Genes Nutr (2010) 5:321–329
DOI 10.1007/s12263-010-0173-y

RESEARCH PAPER

The effects of betaine treatment on rats fed an acute bolus of ethanol at 3 and 12 h post bolus: a microarray analysis

J. Li · F. Bardag-Gorce · J. Oliva · B. A. French · J. Dedes · S. W. French

Received: 24 November 2009 / Accepted: 19 February 2010 / Published online: 19 March 2010

Abstract Betaine, a methyl donor active in methionine metabolism, is effective in preventing and reversing experimental alcohol liver disease. The metabolic and molecular biologic mechanisms involved in this prevention are only partially known. To further investigate how betaine modifies the effects of ethanol on the liver, rats were given an acute ethanol bolus with or without betaine and the results were compared to isocaloric dextrose-fed controls. Livers were subjected to microarray analysis, and functional pathways and individual gene expression changes were analyzed. Experimental groups were compared by Venn diagrams showing that both ethanol and betaine caused a change in the expression of a large number of genes indicating that the changes were global. The bio-informatic analysis showed that all the KEGG functional pathways were affected and mainly down regulated at 3 h post bolus when ethanol plus betaine were compared with ethanol-fed rats. The most profound effect of betaine was on the metabolic pathways both at 3 and 12 h post bolus. At 3 h, the changes in gene expression were mostly down regulated, but at 12 h, the changes were regulated equally up and down. This hypothesis-driven analysis showed that the effects of betaine on the effects of ethanol were partly transient.

Keywords Global change · Gene expression · Methyl donor · Binge drinking · Alcohol

J. Li · F. Bardag-Gorce · J. Oliva · B. A. French · S. W. French (✉)
Department of Pathology, Harbor-UCLA Medical Center, 1000 W. Carson St., Torrance, Los Angeles, CA 90509, USA
e-mail: sfrench@labiomed.org

J. Dedes
LA BioMed, Harbor-UCLA Medical Center, 1124 W. Carson St., Torrance, Los Angeles, CA 90502, USA

Introduction

Ethanol, given as a bolus of 6 g/kg body weight, increases the blood and urinary alcohol to a high level at 3 h post bolus. At 12 h, the blood and urinary alcohol levels return to low a level [2]. At these 2 time points, there are major changes in gene expression in the liver but only minimal evidence of epigenetic changes [2]. Only at 12 h is there a decrease in global DNA methylation [2].

Previously, in using this model, *S*-adenosylmethionine (SAMe) a major methyl donor was fed with ethanol to blunt the effects of ethanol on global gene expression [3]. The effects of betaine on gene expression changes induced by ethanol resembled the effects of SAMe [3]. To further test the role that methyl donors have on the changes in gene expression in the liver after an acute bolus of ethanol, the methyl donor betaine was fed with ethanol. Theoretically, the methylation of DNA and histones that occur by feeding a methyl donor such as betaine could prevent the changes in gene expression observed after an ethanol bolus by causing gene silencing. In the drug-induced liver disease mouse model, betaine feeding prevented the liver pathology from developing when the drug was refed [9]. In this mouse model, betaine feeding prevented the changes in gene expression and the alterations in methionine metabolism by preventing a decrease in betaine homocysteine methyl transferase (BHMT) and glycine *N* methyl transferase [9]. Betaine also prevented the decrease in *S*-adenosylthomocysteine (SAH) [9].

Betaine feeding is also effective in ameliorating experimental alcoholic fatty liver in rats [1] and mice [4]. In mice fed ethanol acutely (three boluses of alcohol), betaine given prior to feeding alcohol, alleviated the liver injury and the impairment of metabolomics [8]. Reduction in *S*-adenosylmethionine (SAMe) and glutathione (GSH) was

prevented, and elevated hypotaurine and taurine were reduced [8]. Proteomic studies have revealed that betaine supplements given to ethanol-fed rats up regulated methionine metabolic pathway enzymes and down regulated carbonic anhydrase III [5]. Betaine, like SAMe in vitro, prevented the shift in the SAMe/SAH ratio that developed in primary liver cultures from rats fed ethanol [6]. Betaine, by supporting the methylation of homocysteine, removes both homocysteine and SAH, restores SAMe levels to normal, prevents and reverses fatty liver, prevents apoptosis and reduces damaged proteins and oxidative stress, all induced by ethanol ingestion [7, 10].

Methods

Animal model of alcoholic liver disease

Male Wistar rats from Harleco (Hollister, CA, USA), weighing 250–300 g, were used. The rats used were from a previously reported study [2] (number 3–4/group). They were fed acutely a bolus of ethanol by gavage (6 g/kg body weight, 20% solution). Controls were fed isocaloric glucose. The rats were killed 3 or 12 h after the alcohol bolus. A second group of rats was fed betaine 1 g/kg body weight by gavage with or without ethanol (6 g/kg) or isocaloric dextrose, and killed at 3 or 12 h (Table 1). Urine and blood were collected at kill to measure alcohol levels. Blood ALT levels were also measured. The urine was collected under toluene to prevent evaporation using metabolic cages (one rat/cage), and the urinary alcohol level was measured using a QED Saliva Alcohol A 150 test kit STC Technologies, (Bethlehem, PA). Blood alcohol and alanine aminotransferase (ALT) were measured using a chemical analyzer. At kill under isofluorane anesthesia, the liver was removed and weighed. A portion of the liver was quick frozen and stored in isopentane in liquid nitrogen, followed by storage at −80°C. Gene microarray analysis was done on RNA extracted from the fast frozen tissue. Microarray analysis results were obtained and were compared with microarrays done on liver tissue from previously reported studies [2]. The rats were maintained according to the Guidelines of Animal Care, as described by the National Academy of Sciences and published by the National Institute of Health (1996).

