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BETAINE PREVENTS MALLORY-DENK BODY FORMATION IN DRUG-PRIMED MICE BY EPIGENETIC MECHANISMS

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Abstract

Previous studies showed that S-Adenosylmethionine (SAMe) prevented MDB formation and the hypomethylation of histones induced by DDC feeding. These results suggest that formation of MDBs is an epigenetic phenomenon. To further test this theory, drug-primed mice were fed the methyl donor, betaine, together with DDC, which was refed for 7 days. Betaine significantly reduced MDB formation, decreased the liver/body weight ratio and decreased the number of FAT10 positive liver cells when they proliferate in response to DDC refeeding. Betaine also significantly prevented the decreased expression of BHMT, AHCY, MAT1a and GNMT and the increased expression of MTHFR, caused by DDC refeeding. S-Adenosylhomocysteine (SAH) levels were reduced by DDC refeeding and this was prevented by betaine. The results support the concept that betaine donates methyl groups, increasing methionine available in the cell. SAMe metabolism was reduced by the decrease in GNMT expression, which prevented the conversion of SAMe to SAH. As a consequence, betaine prevented MDB formation and FAT10 positive cell proliferation by blocking the epigenetic memory expressed by hepatocytes. The results further support the concept that MDB formation is the result of an epigenetic phenomenon, where a change in methionine metabolism causes global gene expression changes in hepatocytes.

INTRODUCTION

Mallory-Denk body (MDB) formation is induced by refeeding diethyl 1-1, 4-dehydro-2,4,6-trimethyl-1,5-pyridine carboxylate (DDC) to drug-primed mice. The MDBs mostly disappear after 1 month of DDC withdrawal (DDC primed hepatocytes). MDB induction by DDC refeeding is prevented by feeding SAMe, a methyl donor (Li et al., 2008). SAMe feeding prevents the switch of SAMe metabolism from the methylating pathway to the decarboxylating methylthioadenosine (MTA) pathway caused by DDC refeeding (Bardag-Gorce et al., 2008b). DDC refeeding causes demethylation of histones. This is prevented by feeding SAMe (Table 1) (Bardag-Gorce et al., 2008b).

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Altered methionine metabolism can contribute to the development of alcoholic liver disease (ALD) and hepatocellular carcinoma (HCC). Both ALD and HCC are major health care problems worldwide (James et al., 2003; Mato et al., 2008). Biosynthesis of S-adenosylmethionine (SAMe) occurs in all mammalian cells as the first step in methionine catabolism in a reaction catalyzed by methionine adenosyltransferase (MAT). The deregulation of SAMe metabolism is linked to liver diseases (Mato et al., 2008). Two genes, MAT1A and MAT2A, encode for the essential enzyme methionine adenosyltransferase (MAT) (Kotb et al., 1997), which catalyzes the biosynthesis of SAMe, the principal methyl donor, from methionine. MAT2A is expressed in various organs, while MAT1A is mostly expressed in the liver (Torre et al., 2000). A change in the ratio of the expression of MAT1A and MAT2A expression occurs in many liver diseases (Lu and Mato, 2005; Martinez-Chantar et al., 2003). In human HCC, MAT1A is replaced by MAT2A (Yang et al., 2001). The decreased ratio of MAT1A/MAT2A leads to a decrease of SAMe in liver tissue (Lu and Mato, 2008). SAMe plays a key role in the methylation of many molecules in the cell: DNA, RNA, biogenic amines, phospholipids and histones (Purohit et al., 2007).

The deregulation of other enzymes involved in methionine metabolism, are involved in liver disease. Methylenetetrahydrofolate reductase (MTHFR) is known to play a role in DNA methylation, synthesis, and repair. Mutations in MTHFR are associated with an increase of HCC (Mu et al., 2007). Glycine N-methyltransferase (GNMT) is the main enzyme responsible for catabolism of hepatic S-adenosylmethionine (SAMe). Point mutations in the GNMT gene, identified in humans, are correlated with liver disease (Luka et al., 2002). GNMT deletion in mice leads to steatosis and hepatocellular carcinoma (Martinez-Chantar et al., 2008). The expression of GNMT is down regulated in human cell lines (Chen et al., 1998) and human HCC (Avila et al., 2000).

Betaine, another methyl donor, might also prevent MDB formation since it increases methionine formation from homocysteine via betaine-homocysteine methyltransferase (BHMT) (Purohit et al., 2007). Betaine functions by removing homocysteine and S-adenosylhomocysteine (SAH) from the liver (Purohit et al., 2007). SAH and the SAMe/SAH ratio levels regulate transmethylation reactions, where an increase in SAH inhibits transmethylation of histones (H3K9 me3 and H3K4 me3). These were reduced when MDBs were induced by refeeding DDC (Bardag-Gorce et al., 2008b). The reduction in the trimethylation of H3K4 and K9 gives rise to global changes in gene expression and a change in epigenetic memory (Jenuwein, 2006).

Why are we testing betaine? Betaine is less expensive and much more stable when ingested (Najm et al., 2004; Schwahn et al., 2003). The hypothesis tested here is that betaine will, like SAMe, prevent MDB formation through epigenetic alterations caused by changes in methionine metabolism.

Materials and Methods

Animals

One-month-old C3H male mice (Harlan Sprague-Dawley, San Diego, CA) were fed DDC (0.1% diethyl-1, 4-dihydro-2,4,6-trimethyl-3,5-pyridinedicarboxylate (Aldrich, St Louis, MD) for 10 weeks to induce MDB formation *in vivo*. The mice were then withdrawn from the drug for 1 month (*n-4*) and refed DDC with or without betaine (2% in the drinking water) for 7 days. The control mice n=4 were fed control diet (Yuan et al., 1996). Mice fed DDC for 10 weeks and then withdrawn from DDC for 1 month (n=4) served as withdrawn controls. All mice were treated in a humane manner as approved by the Animal Care Committee at Harbor-UCLA Laboratory BioMedical Research Institute according to the Guidelines of the National Academy of Science.

Immunohistochemistry

Liver tissue was fixed in 10% buffered zinc formalin. Liver sections were double stained using a mouse monoclonal antibody to CK-8 (Fitzgerald, RDI, Concord, MA) and a rabbit polyclonal antibody to ubiquitin (Dako, Carpinteria, CA) (Table I). Double staining with antibodies to FAT10 (UBD) and PCNA was also done. Quantitation of MDB positive cells, FAT10 positive cells and PCNA positive nuclei were performed using a Nikon morphometric system and a Nikon 400 fluorescent microscope. Texas-red and FITC-conjugated second antibodies were used. DAPI was the nuclear stain.

