changes in clinical chemistry values for over 400 animals. Clustering of the animals on clinical chemistry changes resulted in a clear distinction of the animals displaying hepatotoxicity from those that did not. Additionally, while animals experiencing hepatotoxicity showed many similarities in the observed clinical chemistry alterations, distinct differences were noted in the heat map profile for the different compounds. Using a heat map to visualize complex, high-density clinical chemistry data in a single graphic facilitates the identification of previously unrecognized trends. This method is simple to implement and maintains the biological integrity of the data. The value of this clinical chemistry data transformation and visualization will manifest itself through the integration with other high-density data, such as genomics data, to study physiology at the systems level.

Keywords

Clinical Pathology; Hierarchical Clustering; Rat; Hepatotoxicity; Toxicogenomics

The post-genomic era in science has led to the generation of a number of large, high-density data sets with hundreds to thousands of data points for each tested subject. In the field of toxicology, genomics technologies have been used to investigate how different stresses alter gene expression (see (12) for a recent review). For many *in vivo* studies, gene expression changes are measured in the tissue of interest, but other data are also obtained to give a "phenotypic anchor" (6,11) for the gene changes, including clinical chemistry and histopathology (3). However, for large data sets, including large human clinical/epidemiological studies, it can be problematic to effectively evaluate the phenotypic anchor, due to the sheer number of data points to consider.

Clinical chemistry data are often viewed in a data table or a bar graph, where one can examine the changes that occur for one analyte across the groups of interest. For a study involving only one or a few compounds these types of visualizations help investigators determine how subjects in each group react to the given stressor. However, for large animal data sets involving multiple

*Corresponding Author: 111 T.W. Alexander Drive PO Box 12233, Mail Drop D2-03 Research Triangle Park, NC 27709 Phone: 919-541-3710 Fax: 919-316-4771 paules@niehs.nih.gov.

compounds, dose groups and time points it is very difficult to give a meaningful, visual representation of the data with traditional bar graphs, due to the number of data points that exist in these types of experiments. This is especially true for large human clinical/epidemiological studies, such as the Framingham Heart Study, which has thousands of people enrolled (9). There is obviously a need to visualize high-density clinical chemistry data in a manner that will assist in putting gene expression data, or other high-density data, in the proper biological context. The goal of this study was to develop a method to visualize multiple analytes of clinical chemistry data over many disparate samples (i.e. different compounds, doses and time points) in a single graphic.

Male Fischer rats, approximately 12 to 14 weeks of age, were treated with a single dose from 1 of 8 hepatotoxicants or their respective vehicle (Table 1). Serum (250 μ L) was obtained 6, 24, and 48 hours after dosing for clinical chemistry analysis. Each treatment group contained at least 4 animals. All animals were treated humanely in accordance to guidelines established in the NIH Guide for the Care and Use of Laboratory Animals (1). Clinical chemistry analyses were performed using the Roche Cobas Fara chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ).

The main goal for assessing changes in clinical chemistry across many animals in one graphic is to be able to quickly identify which animals shown signs of organ damage, as evidenced by clinical chemistry alterations. Therefore, each animal's clinical chemistry measurements were analyzed in relation to values observed in the normal population. Since our study set is large, it was possible to obtain the median and standard deviation of the analytes for all of the vehicle-treated animals at all time points (n=103) to define the reference value for each analyte (Table 2) within this study.

After defining the reference value for each analyte, the data were transformed so that the visualization would be an accurate representation of the data. The raw clinical chemistry data needed to be transformed for several reasons. First, several of the analytes have a range of values of over 3 orders of magnitude, thus making log transformation necessary. Second, the various analytes have vastly different dynamic ranges. For instance, significant liver injury is indicated by a several hundred-fold to several thousand-fold changes in normal serum ALT (alanine aminotransferase) levels, while a greater than 2-fold change in serum creatinine levels indicates a significant loss of kidney function (5). Therefore, to put the different analytes on the same scale we performed a Z-score transformation on the log transformed values using the median and standard deviation of the vehicle-treated animals for the basis of the transformation. The Z-score transformation [$Z = (\text{observed value} - \text{baseline median}) / \text{baseline standard deviation}$] ensures that each analyte over the population of animals has a median value of 0 with a standard deviation of 1. We used the median instead of the mean, since the median is less sensitive to statistical outliers. In addition to putting all of the analytes on the same scale, the Z-score transformation also centers the log transformed data on 0, with values greater than the baseline having a positive Z-score and values less than the baseline having a negative Z-score.