Table 1 UAL, BAL, alanine aminotransferase (ALT) were determined for 3 and 12 h feeding of alcohol with or without betaine (mean ± SD, $n = 3$–4)

Groups	Time (h)	*N*	UAL mg %	BAL mg %	ALT U/l
Alcohol*	3	4	453 ± 57	347 ± 68	51 ± 3
Dextrose*	3	4	0	0	41 ± 3
Alcohol*	12	3	33 ± 15	29 ± 17	54 ± 3
Dextrose*	12	3	0	0	50 ± 4
Alcohol + betaine	3	3	255 ± 130	155 ± 110	60 ± 33
Betaine	3	3	0	0	40 ± 3
Alcohol + betaine	12	3	58 ± 29	41 ± 33	43 ± 8
Betaine	12	3	0	0	51 ± 3

Note that betaine reduced the 3 h blood alcohol levels. The 3-h ALT levels were not different compared to the dextrose controls. The alcohol + dextrose-treated results (*) were reprinted from Bardag-Gorce et al. [2], with permission from WILEY InterScience

RNA extraction

Approximately 50–100 μg of frozen liver samples and 1,000 μl of Trizol® (Invitrogen™, Carlsbad, CA, USA) were added to a Bio 101 bead tube containing Lysing Matrix D (MP Biomedicals, LLC, Irvine, CA, USA). Each tissue was homogenized by processing the tubes in the FastPrep centrifuge (MP Biomedicals LLC, Irvine, CA, USA) for 30 s and incubating them on ice for 1 min. Total RNA was extracted following the Trizol® protocol using chloroform, heavy phase lock gel tubes (Eppendorf, Westbury, NY), and isopropanol. RNA quality was confirmed using Agilent's 2100 BioAnalyzer (Santa Clara, CA, USA).

About 5 μg of total RNA was used for preparing biotin-labeled cRNA. Fifteen micrograms of labeled and fragmented cRNA were subsequently hybridized to Rat Genome 230 2.0 Array (Affymetrix, Santa Clara, CA, USA). RNA isolation, labeling, and data analysis were performed at the Microarray Core at Los Angeles Biomedical Research Institute. Hybridization, washing, staining, and scanning of the chips were performed at the Microarray Core at Cedars-Sinai Medical Center.

Microarray analysis

Liver tissue taken from the eight experimental groups (three rats per group), and three rats fed ethanol, betaine, or dextrose were subjected to microarray analysis. Five micrograms of total RNA was used for preparing biotin-labeled cRNA. Labeled and fragmented cRNA was subsequently hybridized to rat Genome Array (Affymetrix, Santa Clara, CA, USA). Labeling, hybridization, image scanning, and initial data analysis were performed at the Microarray Core at Los Angeles Biomedical Research Institute. RNA was converted to cDNA using GeneChip® One-Cycle cDNA Synthesis Kit (Affymetrix, Santa Clara, CA, USA) and then converted to biotinylated cRNA using GeneChip® IVT Labeling Kit (Affymetrix, Santa Clara, CA, USA). The quality of labeled RNA was confirmed with the Affymetrix or Test 3 Array (GeneChip® Rat Exon1.0ST Array [http://www.affymatrix.com]). The

biotinylated cRNA from all samples was hybridized to Affymetrix Rat GeneChip arrays. To perform the hybridization and staining, a hybridization cocktail was prepared, which included controls of the fragmented cRNA. The samples were hybridized in the array at 45°C for 17 h using GeneChip Hybridization Oven 640. Immediately following hybridization, the array underwent an automated washing and staining protocol (R-Phycoerythin Streptavidin conjugated, Molecular Probes, Invitrogen Corp., Carlsbad, CA, USA) on the GeneChip Fluidics Station 400. The array was then scanned with a GeneChip Scanner 3000 (Affymetrix, Santa Clara, CA, USA).

Data preparation, analysis, and integration were performed using Affymetrix's GeneChip Operating Software (GCOS). The software was used to perform image processing, evaluation of data quality, normalization, transformation, and filtering, so that data would be ready for further analysis. Wilcoxon's signed rank test was used in comparison analysis to derive biologically significant results from the raw probe cell intensities on expression arrays. For comparison analysis, each probe set on the experiment array was compared with its counterpart on the control array to calculate the change in P value used to generate the difference call of increase (I; $P < 0.04$), marginal increase (MI; $P < 0.04$ to $P < 0.06$), decrease (D; $P > 0.997$), marginal decrease (MD; $P > 0.992$ to $P > 0.997$), or no change (NC: $P > 0.06$ to $P < 0.997$). Comparison analysis was used to generate a signal log ratio for each probe prior to the experimental array to the corresponding probe pair on the control array. This strategy cancels out differences resulting from different probe-finding coefficients. Signal log ratio was computed by using a one-step Tukey's biweight method by taking a mean of the log ratio of probe pair intensities across the two arrays.

