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Meloxicam Increases EGFR Expression Improving Survival following Hepatic Resection in Diet-induced Obese Mice

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Abstract

Objective—Patients with fatty liver have delayed regenerative responses, increased hepatocellular injury, and increased risk for perioperative mortality. Currently, no clinical therapy exists to prevent liver failure or improving regeneration in patients with fatty liver. Previously we demonstrated that obese mice have markedly reduced levels of EGFR in liver. Herein, we sought to identify pharmacologic agents to increase EGFR expression in order to improve hepatic regeneration in the setting of fatty liver resection.

Methods—Lean (20% calories from fat) and diet-induced obese (DIO) mice (60% calories from fat) were subjected to 70% or 80% hepatectomy.

Results—Using the BaseSpace Correlation Engine of deposited gene arrays we identified agents that increased hepatic EGFR. Meloxicam was identified as inducing EGFR expression across species. Meloxicam improved hepatic steatosis in DIO mice both grossly and histologically. Immunohistochemistry and Western blot analysis demonstrated that meloxicam pretreatment of DIO mice dramatically increased EGFR protein expression in hepatocytes. Following 70% hepatectomy, meloxicam pretreatment ameliorated liver injury and significantly accelerated mitotic rates of hepatocytes in obese mice. Recovery of liver mass was accelerated in obese mice pretreated with meloxicam (by 26% at 24 hrs and 38% at 48 hrs respectively). Following 80% hepatectomy survival was dramatically increased with meloxicam treatment.

Conclusions—Low epidermal growth factor receptor expression is a common feature of fatty liver disease. Meloxicam restores EGFR expression in steatotic hepatocytes. Meloxicam

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pretreatment may be applied to improve outcome following fatty liver resection or transplantation with steatotic graft.

TOC Statement- 20170985

A drug screen of fatty liver identified meloxicam as both increasing EGFR expression and improving recovery following resection in liver. This report identifies meloxicam may clinically improve recovery of fatty liver following resection.

Keywords

liver regeneration; fatty liver; cytokines

Introduction

Hepatic resection in patients with steatosis carries markedly increased rates of perioperative morbidity and mortality^{1,2}. Animal models have demonstrated that steatosis is associated with increased hepatocyte necroapoptosis, delayed entry into the cell cycle, as well as increased rates of cellular dysfunction and failure^{3–7}. Human studies have confirmed a delay in recovery of liver volume in patients with fatty liver[3–7]. To date no effective clinical therapies have been identified to target the abnormalities encountered in steatotic patients who require liver resection. We have recently demonstrated that both overall total body fat and degree of steatosis in both human and animal models is associated with a decrease in hepatocyte epidermal growth factor receptor (EGFR) expression⁸. Moreover, we have also demonstrated that therapeutic targeting of EGFR through gene transfer is associated with improved recovery and survival following hepatectomy in a murine model of diet-induced obesity (DIO)⁸.

Given the critical role EGFR down-regulation plays in the abnormal hepatic regenerative response in steatotic liver we postulated that pharmacologic therapies might be identified to increase EGFR expression and potentially decrease perioperative morbidity and mortality in fatty liver patients undergoing major liver resection. We therefore, undertook a drug discovery approach to find pharmacologic agents that would regulate EGFR. Using such a screen, we identify the cyclooxygenase-2 (cox-2) inhibitor meloxicam as inducing EGFR-expression in DIO mice^{9,10}. We go on to demonstrate that meloxicam pretreatment is effective in improving functional recovery and survival following extensive liver resection in a murine model of diet-induced obesity (DIO). Meloxicam is currently an FDA-approved, non-steroidal anti-inflammatory drug effective in treating osteoarthritis. Meloxicam has fewer gastrointestinal and cardiovascular side effects than previous cox-2 inhibitors[7,9–12]. These data suggest that meloxicam may be a promising agent to augment liver recovery in patients with fatty liver undergoing liver resection or other procedures that require a hepatic regenerative response.