Nuclei Isolation

The isolation of nuclei was carried out according to the method of Umlauf *et al*. (Umlauf et al., 2004). Liver tissue, frozen in isopentane immersed in liquid nitrogen was homogenized in a Dounce homogenizer with 10 strokes in 1 ml of buffer-I. The homogenates were centrifuged for 10 min at 6000×g. The Pellets were then resuspended in buffer-II, placed on ice for 10 min and then centrifuged 20 min at 9000g on a sucrose cushion (buffer III). All buffers used contained protease inhibitors: 10 mM benzamidine, 0.7 μg/ml leupeptin, 50 μg/ml soy bean trypsin inhibitor, 0.2 μg/ml aprotinin, 2 μg/ml antipain, 0.7 μg/ml pepstatin, 0.5 mM PMSF, and 0.5 mM AEBSF (Calbiochem, La Jolla, CA), sodium butyrate 5 mM and DTT 1 mM. Protein concentrations were measured using the Bradford method (Bradford, 1976), and bovine serum albumin was the protein standard.

Histone Isolation

The protocol for Histone isolation was done using the protocol of Shechter *et al*. (Shechter et al., 2007). Briefly, isolated nuclei were mixed with 0.4 N H_2SO_4 and incubated on a rotator for 30 min. at 4°C. Samples were spun in a microcentrifuge at 16,000 g, 10 min. Dissolved histones in the supernatant were then precipitated with 33% TCA. After an acetone wash the histones were dissolved in an appropriate buffer and further analyzed.

Western Blot Analysis

Proteins (50 μg) from liquid nitrogen frozen stored livers and nuclear and histone extracts were separated by SDS-PAGE gels and transferred to a PVDF membrane (Bio-Rad, Hercules, CA) for 1 h in 25 mM Tris-HCl (pH 8.3), 192 mM glycine and 20% methanol. The membranes were stained using primary antibodies to antigens (Table 1). Appropriate species polyclonal and monoclonal HRP-conjugated antibodies were used as the secondary antibodies. The membranes were subjected to chemiluminescence detection using luminal, according to the manufacturer's instructions (Amersham Pharmacia Biotech, Piscataway, NJ). The antibodies used were: Ubiquitin, PCNA (DAKO, Carpinteria, CA), BHMT, AMD1 (Abcam, Cambridge, MA), FAT10 (BioMol, Plymouth, PA), Cytokeratin 8 (RDI, Concord, MA), AHCY (ABR, Golden, CO).

Quantitative Real-time RT-PCR Assay

Total liver RNAs were extracted with Trizol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA) as described previously (Li et al., 2008). The sequences of PCR primers are:

FAT10	NM_023137	Forward	GATTGACAAGGAAACCACTATCCA
		Reverse	ACAAGGGCAGCTCTTCATCAC
GNMT	NM_010321	Forward	TGCTGAAATATGCGCTTAAGGA,
		Reverse	TTGGCTTCTTCAATGACCCAAT
MAT1A	NM_133653	Forward	AGGAGATCAGGGTCTGATGTTTG
		Reverse	GAGCGAGCACGATGGTAAGG

MAT2A	NM_145569	Forward	GTGGGCCTCAGGGTGATG
		Reverse	TCCCCAACCGCCATAAGTATC
AHCY	NM_016661	Forward	GAAGGGTGCTCGCATTGCT
		Reverse	GCCACGAGAGTCTCAATGAGAA
MTHFR	NM_010840	Forward	CATCCGGACCGAGTTTGCT
		Reverse	CGGCGCCTGCAGATACC

Microarray analysis

Liver tissue from three mouse of each of the 4 treatment groups was subjected to microarray analysis. Total liver RNA is extracted with Ultraspec™ RNAs Isolation Systemic (Biotecz Laboratories, Houston, TX) and are cleaned up with Rneasy columns (Qiagen, Valencia, CA). Five micrograms of total RNA was used for preparing biotin-labeled cRNA. Labeled and fragmented cRNA is subsequently hybridized to Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA). Labeling, hybridization, image scanning and initial data analysis are performed by the Microarray Core at Los Angeles Biomedical Research Institute.

Sample preparation and loading

Equal amounts of RNA (5 μg) from each sample were used for Affymetrix GeneChip analysis. RNA was converted to cDNA using GeneChip® One-Cycle cDNA Synthesis Kit (Affymetrix) and then to biotinylated cRNA using GeneChip® IVT Labeling Kit (Affymetrix). The quality of labeled RNA was confirmed with the Affymetrix Test 3 Array. The biotinylated cRNA from all samples was hybridized to Affymetrix Mouse 430 2.0 GeneChip arrays.

Hybridization and staining

Hybridization cocktail was prepared, which includes controls at 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) on the GeneChip Fluidics Station 400. The arrays were then scanned with a GeneChip Scanner 3000 (Affymetrix).

Microarray data analysis

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 was ready for further analysis. Wilcoxon's 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 that was 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 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) and GeneSpring (Silicon Genetics). In the BULLFROG analysis, the filtering criteria used to find genes unique to DDC 7 days was the following: 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 "Per 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 < -0.08 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 and K-means and GeneTree to find similar patterns of gene expression. The lists of transcripts were also uploaded into GenMapp (Gene Micro Array 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.htm1) and blasting the list of total changed genes issued from our experiment for analysis. The website calculates the number of up-regulated and down-regulated genes for each pathway shown in the KEGG graph. To determine the present gene change in each pathway, the number of changed genes present in each pathway was divided by the total number of genes in the same pathway.

The same calculation was performed in the ABI panther (http://www.pantherdb.org/genes) website to illustrate the pie chart. To determine the percent gene change in each pathway, the number of genes present in each pathway was divided by the total number of changed genes.

HPLC

SAH, SAMe and MTA levels were measured as described (Huang et al., 1998; Huang et al., 1999) with slight modification. Liver specimens were homogenized in 0.5M perchloric acid and centrifuged at 1,000g (Beckman GPR centrifuge) for 15 minutes. The aqueous layer was quantitatively removed and neutralized with 3M KOH. SAH levels were determined in the neutralized perchloric acid extracts by high-performance liquid chromatography (Series 410 LC pump, Perkin Elmer, Waltham, MA) with a LC-90 UV detector and a LC-100 integrator (Perkin Elmer, Waltham, MA) using a Partisil SCX 10-μm column (25 30.44-cm inner diameter; Whatman Chem. Sep. Maidstone). SAH, SAMe and MTA were eluted isocratically at 1 ml/mn with 0.03 M $NH_4H_2PO_4$ containing 2% acetonitrile (vol/vol) and adjusted to pH 2.6 with 2 M H_3PO_4. SAH was identified by measuring absorbance at 254 nm at a sensitivity scale of 0.01. The amount of SAH, SAMe and MTA in each sample was calculated from standard curves. The identity of SAH peaks was also confirmed by spiking the sample with known standards. Their levels were reported as μM/100 mg net weight liver.