Once the absolute and comparison data files were created in GCOS, genes were identified with signal intensity differences using BULLFROG v12.3 TG Lockhart and Lockhart, (San Diego, CA, USA) and GeneSpring Silicon Genetics, Agilent Technologies, (Santa Clara, CA, USA). In the BULLFROG analysis, the pair-wise comparisons were used to find consistent differences among all of the samples compared. The criteria used were as follows: a change call of increase/marginal increase or decrease/marginal decrease, fold change >1.7, and a present call in at least one of the arrays. In GeneSpring, the probes were first normalized using "Peer Gene: Normalize to median." Next, transcripts were determined to be differentially expressed based on the following criteria: a Change Call of Increase, Marginal Increase, Decrease, or Marginal Decrease with a Change P value <.0.006 or >0.994, a Signal Log Ratio <−0.8 or >0.8, a Present Call for the probe set in either or both experimental conditions, and a minimum signal intensity of 50 of a probe in either or both of the experimental files.

After generating a list of differentially expressed genes, downstream analysis was performed. The filtered transcripts were clustered in GeneSpring using SOM, K-means, and GeneTree to find similar patterns of gene expression. The lists of transcripts were also uploaded into GenMapp (Gene MicroArray Pathway Profiler, Gladstone Institutes University of California at San Francisco). GenMapp clusters the transcripts based on biological function.

The data illustrated were obtained by using the KEGG web site (http://www.genome.jp/kegg/pathway.html) and blasting the list of total changed genes issued from our experiment for analysis. The web site calculates the number of up regulated and down regulated genes for each pathway shown in the KEGG graph. To determine the percent gene change in each pathway, the number of changed genes present in each pathway was divided by the total number of genes present in the same pathway. The excel graph reports the total number of genes present in each pathway (left near the pathway name), the number of genes up regulated (red), the number of genes down regulated (green), and the calculated percent of genes changed in each pathway.

Statistical analysis

Array data were all-median normalized and log2 transformed using GeneSifter software (GeneSifter.Net, V12X Laboratories, Seattle, WA, USA). Statistical analyses of array data were performed using GeneSifter software.

Results and discussion

Animal feeding experiment (Table 1)

The effect of betaine on the acute ethanol bolus was measured. Eight groups of rats were studied ($n = 3$), i.e., (ethanol + betaine; dextrose + betaine 3 h); (ethanol + betaine; dextrose + betaine 12 h); (ethanol or dextrose 3 h); (ethanol or dextrose 12 h) (see Table 1).

Microarray analysis

The effect of ethanol, betaine, and the combination of ethanol and betaine on gene expression changes at 3 and 12 h were compared by functional clustering analysis (heat map) (Fig. 1a, b). The dextrose control results were quite homogeneous when the 3-h control rats and 12-h control rats were compared. The effects on gene expression changes at 3 h for the ethanol-, betaine-, and

ethanol + betaine-treated groups (Fig. 1a) were all similar when compared to the controls. However, the changes induced by ethanol at 12 h compared with controls were largely prevented by betaine when fed with ethanol (Fig. 1b).

The Venn diagrams illustrated in Fig. 2a show the number of genes that underwent changes at 3 h and the comparison between treatment groups. When the ethanol-fed rats were compared to the controls, the expression of 646 genes was changed, 272 unique to the 3 h ethanol bolus when blood alcohol levels were high. When the betaine-fed rats were compared to controls at 3 h, the expression of 1,849 genes was changed, 1,183 unique to betaine. When the betaine plus ethanol-fed rats were compared with ethanol-fed rats, the expression of 698 genes was changed, 230 unique to ethanol + betaine. The results indicate the complex global nature of the gene expression changes that were caused by high BAL and betaine (3 h) on the liver. Only the changes in expression of 24 genes were shared between the 3 comparisons.

The Venn diagram at 12 h post ethanol bolus (Fig. 2b) showed fewer changes in gene expression when 3 h post bolus ethanol was compared to the controls. Two hundred and ninety-two changes in gene expression unique to ethanol were found (Fig. 2b). When betaine + ethanol treatment was compared to ethanol, 472 unique changes in gene expression were found. However, when betaine treatment was compared to the dextrose controls, 697 unique changes in gene expression were noted Fig. 2b. Only the changes in the expression of 37 genes were shared by the three comparisons. The results further indicate the complex global nature of the changes in gene expression that were caused by low BAL and betaine (12 h) on the liver. They also differed between the 3- and 12-h intervals.