Materials and methods

Pharmacologic agent search

In silico discovery of EGFR modulators used BaseSpace Correlation Engine (Illumina) querying for EGFR in all curated studies. Data were filtered as described in the results. Identified studies in Table 1 are from the Gene Expression Omnibus repository, including GSE49000: Liver and skeletal muscle expression profile of mice fed SRT2104¹¹, GSE8858: Liver Pharmacology and Xenobiotic Response Repertoire¹², GSE13992: Liver from c-Met null and wildtype mice treated with hepatocyte growth factor¹³, and GSE8251 Non-genotoxic Hepatocarcinogens (Iconix study)¹⁴, and from the Chemical Effects in Biological Systems (CEBS) database Study ID 004-00004-0100-000-9: Drug Matrix In Vivo Toxicogenomic Study - Rat Liver [Codelink]¹². BaseSpace data were last accessed on 07/06/2017, and the results from GSE49000 had not been reported at the time of the experiments reported here. Thus, at that time the top compound for increased EGFR expression in liver was meloxicam.

Mouse Studies and Reagents

All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Protocols for the care and use of animals were approved by the Indiana University-Purdue University Indianapolis Animal Care and Use Committee.

As previously described⁸, diet-induced obese (DIO) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Male C57BLI/6J mice were initially fed with a 60% fat diet at 5 weeks of age. Mice were ordered at 12–14 weeks of age and the 60% fat diet was continued to 16–18 weeks of age when all experiments were performed.

DIO mice were treated with 10 mg/kg intraperitoneal injections of meloxicam dissolved in DMSO 48 hours and 24 hours before PH as well as immediately after PH. Mice injected with DMSO vehicle control (control) or Meloxicam were subjected to 70% hepatectomy, 80% hepatectomy, or sham surgery, which were performed as previously described^{15–17}. Mice procedures and euthanasia was performed under general anesthesia¹⁸. At necropsy tissues were collected and weighed. Liver samples were obtained and processed in pairs by either snap frozen in liquid nitrogen or fixing in 10% neutral buffered formalin. Meloxicam, (4-Hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide), (SKU 1379401), ALT activity kit, AST activity kit, and Bilirubin kit were purchased from Sigma-Aldrich (St. Louis, MO). Meloxicam was dissolved in DMSO 15 mg/ml, diluted with Lactated Ringer's solution to 0.5 mg/ml. The meloxicam solution was made fresh prior to each use.

Immunohistochemistry

Hematoxylin and eosin (H&E) staining and periodic acid-Schiff (PAS) staining were used to evaluate fat and glycogen content respectively of liver tissue. Formalin-fixed, paraffin-embedded, dehydrated and cleared liver sections were subjected to standard procedures of immunohistochemistry¹⁹. The following primary antibodies were used for

immunohistochemistry: anti-EGFR (D38B1) XP (1:50 dilution; Cell Signaling Technology, Danvers, MA) and anti-PCNA (1:100 dilution; Sigma WH000511M2). EGFR staining was performed as described by Cell Signaling Technology EGFR antibody IHC data sheet. PCNA staining was performed using routine histological protocol. Briefly, slides were passed through xylene, graded alcohol, and rinsed in phosphate buffered saline (PBS). Liver section antigens were retrieved in sodium citrate buffer (10mM Sodium citrate, 0.05% Tween 20, pH6.0). Endogenous peroxide was inactivated using 3% hydrogen peroxide, followed by a one hour incubation at room temperature with the primary antibody, incubated in Dako EnVision+ System- HRP labeled Polymer secondary antibody (Dako North America, Inc) for 30min at room temperature. Signal was detected and developed using the Dako liquid DAB+Substrate Chromogen System (Dako North America, Inc).

Whole Slide Digital Imaging

Imaging was performed using the Aperio Whole Slide Digital Imaging System. The Aperio Scanscope CS system was used to image each slide. (360 Park Center Drive Vista, CA 92081). All slides were imaged at 20× magnification. The scan time ranged from 1.5 minutes to 2.5 minutes. The whole images were stored and housed in the Spectrum software system.

Image Analysis/Automatic Image Quantification

Images were analyzed with three different modalities. The first was histologically using an H&E stain. Each sample, four or five per slide, was scored. The scoring was ranked by the degree of fat in the liver tissue, 0.5 being very little or miniscule fat to 4 which is an extremely fatty liver. The second method was using the Aperio Imagescope. This uses a computer-assisted morphometric analysis of digital images. Software capabilities were used to quantitate the degree of Epidermal Growth Factor Receptor (EGFR) staining in each liver sample. Using the Aperio software, an area was selected on each sample encompassing the most positively stained areas of EFGR. The selected areas were roughly the same size and were evaluated using a standard FDA positive pixel algorithm (hue value: 0.1, Hue width: 0.5, and color saturation threshold:0.04). The third method was counting the number of PCNA positive staining cell nuclei and the number of total nuclei in a standardized area using light microscopy.