Statistical analysis

P values were determined by ANOVA and Student-Newman-Keuls for multiple group comparisons (Sigma-Stat software, San Francisco, CA).

RESULTS

The Heatmap comparing mice refed DDC with mice refed DDC (3 mice/group), plus betaine, showed that betaine prevented many of the changes in gene expression induced by DDC refeeding. The expression of genes is almost restored to control levels after 1 month of withdrawal (Fig1A). This change was reflected by changes in expression, which were compared by the kegg functional pathway analysis (Fig1B). The liver/body weight ratio increased after DDC refeeding (Fig2) ($p<0.001$) and betaine feeding reduced this increase ($p<0.001$). The ratio was also increased in the DDC withdrawal group when compared to the controls ($p<0.025$). The DDC+betaine group had a higher ratio than the DDC withdrawal group ($p<0.021$). Although betaine reduced the increased ratio of liver/body weight compared with the DDC refed group, it didn't reduce the increase to the level of the DDC withdrawal group baseline.

Betaine treatment reduced the numbers of MDBs formed when fed to the DDC refed group (Fig 3). Betaine reduced the number of MDBs to the level of the DDC withdrawal group. PCNA positive nuclei were counted in the 4 groups (Fig 4). Refeeding DDC increased the number of PCNA positive nuclei compared with controls ($p<0.001$), and the DDC withdrawal group ($p<0.001$). The DDC refed plus betaine group did not differ significantly from the two controls. It was concluded that betaine prevented the increase in liver cell proliferation induced by DDC refeeding. Refeeding DDC increased the number of FAT10 positive cells and betaine reduced the number of FAT10 positive cell levels to control levels (Fig 5). However, after 1 month withdrawal, FAT10 cells were still increased in the liver tissue from drug-primed mice.

Real-Time PCR confirms these results, except for 1 month withdrawal where the FAT10 mRNA expression recovered to control levels (Fig 5). The expression of MAT1A tended to decrease, as the result of DDC refeeding. Betaine treatment prevented this (Fig 6). MAT2A expression was not increased in DCC refed mice or changed by betaine (Fig 6). However, GNMT expression was reduced by DDC refeeding and this was prevented by betaine treatment (Fig 6). After 1 month withdrawal, GNMT expression didn't return to the control levels of expression. Same results were observed with AHCY (Fig6). The expression of MTHFR was increased by DDC refeeding and betaine treatment partially prevented this induction (Fig 6). There remained a significant difference between the betaine treated groups, the control groups and the 1 month withdrawal group.

In parallel, the BHMT expression was analyzed by Western blot. The level of BHMT and AHCY proteins were down regulated by DDC and this change was prevented by betaine. After 1 month withdrawal, the expression of the two proteins recovered to the control level of expression. DDC decreased the expression of adenosylmethionine decarboxylase (AMD1) and the expression of GNMT, and this was partially prevented by betaine feeding. AMD1 and GNMT expression was similar when control and 1 month withdrawal groups were compared (Fig 7). This was also true for AHCY and BHMT. The reduction of GNMT expression induced by refeeding DDC lead to a decrease of SAH levels in the liver tissue, which was prevented by betaine feeding (Fig 8). SAMe and MTA levels were not changed by the treatments.

DISCUSSION

Betaine, as a methyl donor, reduced the increase in liver/body weight ratio, MDB formation, FAT10 expression and the mitotic index (PCNA positive nuclei) caused by DDC refeeding. Betaine attenuated the decrease of MAT1A, AHCY, BHMT and AMD1 expression, and prevented the increase of MTHFR expression, caused by refeeding DDC for 7 days (Fig 9).

The results are similar to those observed after feeding SAMe (Bardag-Gorce et al., 2008b; Li et al., 2008; Oliva et al., 2008). GNMT expression was also reduced by DDC refeeding and this was prevented by betaine (Fig 6 and 7). A reduction in GNMT could explain how DDC refeeding reduced the levels of SAH. The increased expression of MTHFR caused by DDC refeeding could further inhibit GNMT activity (Purohit et al., 2007).

Not previously observed was the decrease in the expression of BHMT in mice refed DDC and this change was prevented by betaine. The reduction in levels of BHMT could have impeded the maintenance of methionine and SAMe levels when DDC was refed and could contribute to the reduced methylation of the histones 3 lysine 4 and 9 (Bardag-Gorce et al., 2008b). However, the decrease of the GNMT expression should induce an increase of SAMe levels but it didn't happen. The SAMe level was stable, as was the MTA level. The decrease of AMD1 expression should increase the SAMe levels in the cells. However, AMD2 could balance the down expression of AMD1 to maintain MTA and SAMe.

The liver phenotype changed after DDC refeeding (liver weight, MDB formation, FAT10 expression) where the DDC primed liver cells retained the memory of the epigenetic modifications induced by DDC, after drug withdrawal (Oliva et al., 2008). SAMe deficiency increases the susceptibility of the liver to steatosis, oxidative damage, and spontaneous development of HCC (Lu and Mato, 2008). The increase of PCNA in FAT10 positive cells suggests that the phenotype changed liver cells have a growth advantage over the unchanged liver cells, which did not express FAT10. Although SAMe level were stable in the liver, SAMe could be decreased in individual cells which were over expressing FAT10 and MDBs. These cells showed a high frequency of PCNA expression and could indicate that they have an increased in growth advantage. Betaine prevents FAT10 over expression and MDBs formation similar to SAMe treatment (Li et al., 2008; Oliva et al., 2008). These results also correlated with the inhibitor effects of SAMe on the growth factor pathway and the decrease in SAMe observed in regenerating liver tissue (Lu and Mato, 2008).