When the changes in gene expression in rats fed ethanol and betaine were compared with ethanol alone by KEGG functional pathways, most pathways affected by ethanol were down regulated by betaine 3 h post bolus (Fig. 3). This was especially true for metabolic pathways. Other pathways that were predominantly down regulated were cancer, focal adhesion, cytokine-cytokine receptor interaction, wnt signaling, tight junction, MAPK signaling, and retinol metabolism pathways (Fig. 3).

When the changes in gene expression in rats fed betaine were compared with the dextrose controls and analyzed by KEGG functional pathways, most pathways were equally up and down regulated except for MAPK signaling, biosynthesis of steroids, and metabolic pathways at 3 h post bolus (Fig. 4).

At 12 h, the KEGG functional pathways differed from those at 3 h. When the changes in gene expression in rats fed ethanol and betaine were compared with ethanol alone and analyzed by KEGG functional pathways, the metabolic

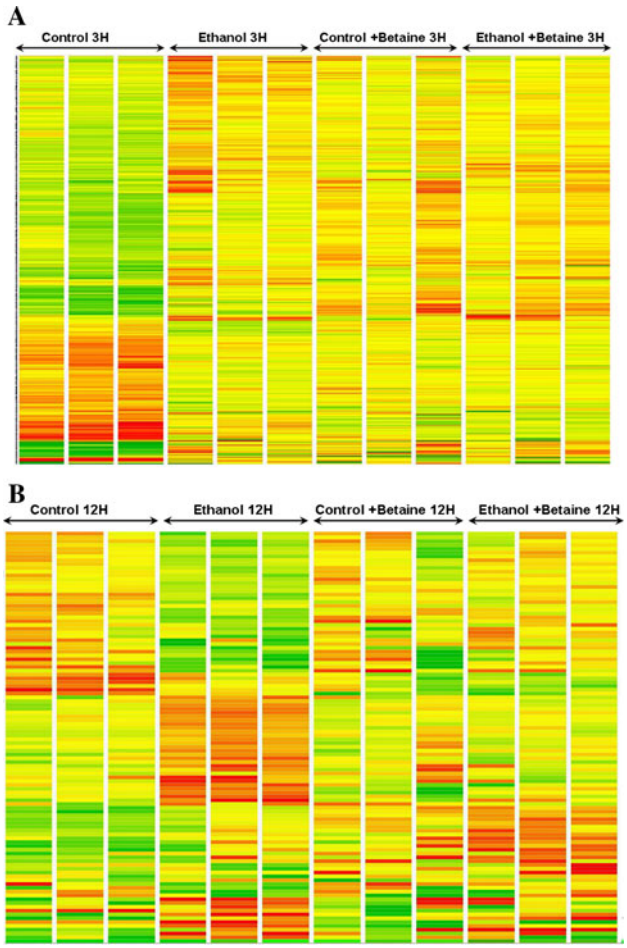

Fig. 1 Heat map comparing the changes in gene expression of functional clusters at 3 h (**a**) and 12 h (**b**) ($n = 3$)

pathways were still largely down regulated to the same extent as at 3 h post bolus. However, the number of up regulated genes more than doubled. The expression of the number of genes in the MAPK pathway was markedly increased at 12 h compared with 3 h. Other pathways up regulated at 12 h compared to 3 h were the chemokine signaling pathway and pathway to cancer.

When the changes in gene expression in rats fed betaine at 12 h were compared with the dextrose controls and analyzed by KEGG functional pathways, the changes were equally up and down regulated (Fig. 6). The main exceptions were the MAPK signaling pathway, which was mainly up regulated as was the chemokine signaling pathway.

The 3 h betaine plus ethanol versus ethanol comparison was subjected to data mining to determine which specific genes were down regulated in the metabolic pathways and other pathways (Fig. 4). Two enzymes involved in the metabolism of methionine were affected. Glycine *N*-methyl-transferase (GNMT) was down regulated. It is the major enzyme in the liver dealing with transmethylation