EchoMRI

Fresh liver tissue was scanned using an EchoMRI Body Composition Analyzer (EchoMRI, LLC) to measure liver samples fat percentage and mass ¹⁷.

Western blot analysis

For analysis of whole tissue lysates, snap-frozen liver pieces were homogenized in modified RIPA buffer. Protein was quantified using the Pierce BCA protein assay kit (Thermo scientific, Rockford, IL). Liver homogenates were separated by polyacrylamide gel (Bio-Rad) electrophoresis under reducing conditions. Proteins from the gels were electrophoretically transferred to Nitrocellulose membranes (Bio-Rad). Immunoblotting was performed using the following antibodies: phosphor-EGFR, EGFR XP, phosphor-eIF2a,

eIF2a, phosphor-Erk1/2, Erk1/2, phospho-eIF2a, GAPDH (Cell Signaling Technology, Danvers, MA); COX2 (Santa Cruz Biotechnology, Santa Cruz, CA), and PCNA (Sigma WH0005111M2). Antibody binding was detected following appropriate secondary antibody methods using SuperSignal West Femto (Thermo scientific) or Odyssey CLx western blot detection system (Li-Cor, Lincoln, NE).

ALT activity, AST activity, and Bilirubin Assay

ALT activity, AST activity, and bilirubin assays were performed and calculated according to the manufacturer's instructions (Sigma-Aldrich). The mice were euthanized following 48-hour meloxicam treatment. Blood samples were taken from the heart using cardiac puncture then centrifuged at 3500 rpm for 15 min at room temperature. After collecting the serum, the samples were immediately stored at -80°C .

Statistical Analysis

The results are presented as the mean $\pm$ standard error for each group. Data were analyzed for significance using Student *t* test. For compare multiple means, the one-way ANOVA test was used (Prism software). The log rank test was applied to compare survival curves. Differences between groups were considered significant if **p* 0.05; ***p*<0.01; ****p*<0.001.

Results

Discovery of drugs to increase EGFR expression

In order to discover compounds that we could deliver to mice to increase EGFR expression, we queried gene expression profiles of 21,196 publicly available, curated datasets. Querying for EGFR, liver and RNA expression produced 812 studies and 3,898 biosets (within-study pairwise comparisons of treated and reference groups). Of these, 1,465 showed an increase in EGFR expression >1.2 fold (Supplemental Table 1). Restricting the top 100 studies to only those using wild-type whole organisms and treatment versus vehicle/untreated design yielded 10 studies (Table 1). The top study, GSE49000, demonstrated >20-fold increased EGFR with either caloric restriction over 13 weeks or chronic administration of the SIRT1 activator SRT2104 and normal diet. In acute compound administration studies, five days of meloxicam or torsemide treatment of rats or 2 hours of hepatocyte growth factor (HGF) treatment in mice all showed increased expression of EGFR. We sought to apply acute administration of an FDA-approved drug and thus chose meloxicam for further study.

Meloxicam increases EGFR expression in mice with fatty liver

Following identification of meloxicam as inducing EGFR in gene array studies, we next investigated the ability of meloxicam therapy to increase EGFR in the DIO model of fatty liver. An in vivo dose response of meloxicam was completed and the smallest observed dose to induce EGFR expression was identified as 10 mg/ml (supplemental Figure 1A). We also examined if two doses of meloxicam increased EGFR for a longer period. More prolonged induction of EGFR was observed with multiple meloxicam doses, thus for subsequent surgical studies 2 pre-resective doses were used (supplemental Figure 1B).

DIO mice were next treated with meloxicam 10mg/kg in DMSO at t=0 and 24 hrs and sacrificed at 48 hrs. Meloxicam treatment increased hepatic EGFR expression. IHC microrgraphs of liver tissue collected after treatment with DMSO control versus meloxicam were stained for presence of EGFR. An approximate two-fold increase in EGFR expression (2.04 fold, $p<0.05$) after meloxicam treatment was observed (Figure 1 A, B). Furthermore, Western blot analysis showed a significant increased EGFR, p-EGFR expression, and increased PCNA expression of 3.7, 3.3, and 1.8-fold respectively. COX2 expression was increased 1.8-fold as well (Figure 1 C, D)

Meloxicam reduces hepatic lipid content, but has no effect on organ mass and does not cause liver injury