DDC induced the formation of MDBs and hypomethylation of DNA in the MDB forming FAT10 positive cells (Oliva et al., 2008). Hypomethylation of DNA is a common tumor marker (Vucic et al., 2008). Many studies have shown that a methyl-deficient diet causes hypomethylation of DNA (Ghoshal et al., 2006; James et al., 2003). Previously reported studies on DDC refed mice showed an increase in the expression of tumor markers such as AFP, GSTmu2 and FAT10 (Nan et al., 2006; Oliva et al., 2008). The combination of the epigenetic modifications observed, such as hypomethylation of the DNA and the over expression of PCNA and FAT10, favors tumorgenesis in the DDC fed model studied here (Bardag-Gorce et al., 2008b; Lee et al., 2003; Oliva et al., 2008; Roomi et al., 2006). After 9 months of withdrawal from DDC, the liver cell population that forms MDBs in response to DDC toxicity develops liver tumors (Nan et al., 2006). The question remains: Can betaine treatment prevent the formation of tumors in DDC-primed mice studied here?

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Glossary

MDB	Mallory-Denk bodies
SAMe	S-Adenosylmethionine
SAH	S-adenosylhomocysteine
MAT1a and 2a	Methionine adenosyl-transferase 1a and 2a
MTHFR	Methylenetetrahydrofolate reductase
GNMT	glycine N methyltransferase
AHCY	S-adenosyl homocysteine hydrolase
BHMT	Betaine homocysteine methyltransferase

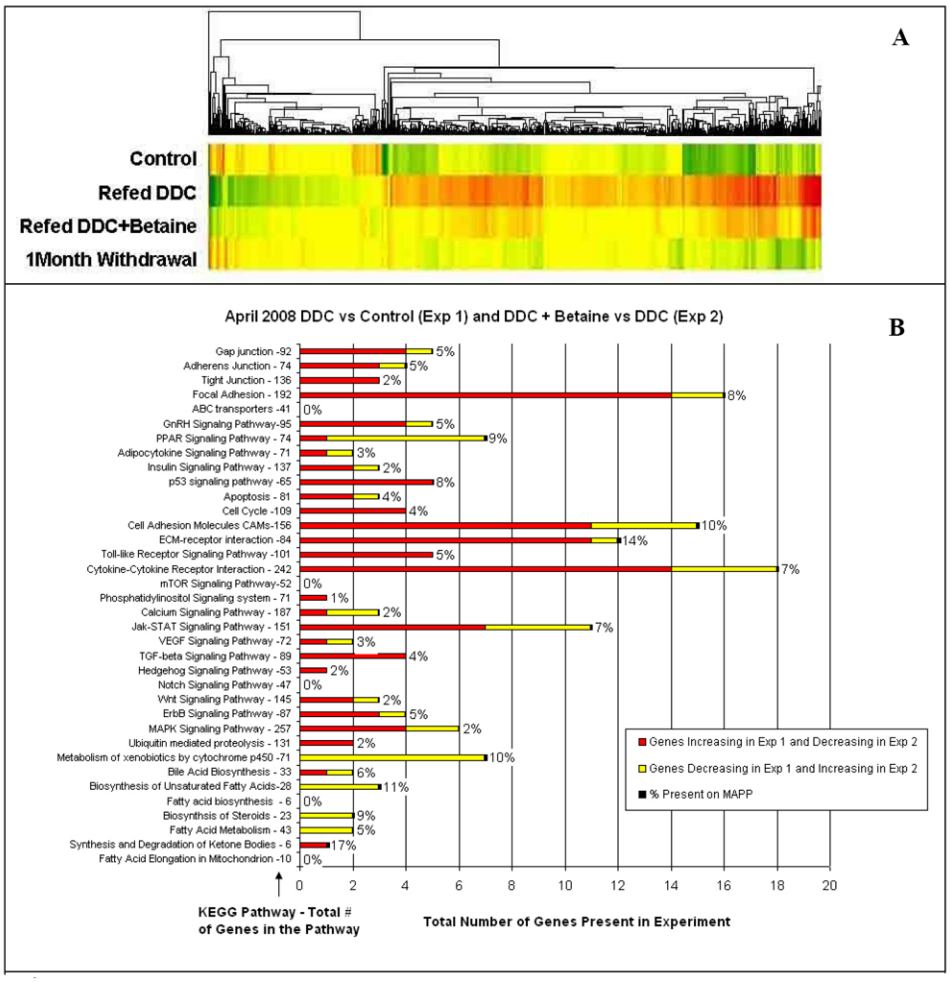

Fig 1.

A) Heatmap showing changes in gene expression when DDC is refed to drug-primed mice (5050 genes were identified). The microarrays datas are an average of three mice B) Functional pathways showed that the expression of 3364 genes changed when the DDC refed mice were compared with DDC refed + betaine fed mouse, using KEGG software.

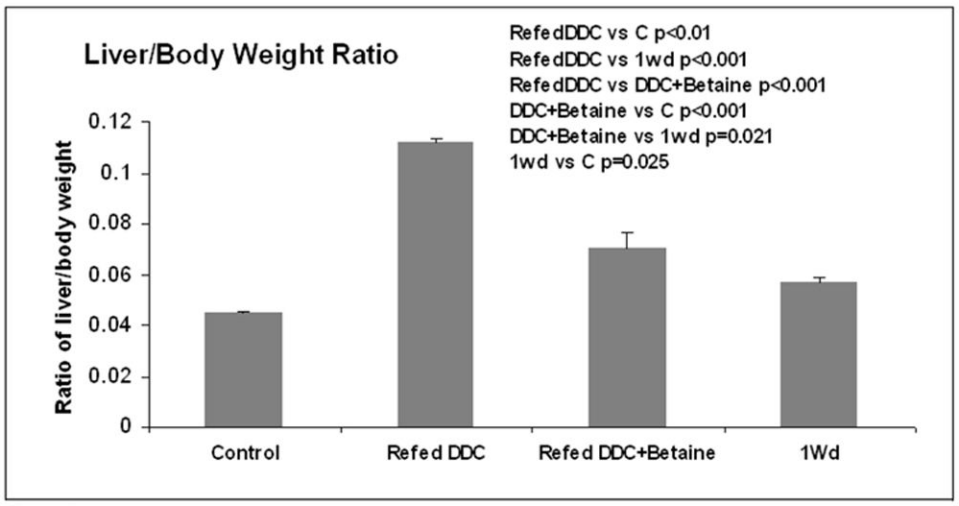

Fig 2.

Liver/body weight ratios of the 4 treatment groups of mice. (Mean±SEM, n=4). All groups are significantly different from each other ($p<0.05$).