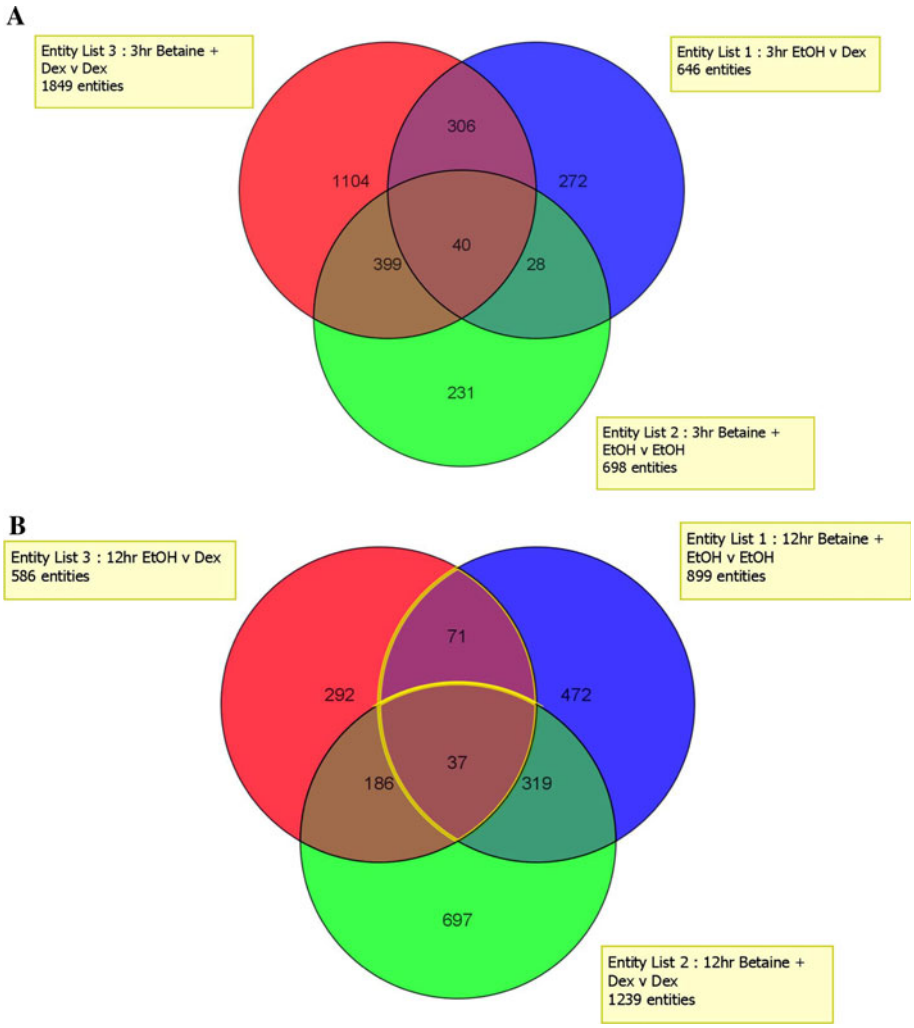

Fig. 2 Venn diagram comparing the changes in gene expression when rats were fed ethanol or betaine or the combination of ethanol and betaine 3 h (**a**) and 12 h (**b**) post bolus ($n = 3$)

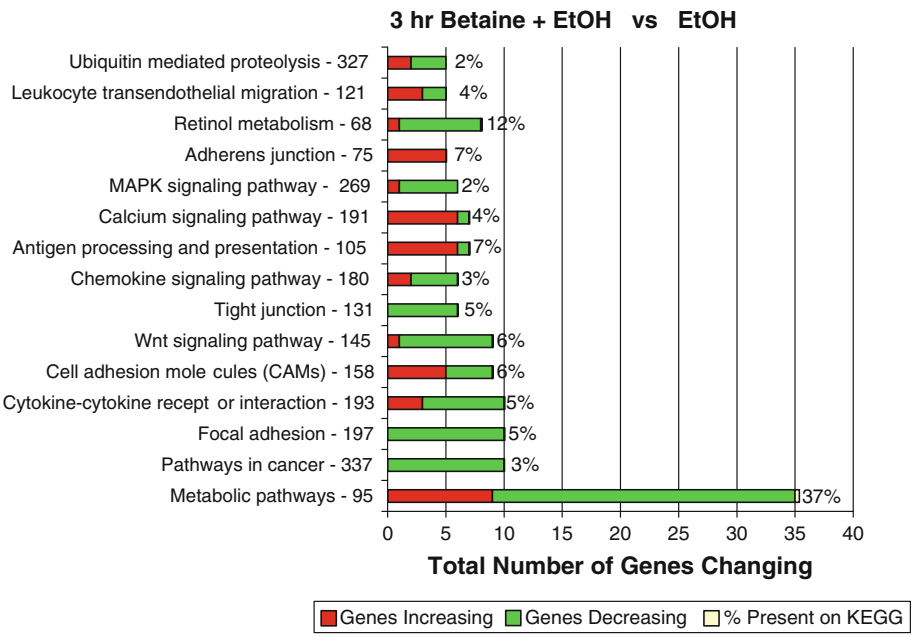

Fig. 3 KEGG functional pathways affected 3 h post bolus when rats fed betaine and ethanol were compared with rats fed ethanol alone ($n = 3$, the total number of genes in each pathway is listed on the x axis)