Samples collected after treatment with DMSO (control) or meloxicam were further examined. Meloxicam treatment decreased fatty vacuolar change in DIO mice utilizing hematoxylin and eosin (H&E) stain (Figure 2A). PAS staining also showed a dramatic decrease in glycogen stores in DIO mice treated with meloxicam (Figure 2 B). Liver fat mass was evaluated with EchoMRI. A 17% reduction in hepatic fat content after meloxicam treatment without change in overall liver mass was observed suggesting a decrease in hepatic steatosis (Figure 2 C, D). Examination of other organs demonstrated that the administration of meloxicam did not have an effect on heart, spleen, fat tissue, nor muscle mass (Figure 2E). To determine whether meloxicam induced liver injury, we evaluated serum levels of AST, ALT, and bilirubin. There was no significant change in serum levels observed 48 hrs after first meloxicam administration (Figure 2F).

Meloxicam accelerates liver regeneration after 70% hepatectomy in DIO mice

14–16 week old DIO mice were treated with 10 mg/kg intraperitoneal injections of meloxicam dissolved in DMSO 48 hours and 24 hours before PH. Liver samples were taken 24 and 48 hours after 70% hepatectomy in DIO mice treated with either DMSO or meloxicam to evaluate whether meloxicam would aid fatty liver in recovery after partial hepatectomy. Previously we have reported that although delayed, we observe approximately a 100% survival rate in DIO mice undergoing 70% hepatectomy⁸. Compared to DIO control mice, meloxicam dramatically decreased liver intrahepatic hemorrhage, and regions of bilious discoloration, particularly at 48 hours after surgery (Figure 3A). Meloxicam did not increase liver size alone (Figure 2D and 3A), but significantly accelerated hepatic regeneration as measured by return of mass by 26% and 38% at 24 hrs and 48 hrs after 70% hepatectomy in DIO mice (Figure 3B). H&E staining showed a decrease in vacuolar changes in meloxicam treated mice at both 24 and 48 hours after hepatectomy, confirmed by histology score (Figure 4A). EGFR staining demonstrated significant increase in expression in meloxicam treated mice at 24 hours, and mildly increased expression at 48 hours (Figure 4B). PCNA staining was increased as well by meloxicam administration at both 24 and 48 hours (Figure 4C).

Effect of meloxicam on mortality after partial hepatectomy

As hepatic regeneration following 70% hepatectomy is associated with excellent survival in DIO mice, we examined the ability of meloxicam to improve survival after 80% hepatectomy, a model of high mortality rates in DIO mice. 14–16 week old DIO mice were

treated with intraperitoneal injection of 15 mg/kg meloxicam 48 hours and 24 hours prior to surgery and then subjected to extended, 80% PH. Survival after 80% hepatectomy was charted with a Kaplan-Meier plot. A dramatically improved survival was observed in DIO mice pretreated with meloxicam. Survival at 150 hours was shown to increase from 20% to 40% after pretreatment with meloxicam (Figure 5).

Discussion

Steatosis and obesity are significantly increasing problems in both the Western and developing world. Patients with steatosis and obesity requiring liver resection or transplant for either primary or metastatic liver tumors demonstrate delayed regeneration and a substantially increased risk for morbidity and mortality^{20, 21}. While the EGFR pathway is not essential in lean animals, when lost there is impairment of liver regeneration and increased necroapoptosis^{22–24}. Previous work from our group demonstrated a down-regulation of EGFR in the setting of obesity and steatosis as a contributory mechanism to this delayed regenerative response and increased mortality observed in fatty liver undergoing resection⁸. Previously we also demonstrated that correction of the decreased EGFR expression through plasmid hydrodynamic injection in mice improves both the delayed regenerative response and decreased survival observed in models of regenerating fatty liver and obesity. Herein, we undertook a drug discovery approach to identify in vivo pharmacologic therapies that might be used clinically to correct the abnormal regenerative response of fatty liver. Our search for potential pharmacologic agents identified the cyclooxygenase (cox-2) inhibitor, meloxicam, as a potent in vivo inducer of EGFR in liver. Given that meloxicam is already a Food and Drug Administration (FDA) approved anti-inflammatory agent we sought to focus on its potential to improve the hepatic regenerative response in the setting of obesity and steatosis.