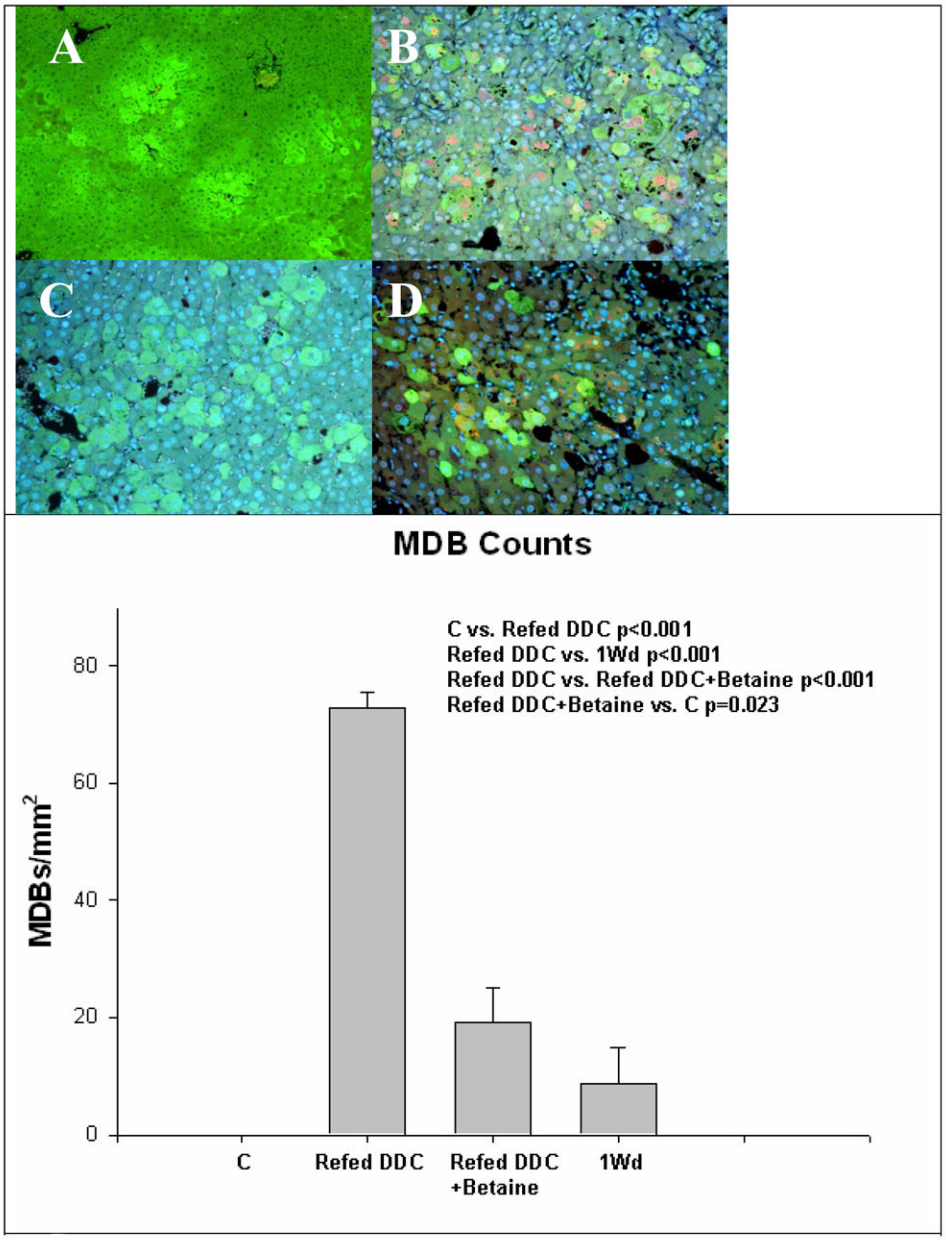

Fig 3.

A) Control, B) DDC refed, C) DDC refed + betaine, D) 1 month withdrawal (1MW). Refeeding DDC induced the formation of MDBs in hepatocytes (B). Betaine partially prevented the formation of MDBs (C). MDBs were stained yellow-red (B and D). The control shown (A) had no MDBs. MDBs were inconspicuous in the betaine treated livers (C). (Mean±SEM, n=4)