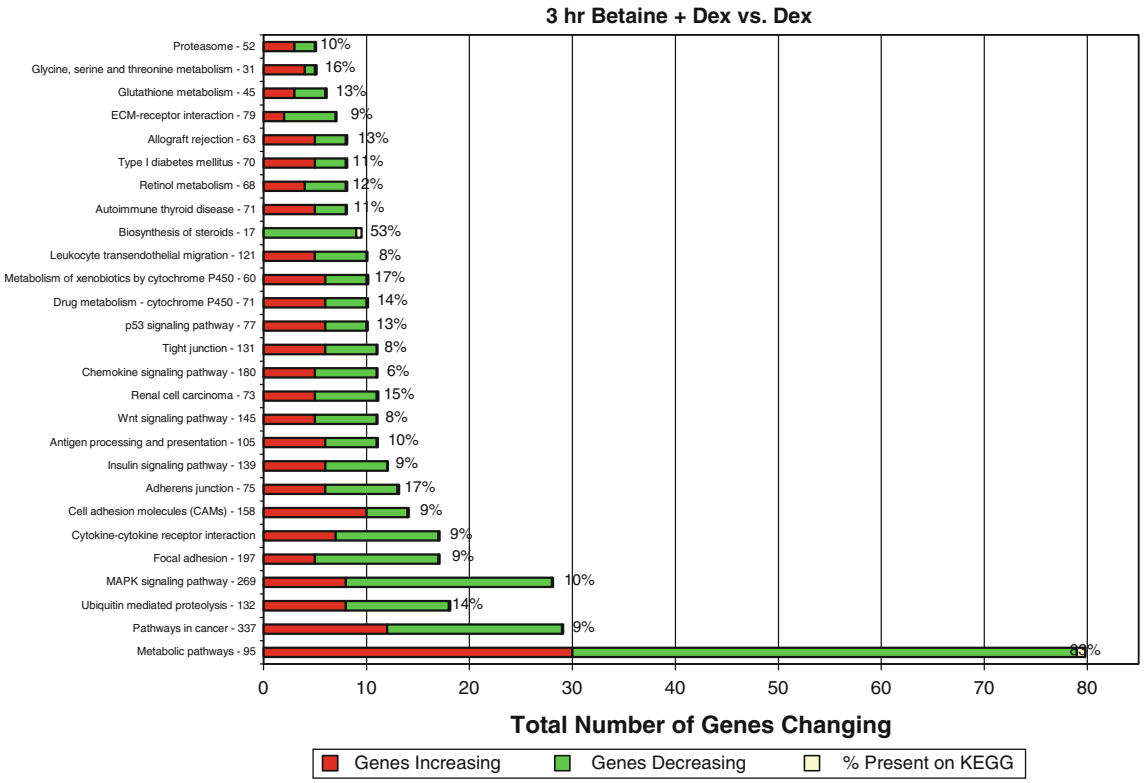

Fig. 4 KEGG functional pathways affected 3 h post bolus when rats fed betaine were compared to rats fed dextrose ($n = 3$ the total number of genes on each pathway is listed on the y axis)

Table 2 Dextrose + betaine versus dextrose 3 h gene expression regulation

Gene symbol	Ave ratio (FC)	±SD	Gene title
Cxcl1	138.14	3.79	Chemikine (CXC) ligand 1
Socs3	4.23	1.44	Suppressor of cytokine signaling 3
Hmox1	4.08	1.64	Heme oxygenase 1
Rdh2	3.71	3.61	Retinol dehydrogenase 2
Igfbp1	3.71	4.41	Insulin-like growth factor–binding protein 1
Prkce	2.06	1.26	Protein kinase C epsilon
Amd1	1.77	1.43	Adenosylmethionine decarboxylase 1
Scap	−1.69	−1.13	SREBP chaperone
Sirt4	−1.88	−1.17	Sirtuin 4
Prkci	−1.95	−1.13	Protein kinase C iota
Adh1	−2.03	−1.43	Alcoholic dehydrogenase 1
Car3	−2.1	−1.42	Carbonic anhydrase 3
Car2	−2.1	−1.57	Carbonic anhydrase 2
Car5a	−2.19	−1.64	Carbonic anhydrase 5a mitochondria
Cxcl12	−2.33	−1.25	Chemokine (C-X-C) ligand 12
Cxcl13	−2.36	−1.48	Chemokine (C-X-C) ligand 13
Adcy5	−2.5	−1.38	Adenylate 5
Map3k10	−2.51	−1.34	Mitogen-activated protein-3 kinase 10
Aldh1a1	−3.71	−1.44	Aldehyde dehydrogenase 1A1

FC fold change

reactions where SAMe is converted to SAH. Ahcy, which catalyzes the reversible hydrolysis of SAH to yield homocysteine and adenosine, was also down regulated. Carbonic anhydrase was down regulated, which has been previously reported to be down regulated in betaine-supplemented ethanol-fed rats [7]. It was down regulated by

Table 3 Ethanol + betaine versus ethanol 3 h gene expression down regulated

Gene symbol	Avg ratio (FC)	±SD	Gene title
Cth	−1.72	−1.07	Cystathionase
Gnmt	−1.74	−1.19	Glycine A-methyltransferase
Ahcyl1	−1.84	−1.15	*S*-adenosylhomocysteine hydrolase-like
Car2	−1.96	−1.33	Carbonic anhydrase II
Cxcl13	−2.01	−1.44	Chemokine (C-X-C) ligand 13
Retsat	−2.03	−1.78	Retinol saturase
Jam2	−2.19	−1.55	Junctional adhesion molecule
Prkci	−2.43	−1.27	Protein kinase C Iota
Aldh1a1	−2.53	−1.56	Aldehyde dehydrogenase 1A1
Igfbp2	−4.35	−1.71	Insulin-like growth–binding protein 2