Using an in vivo model of murine diet-induced obesity (DIO) and steatosis we observed that meloxicam therapy was associated with a dramatic reduction of steatosis and induction of hepatocyte EGFR, similar to what we have observed following hydrodynamic EGFR transfection⁸. Histologically liver samples demonstrated an improved histology score for degree of steatosis after meloxicam treatment and decreased fat as measured by echo MRI evaluation. Meloxicam effects occurred without influencing of the overall organ mass, injuring hepatocytes or worsening liver function. Pretreatment of DIO mice with meloxicam induced an accelerated hepatic regenerative response following 70% hepatectomy, and a dramatic improvement in survival after extended (80%) hepatectomy. These observations allow us to conclude that meloxicam improves the dysregulated and delayed regenerative response in fatty liver at least partially through the EGFR pathway.

A number of authors to date have focused on the inhibitory effects of meloxicam on cox-2 expression in fibrotic liver disease. These studies have demonstrated a beneficial effect in regenerating fibrotic and cirrhotic livers. Cox-2 is, in part, responsible for progression to end stage liver disease. In rat models of chronic liver injury and fibrosis meloxicam has been observed to ameliorate ischemia reperfusion injury of the liver and small bowel after liver resection^{25–27}. Furthermore, it has been observed to protect livers from aluminum-overload

²⁸, protect against liver fibrosis ²⁹, carbon tetrachloride exposure and oxidative injury ^{9, 26} in rat models.

In human subjects meloxicam has also been observed to reduce fibrosis in newborn patients prior to Kasai portoenterostomy ³⁰. A single dose of meloxicam has also been observed to improve the regenerative response in adult patients with fibrotic liver undergoing resection ²⁵. Other data has suggested that meloxicam does not increase and may, in fact, reduce proliferation of hepatocellular cancer or cholangiocarcinoma ^{31 32}. There is conflicting data however if prolonged meloxicam therapy may cause intestinal, gastric and hepatic toxicity ^{33, 34}.

The interactions of cox-2 regulation with EGFR and other ERB-2 family members has been proposed primarily in intestinal conditions such a familial adenomatous poliposis and ovarian cancer ³⁵. Studies in enterocytes have implicated EGFR as potentially upstream of COX-2 in models of lipopolysaccharide (LPS) induced necrotizing enterocylsis ³⁶. Given a potential early role for LPS in liver regeneration future studies are indicated to better understand the signaling interaction of EGFR and cox-2. Further studies are also needed to determine if meloxicam, in addition to decreasing hepatic steatosis, decreasing cox-2 function and increasing EGFR expression may also work through other yet to be defined signaling pathways.

The data presented herein, suggest that short-term meloxicam may be used to reduce steatosis of the liver and enhance regenerative capacity in patients with fatty liver. The mechanism for reversal of fatty liver changes likely is, in part, mediated by the upregulation of EGFR but may also be due to effects on cox-2. These results gives further insight into pathogenesis of steatosis and implies that meloxicam, and potentially other methods of restoring EGFR, may be advantageous in instances when liver resection or transplantation is needed in the setting of steatosis and obesity. Specifically, these data in addition to an already impressive number of beneficial studies in both animal models and humans support the initiation of clinical trials to determine the potential peri-operative benefit of meloxicam for obese patients undergoing major liver resection. These data also suggest investigating meloxicam as a potential application of fatty livers for transplantation.

In conclusion, we have used a drug discovery approach to identify a potential in vivo therapy to improve recovery following liver resection in the setting of fatty liver. We identify that meloxicam, a cox-2 inhibitor, improves outcomes following extreme liver resection associated with the restoration of hepatic EGFR expression. This data supports existing clinical data that has suggested its benefit in acute liver injury and explains the likely mechanism through which it impacts the hepatic regenerative response. Further investigation is warranted to evaluate drug and other potential EGFR enhancing compounds as adjuncts to improve outcomes after hepatectomy in the setting of fatty liver.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of Non-Standard Abbreviations

DIO diet-induced obese

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Figure 1.

Meloxicam increases EGFR expression in diet-induced obese mice (a) Representative immunohistochemistry of meloxicam and control (DMSO carrier only) treated diet-induced obese mouse liver demonstrates increased EGFR membrane staining in meloxicam treated livers. (b) Quantification of representative sections ($n > 5$ per group, repeated at least twice) demonstrate a two-fold increased staining for EGFR in meloxicam treated liver. (c) Western blot analysis demonstrates increased expression of phosphorylated-EGFR (p-EGFR), EGFR, PCNA, and COX-2 with meloxicam treatment in fatty liver. (d) Quantification of Western blots demonstrating Meloxicam was associated with fold increases 3.67 for phosphorylated-

EGFR, 8.36 for EGFR, 1.82 for PCNA, and 1.77 for cox-2 respectively. Representative Western blot of n>5 per group, repeated at least twice, * p<0.05, ** p<0.01, and *** p<.001.