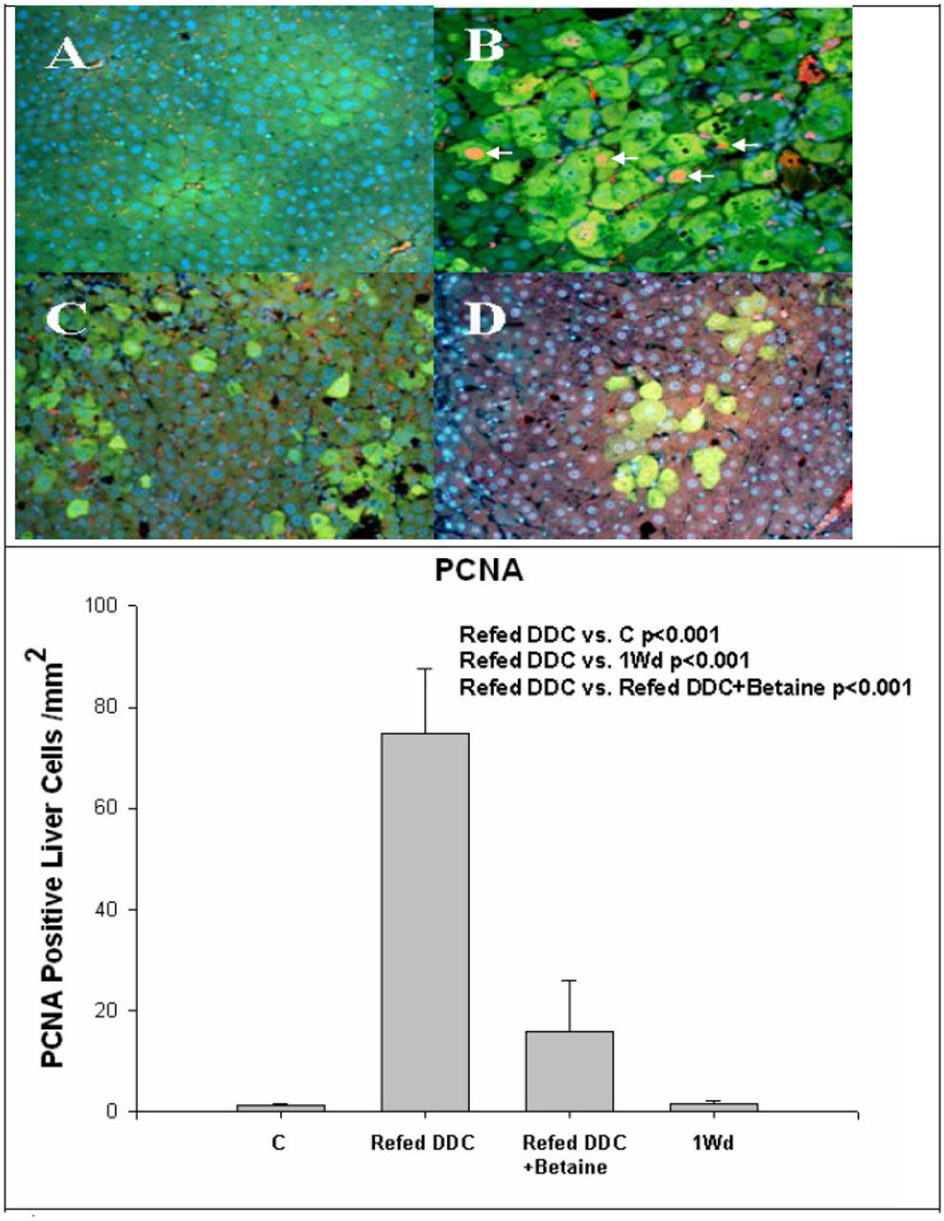

Fig 4.

A) Control, B) Refed DDC, C) Refed DDC+betaine, D) 1 month withdrawal. Refeeding DDC induced the expression of PCNA (Arrows) (B), and this was partially prevented by betaine. (Mean±SEM, n=4) FAT10 is green, PCNA is red-yellow. PCNA-FAT10 double stain x260.

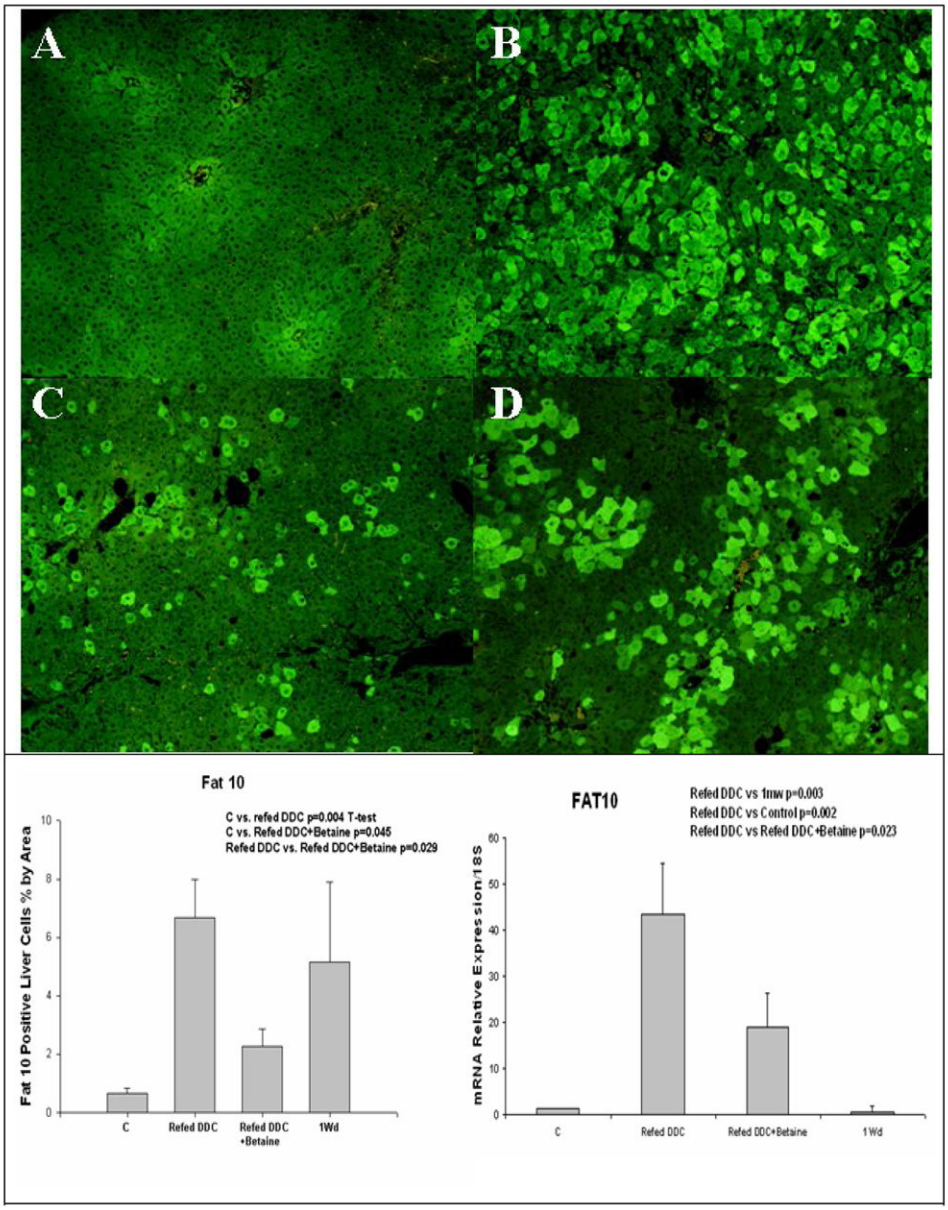

Fig 5.

A) Control, B) Refed DDC, C) Refed DDC+betaine, D) 1 month withdrawn. Refeeding DDC induces the expression of FAT10, prevented by betaine. Real-Time PCR on FAT10. Real-Time PCR confirms results obtained by immunohistochemistry, except for 1 month withdrawal. FAT10 stain x130. (Mean±SEM, n=4)