FC fold change

Table 4 Ethanol + betaine versus ethanol 12 h post bolus gene expression

Gene symbol	Avg ratio (FC)	±SD	Gene title
CXC1	10.1	1.94	Chemokine (C-X-C) ligand 1
Car12	3.7	1.4	Carbonic anhydrase 12
Adrm	3	1.22	Adhesion regulating molecule
Scap	2.4	1.53	SREBP chaperone
Rdh10	2.3	1.27	Retinol dehydrogenase
Cth	−2.3	−1.4	Cystathionase
ILgfbpl	−2.35	−2.25	Insulin-like growth factor–binding protein 1
Lepr	−2.5	−2.84	Leptin receptor

FC fold change

Table 5 Dextrose + betaine versus dextrose 12 h post bolus expression

Gene symbol	Avg ratio (FC)	±SD	Gene title
Rdh2	2.3	1.55	Retinol dehydrogenase 2
Map3k3	1.92	1.47	Mitogen-activated protein 3 kinase 3
Rdh10	1.87	1.45	Retinol dehydrogenase 10
Car14	−1.88	−1.56	Carbonic anhydrase 14
Cxcl12	−2.17	−1.64	Chemokine (C-X-C) ligand 12
Car3	−2.27	−1.61	Chemokine (C-X-C) ligand 3
Car1	−2.5	−1.59	Carbonic anhydrase 1
Ubd	−2.81	−1.85	Ubiquitin D

FC fold change

betaine alone without ethanol ingestion in our study at both 3 and 12 h post bolus (Tables 2 and 5). Retinol saturase was down regulated (Table 3). The junctional adhesion molecule pathway was down regulated (Table 3), which is a reflection of the down regulation of cell adhesions (Fig. 3). Cystathionase, which generates cysteine and aldehyde dehydrogenase, which oxidizes acetaldehyde, were both down regulated (Table 3) as were many other enzymes in the metabolic pathway (Fig. 3). Chemokine (c-x-c) ligand 13 was down regulated (Table 3), which is a reflection of the down regulation of the chemokine signaling pathway (Fig. 3). Insulin-like growth factor–binding protein 2 was down regulated (Table 3), which is a reflection of the down regulation of the pathways in cancer (Table 3; Fig. 3).

Betaine fed with dextrose at 3 h post bolus caused a balanced up and down regulation of genes (Table 2) as was revealed by data mining. The results correlated with the up and down regulation of the pathways seen in Fig. 4. Of note was the up regulation of adenosylmethionine decarboxylase, which is involved in the metabolism of SAMe to form methylthioadenosine (MTA) and polyamines [9]. Igfbp1 and Map3k5 were up regulated, which correlate with the up regulation of the pathway to cancer (Fig. 4). Both Adh1 and Aldh1a1 were down regulated (Table 2), which could reduce the elimination rate of ethanol. This result does not explain why the UAL and BAL were lower when betaine was fed with ethanol compared to when ethanol was fed alone at 3 h post bolus (Table 1).

The 12-h ethanol plus betaine versus ethanol comparison was subjected to data mining for changes in the individual gene expressions (Table 4), as predicted from the changes in the KEGG functional pathways (Fig. 5). There was an increase in the chemokine signaling pathway where