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Figure 2.

Meloxicam decreases hepatic lipid content, has no effect on organ mass and does not cause liver injury. Mice euthanized on day2 after Meloxicam 10mg/kg 2 doses administration. (A) H&E stained sections of diet-induced obese mouse liver demonstrates decreased fatty vacuolar changes in mice treated with meloxicam versus DMSO control. (B) PAS staining shows decrease in glycogen stores following meloxicam treatment. Quantification of changes in diet-induced obese liver : (C) Echo MRI quantification of liver fat. Liver fat content decreased by 13%, while the overall liver weight remained unchanged (D). (E) The mass of other organs remained unchanged as well after treatment with meloxicam. (F) Serum AST, ALT, and total bilirubin were unchanged by treatment with meloxicam. N>5 mice per point, repeated at least twice, * p<0.05, ** p<0.01, and *** p<0.001. Abbreviations used: GSN=gastrocnemius, Tibi=tibialis, Quad=quadracept.

Figure 3.

Meloxicam treatment improves recovery following 70% hepatectomy. (A) Gross examination of liver 24 or 48 hrs after 70% PH shows decreased bilious discoloration and hemorrhage, as well as improved recovery of liver mass with meloxicam treatment. Representative images at necropsy demonstrating increased yellow discoloration of diet-induce obese control liver at 48 hrs following hepatectomy. (B) Liver mass as a proportion of body weight shows 26% increase at 24 hours and 38% increase at 48 hours in the meloxicam treatment group compared to the DMSO-control group.

Figure 4.

Meloxicam decreases fat content, increases EGFR and PCNA expression after partial hepatectomy. (A) Treatment with meloxicam demonstrated decreased vacuolar changes, intrahepatic hemorrhage and regions of bilious staining. Histologic scoring for liver injury demonstrated meloxicam treatment was associated with a 53% reduction of injury score at 24 hours and 29% reduction of injury score at 48 hours. (B) Representative immunohistochemistry demonstrating meloxicam treatment was associated with a persistent increase in EGFR expression after partial hepatectomy. Quantification of EGFR expression demonstrates a 3.65 fold increase of EGFR at 24 hours with equivalent expression at 48 hours. (C) Meloxicam therapy is associated with increased PCNA expression at both 24 and 48 hours after hepatectomy. Representative immunohistochemistry section demonstrating increased PCNA expression following meloxicam treatment. Data based on N>5 mice per timepoint. Representative results of at least two separate experiments. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

* : $p < 0.05$, Log-rank test and Gehan-Breslow-Wilcoxon Test

Figure 5.

Meloxicam therapy improves survival in mice with fatty liver undergoing extended 80% hepatectomy. Kaplan-Meier plot to moribund after 80% hepatectomy for DIO mice by Meloxicam 48 hours treatment before surgery. Meloxicam treated mice rate of survival dramatically increased from 20% to 50% at 168hrs. (* $p < 0.05$).

Table 1

Top agents by gene array associated with induction of hepatic EGFR.

Geneset ID	Species	Experiment	P-value	N	Fold Change
GSE49000	Mouse	Caloric restricted diet for 13 wks vs control	1.00E-09	5	23
GSE49000	Mouse	SRT2104 100mg/kg in standard diet 13 wks vs untreated	5.50E-05	5	20.8
*CEBS	Rat	MELOXICAM 6mg/kg 5 days vs vehicle	8.30E-12	3	8.27
GSE8858	Rat	MELOXICAM 0.6mg/kg 5 day vs vehicle	3.60E-09	3	7.27
*CEBS	Rat	MELOXICAM at 33 mg/kg 5 day vs vehicle	4.60E-10	3	5.79
*CEBS	Rat	TORSEMIDE 110 mg/kg 5 days vs vehicle	1.01E-02	3	5.72
GSE8858	Rat	TORSEMIDE 100 mg/kg 5 days vs vehicle	1.00E-02	3	5.11
GSE8858	Rat	MELOXICAM 33 mg/kg 5 days vs vehicle	7.80E-08	3	5.07
GSE13992	Mouse	HGF 2ug per mouse for 2h vs untreated	0.0141	3	5.07
GSE8251	Rat	MELOXICAM for 5d vs vehicle	7.60E-08		4.94

* CEBS 004-00004-0100-000-9