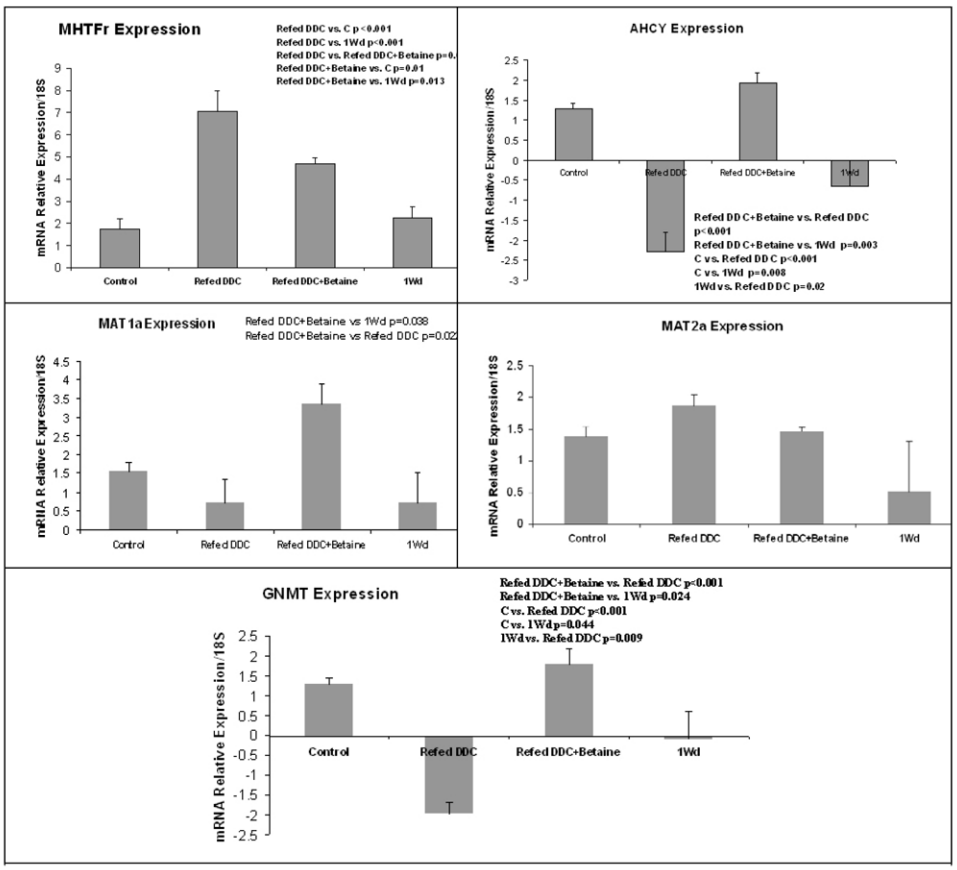

Fig 6.

The expression of enzymes involved in SAMe metabolism are quantified by Real-time PCR. The expressions are relative to the controls values. The expression of MAT1A decreased in mice refed DDC compared to the DDC one month withdrawal mice. Betaine treatment restored MAT1A expression compared to the DDC refed group (Mean±SEM, n=4).

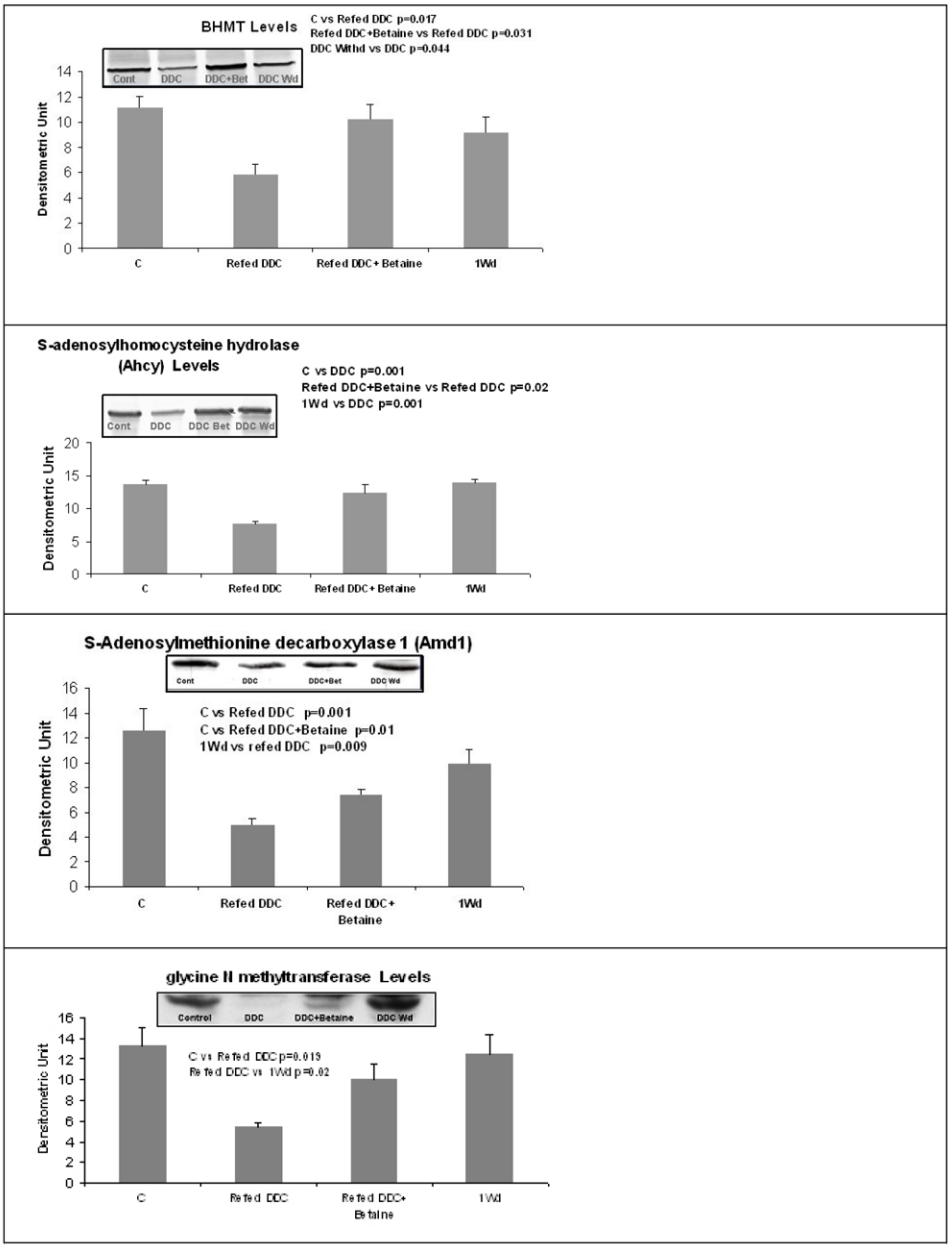

Fig 7.