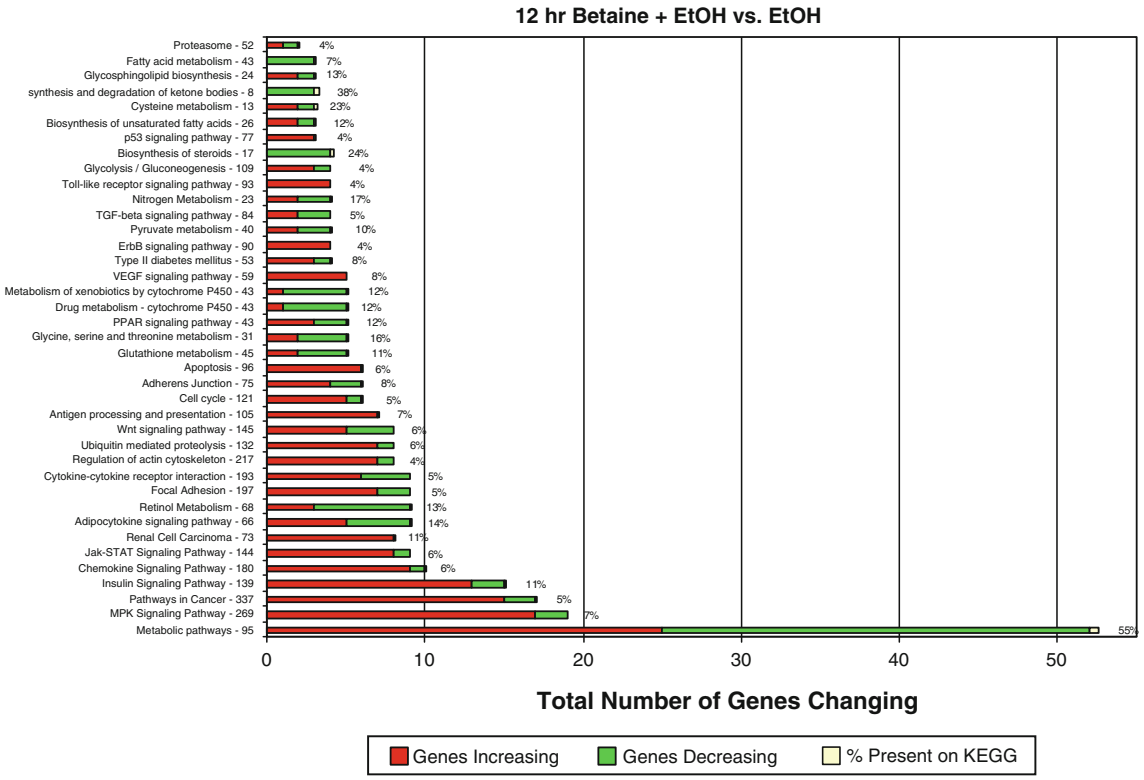

Fig. 5 KEGG functional pathways affected the 12 h post bolus when rats fed ethanol and betaine were compared to rats fed ethanol only ($n = 3$, the total number of genes in each pathway is listed on the y axis)

Fig. 6 KEGG function pathways affected at 12 h post bolus when rats fed betaine were compared to rats fed dextrose ($n = 3$, the number of genes on each pathway is listed on the x axis)

CXC1 was up regulated 10.1-fold. Adrm was up regulated threefold (Table 4) where focal adhesion molecules were up regulated as indicated by the KEGG functional pathway (Fig. 5). The metabolic pathway was up regulated (Fig. 5), and this was reflected by the up regulation of Rdh6, Scap, and Car 12 (increased 3.7-fold) (Table 4). Igfbp1 was down regulated.

Data mining results of the betaine versus dextrose comparison at 12 h post bolus (Fig. 6) showed a balance in genes that were up or down regulated. Metabolic genes such as Car 1 and 3 were down regulated, where as, Rdh2 and Rdh10 were up regulated (Table 5). The MAPK signaling pathway was markedly up regulated, and this was reflected by the 1.9-fold increase in the expression of Map3k3.

Thus, the response of gene expression changes observed after both acute ethanol and betaine bolus was global, involving almost all functional pathways and changing between 3 and 12 h post bolus. Betaine modified the effects on gene expression induced by ethanol at 3 h post bolus but had less effect at 12 h post bolus.

Acknowledgments The authors thank Adriana Flores for typing the manuscript. The study was supported by NIH/NIAAA Grant 8116.

References

1. Barak AJ, Beckenhauer HC, Junnila M, Tuma DJ (1993) Dietary betaine promotes generation of S-adenosylmethionine and protects the liver from ethanol-induced fatty infiltration. Alcohol Clin Exp Res 17:552–555
2. Bardag-Gorce F, Oliva J, Dedes J, Li J, French BA, French SW (2009) Chronic ethanol feeding alters hepatocyte memory which is not altered by acute feeding. Alcohol Clin Exp Res 33:684–692
3. Li J, Bardag-Gorce F, Oliva J, Dedes J, French BA, French SW (2009) Gene expression modifications in the liver caused by binge drinking and S-adenosylmethionine feeding. The role of epigenetic changes. Genes Nutr. PMID: 19960281
4. Ji C, Kaplowitz A (2003) Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress and liver injury in alcohol-fed mice. Gastroenterology 124:1488–1499
5. Kharbanda KK (2009) Alcoholic liver disease and methionine metabolism. Semin Liver Dis 9:155–165
6. Kharbanda KK, Rogers DD II, Mailliard ME, Siford GL, Barak AJ, Bechenhauer HC, Sorrell MF, Tuma DJ (2005) A comparison of the effects of betaine and S-adenosylmethionine on ethanol-induced changes in methionine metabolism and steatosis in rat hepatocytes. J Nutr 135:519–524
7. Kharbanda KK, Vigneswara V, McVicker BL, Newlaczyl AU, Bailey K, Tuma D, Ray DE, Carter WG (2009) Proteomics reveal a concerted upregulation of methionine metabolic pathway enzymes, and down regulation of carbonic anhydrase-III in betaine supplemented ethanol-fed rats. Biochem Biophys Res Commun 381:523–527
8. Kim SJ, Jung YS, Kwon DY, Kim YC (2008) Alleviation of acute ethanol-induced liver injury and impaired metabolomics of S-containing substances by betaine supplementation. Biochem Biophys Res Commun 368:893–896
9. Oliva J, Bardag-Gorce F, Li J, French BA, Nguyen SK, Lu SC, French SW (2009) Betaine prevents Mallory-Denk body formation in drug-primed mice by epigenetic mechanisms. Exp Mol Pathol 86:77–86
10. Purohit V, Abdelmalek MF, Barve S, Benevenga NJ, Halsted CH, Kaplowitz A, Kahrbanda KK, Liu Q-Y, Lu SC, McClain CJ, Swanson C, Zakhari S (2007) Role of S-adenosylmethionine, folate, and betaine in the treatment of alcoholic liver disease: summary of a symposium. Am J Clin Nutr 86:14–24