The clinical chemistry Z-scores were then used to perform hierarchical clustering using Eisen's Cluster program (2). Eisen's TreeView program (2) was used to visualize the data in a heat map, with yellow indicating Z-scores > 0 , blue indicating Z-scores < 0 , black indicating Z-scores ≈ 0 and grey indicating data not present. Figure 1 shows the cluster and heat map of all treated animals in this 8 compound study (See supplemental Figure 1 for the fully annotated cluster). Looking at the dendrogram for the analytes, it is evident that the liver enzymes cluster tightly together (ALT, AST, LDH, SDH; see table 2 for full names), providing support that the data transformation is valid since these analytes are markers of hepatocellular damage and increase with liver injury (5). The middle portion of the heat map consists of animals displaying

evidence of hepatotoxicity based on the elevation of liver enzymes (ALT, AST, SDH, LDH). In general, these animals were exposed to either the high or moderate dose of the hepatotoxic compounds, with the notable exception of the animals dosed with the non-hepatotoxic compound 1,4-dichlorobenzene (8), which did not elicit clinical chemistry changes associated with hepatotoxicity. Examination of several subclusters reveals that hepatotoxic doses of the administered compounds elicit similar alterations in the clinical chemistry panel profile; however, each compound elicits a pattern of change that is distinct. Thioacetamide shows indications of eliciting nephrotoxicity, in addition to hepatotoxicity, as evidenced by the elevation of blood urea nitrogen and creatinine 48 hours after a 150 mg/kg dose (Subcluster A, Figure 1). Subcluster B (Figure 1) shows a group of animals dosed with 5 different hepatotoxins exhibiting similar elevations of ALT, AST, LDH, SDH and TBA (total bile acids), but dissimilar reductions in serum triglycerides. Diquat appears to elicit a different hepatotoxic response from the other hepatotoxins, in that no elevations in SDH or TBA are apparent (Subcluster C, Figure 1). Based on this heat map of clinical chemistry alterations, it can be determined that the experimental groups have similar, but distinct pathologies.

Described here, for the first time, is a method that can be used to visualize high-density clinical chemistry data in a single graphic by using a heat map. The need for this type of visualization arises from the prevalence of large experimental data sets that contain hundreds, if not thousands, of data points. Our method makes use of the Z-score transformation to put each animal's clinical chemistry data in the framework of what is normal for an untreated rat, which means a bank of historical data or a large group of concurrent control animals is needed for this type of transformation to work. For animal studies, the reference control should be of the same sex, strain and age as the test animals, with all animals on the same diet, since these factors significantly influence individual animals' clinical chemistry values (5,7). This type of data transformation should also prove useful for large human clinical data sets, as there is considerable historical data on the reference values for the different clinical chemistry analytes in human populations (10).

The most important facet of this normalization procedure is that the biological context of the clinical chemistry data is maintained. This can be seen in Figure 1 by the high degree of similarity seen between individual rats in the different treatment groups. Also the tight clustering of ALT, AST, LDH, SDH and TBA indicates that the biological context of the data is preserved following the data transformation, since these analytes are released into the blood following liver injury (5).

An important advantage of using a heat map to visualize clinical chemistry data across multiple animals and compounds is that patterns in the data can be identified that were not readily discernible when looking at each clinical chemistry parameter or treatment group individually. For instance, it is apparent the serum triglyceride levels often decrease with hepatic damage; however as can be seen in the heat map, some compounds, interestingly, do not elicit the concomitant decrease in serum triglycerides. Additionally, examination of the heat map indicates that exposure to the highest doses of thioacetamide and N-nitrosomorpholine elicited kidney damage at 48 hours after dosing, as seen by the elevation in blood urea nitrogen levels and was confirmed by histopathology (4). However, the two compounds appear to elicit different types of kidney damage, since thioacetamide administration led to an increase in serum creatinine, while N-nitrosomorpholine administration did not.

The greatest value of this clinical chemistry data transformation and visualization likely resides in its integration with other high-density data, such as genomics, proteomics and metabolomics data. By integrating disparate types of data effectively, while ensuring to maintain the biological meaning in the data, greater knowledge and insight should be achieved than what can be attained from each type of data by itself. We, and others (4), have started the process

of integrating disparate data types which will hopefully provide a clear benefit for the interpretation of high-density data sets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Drs. Robert Maronpot and Jack Taylor for critical review of this manuscript. This research was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences, and also funded in part with Federal funds from the National Institute of Environmental Health Sciences, National Institutes of Health, under Contract N01-ES-35513.