Expression of BHMT was decreased by DDC refeeding as determined by Western blot. This was prevented by betaine. The same results were found for AHCY, AMD1 and GNMT. (Mean±SEM, n=4)

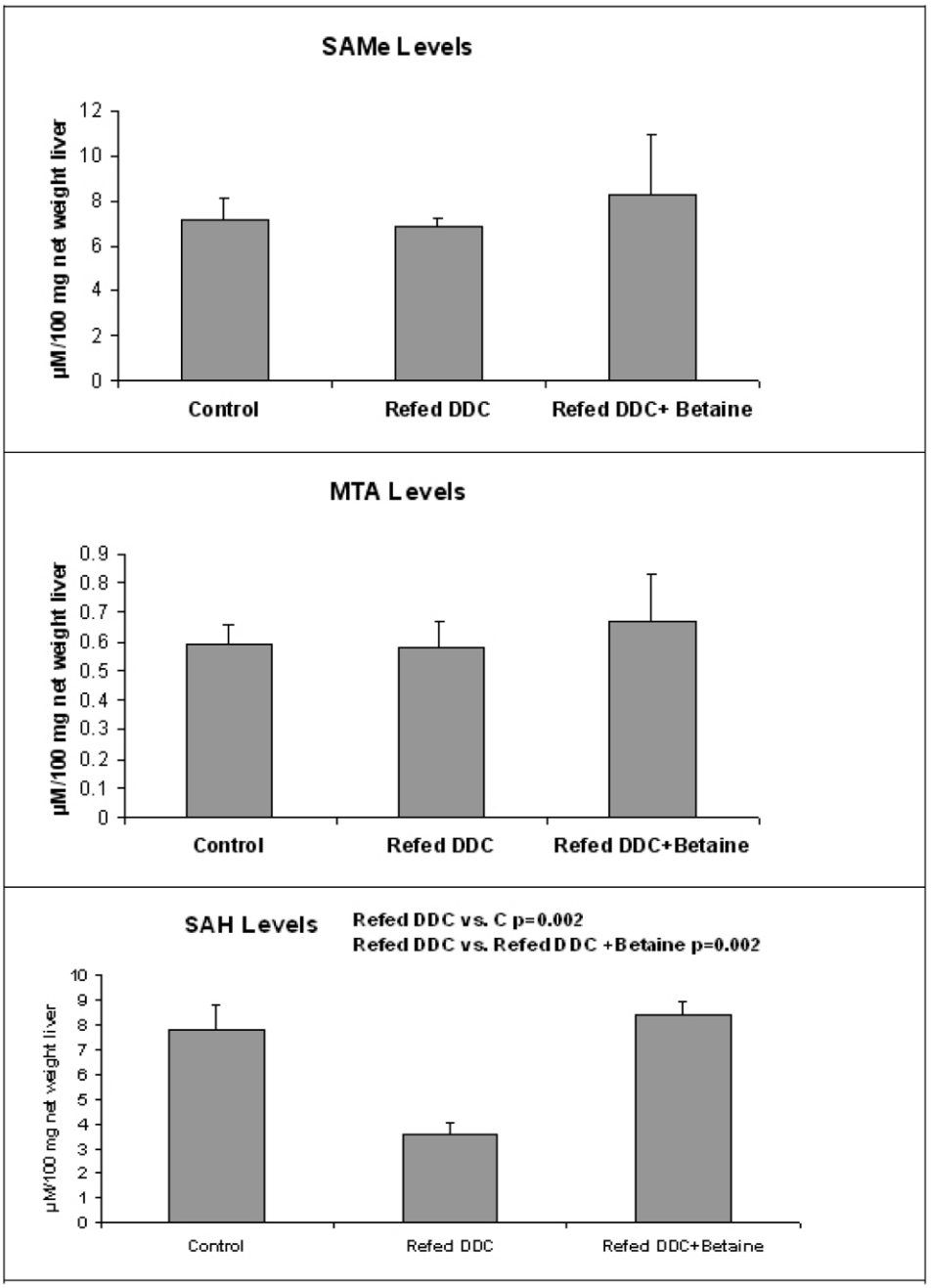

Fig 8.

SAMe and MTA levels were not changed by DDC refeeding. DDC refeeding decreased SAH levels and this was prevented by feeding betaine. SAH levels by HPLC. (Mean±SEM, n=3-4)

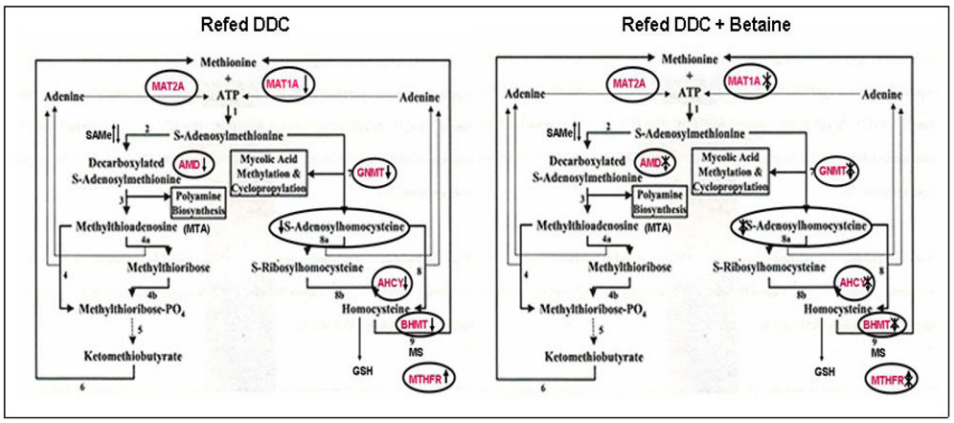

Fig 9.

DDC Refeeding decreased AMD, AHCY GNMT and MAT1A expression, and increased the expression of MTHFR. Betaine treatment prevented these changes. The arrows indicate the increase or decrease of the gene expression. The double cross indicated betaine treatment prevents the DDC effect. (Diagram modified from Berger et al. BMC Microbiology 2003 3:12)

Table 1

SAMe REVERSED DDC INDUCED EPIGENETIC CHANGES

Histone Modification	**Residue**	**DDC Refed**	**DDC refed + SAMe**
Acetylation	Histone 3 lysine 9 (AcH3K9)	↑	↑
	Histone 3 lysine 18 (AcH3K18)	↓	↓
Trimethylation	Histone 3 lysine 4 (H3K4me3)	↓	nl
	Histone 3 lysine 9 (H3K9me3)	↓	nl
Ubiquitinylation	H2A / H2B	↑	nl
Histone Modifying Enzyme	**Enzyme**	**DDC Refed**	**DDC refed + SAMe**
Histone Acetyl Transferase	GCN5	↑	nl
	Pcaf	↓	
	Cbp/p300	↓	
Histone Deacetylase	Sirtuin 1	↓	↓
	HDAC9	↑	↓
	HDAC7a	↑	nl
	HDAC11	↓	
Histone Methyltransferase	SET 7/9	↑	nl
	SUV 39H1	↓	nl
Histone Demethylase	LSD1	nl	nl