References

1. Council NR and (U.S.) IoLAR. Guide for the Care and Use of Laboratory Animals. National Academy Press; Washington, D.C.: 1996.
2. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proc Nat Acad Sci USA* 1998;95:14863–14868. [PubMed: 9843981]
3. Hamadeh HK, Knight BL, Haugen AC, Sieber S, Amin RP, Bushel PR, Stoll R, Blanchard K, Jayadev S, Tennant RW, Cunningham ML, Afshari CA, Paules RS. Methapyriline toxicity: Anchorage of pathologic observations to gene expression alterations. *Toxicol Pathol* 2002;30:470–482. [PubMed: 12187938]
4. Lobenhofer EK, Boorman GA, Phillips KL, Heinloth AN, Malarkey DE, Blackshear PE, Houle C, Hurban P. Application of visualization tools to the analysis of histopathology data enhances biological insight and interpretation. *Toxicol Pathol*. in press.
5. Loeb, WF. The Clinical Chemistry of Laboratory Animals. Taylor & Francis; Philadelphia: 1999.
6. Paules R. Phenotypic anchoring: linking cause and effect. *Environ Health Perspect* 2003;111:A338–339. [PubMed: 12760838]
7. Rao GN. New nonpurified diet (NTP-2000) for rodents in the National Toxicology Program's toxicology and carcinogenesis studies. *J Nutr* 1997;127:842S–846S. [PubMed: 9164250]
8. Stine ER, Gunawardhana L, Sipes IG. The acute hepatotoxicity of the isomers of dichlorobenzene in Fischer-344 and Sprague-Dawley rats: isomer-specific and strain specific differential toxicity. *Toxicol Appl Pharmacol* 1991;109:472–481. [PubMed: 1853346]
9. Study, FH. <http://www.nhlbi.nih.gov/about/framingham/index.html>
10. Sunderman FW Jr. Current Concepts of “Normal Values,” “Reference Values,” and “Discrimination Values” in Clinical Chemistry. *Clin Chem* 1975;21:1873–1877. [PubMed: 1192578]
11. Tennant RW. The National Center for Toxicogenomics: using new technologies to inform mechanistic toxicology. *Environ Health Perspect* 2002;110:A8–A10. [PubMed: 11781174]
12. Waters MD, Fostel JM. Toxicogenomics and systems toxicology: aims and prospects. *Nat Rev Genet* 2004;5:936–948. [PubMed: 15573125]

Table 1**Hepatotoxicants used in this study**

Compound	Doses	Vehicle
Bromobenzene	L: 25 mg/kg, M: 75 mg/kg, H: 250 mg/kg	Corn oil
1,2-Dichlorobenzene	L: 15 mg/kg, M: 150 mg/kg, H: 1500 mg/kg	Corn oil
1,4-Dichlorobenzene	L: 15 mg/kg, M: 150 mg/kg, H: 1500 mg/kg	Corn oil
Diquat	L: 5 mg/kg, M-L: 10 mg/kg, M-H: 20 mg/kg, H: 25 mg/kg	PBS, pH 7.4
Galactosamine	L: 25 mg/kg, M: 100 mg/kg, H: 400 mg/kg	PBS, pH 7.4
Monocrotaline	L: 10 mg/kg, M: 50 mg/kg, H: 300 mg/kg	PBS, pH 7.4
N-Nitrosomorpholine	L: 10 mg/kg, M: 50 mg/kg, H: 300 mg/kg	PBS, pH 7.4
Thioacetamide	L: 15 mg/kg, M: 50 mg/kg, H: 150 mg/kg	PBS, pH 7.4

All compounds were administered via a single oral dose (5 mL/kg BW) to male Fischer rats (approximately 12 to 14 weeks of age), except for diquat and galactosamine, which were administered i.p (n = 4 animals per treatment group). The dose levels for each hepatotoxicant (except for 1,4-dichlorobenzene, a non-toxic isomer of 1,2-dichlorobenzene) were determined to give the following information: lowest dose (L) – little or no toxicity, moderate dose (M) – mild to moderate toxicity, highest dose (H) – moderate to marked toxicity. For diquat, M-L stands for moderate-low and M-H stands for moderate-high. All animals were fasted 12-18 hours prior to dosing. PBS = phosphate buffered saline.

Table 2

Clinical chemistry values for vehicle-treated animals

Clinical Chemistry Parameter	Units	Median	Standard Deviation
Alanine Aminotransferase (ALT)	U/L	58	48
Aspartate Aminotransferase (AST)	U/L	103	101
Lactate Dehydrogenase (LDH)	U/L	529	533
Sorbitol Dehydrogenase (SDH)	U/L	3.1	2.8
Alkaline Phosphatase (ALP)	U/L	318	65
Total Bile Acids (TBA)	μmol/L	11.6	10.4
Cholesterol	mg/dL	52	14
Triglycerides	mg/dL	193	61
Total Protein	g/dL	6.4	0.8
Albumin	g/dL	2.9	0.5
Creatinine	mg/dL	0.3	0.1
Blood Urea Nitrogen (BUN)	mg/dL	18	3

Clinical chemistry measurements were obtained from male Fischer rats 6, 24 and 48 hours after a single dose of vehicle. The vehicle was either corn oil or phosphate buffered saline, pH 7.4. Medians and standard deviations were calculated for each clinical chemistry analytes based on all vehicle treated animals at all time points (n = 103). These values were log transformed and then used as the baseline values for the Z-score transformation. All rats were between 12-14 weeks in age and fasted for 12-18 hours prior to dosing.