Mesenchymal Stem Cell Microvesicles Assist in Recovery from Acetaminophen-Induced Liver Injury

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Abstract

Acetaminophen, found in Tylenol, a common household pain and fever reducer, can cause liver damage when more than the recommended dose is taken. At the turn of the millennia, 48% of acute liver failure cases were attributed to accidental overdoses of this drug. Mesenchymal stem cells (MSC), a stem cell in compact bone capable of changing into many cell types, is being researched as an alternative to induced pluripotent stem cells and embryonic stem cells in regenerative medicine. Cell-therapies are problematic since they elicit an immune response in transplant recipients, have limited survival, and potentially transform into tumors. Microvesicles (MV), small particles released by cells as a form of communication, influence the gene and protein expression of cells that internalize them. MV provide a cell-free alternative for tissue restoration as they avoid many stem cell therapy related issues. When using murine-derived MSC MV and human-derived MSC MV to treat APAP injury, we have found that Cdkn1a and Cdc14b, regulatory genes in cell proliferation, began to normalize 48 hours post APAP administration suggesting an increase in cell proliferation. We hypothesize that MSC MV may facilitate liver recovery from acetaminophen overdose.

Introduction

Acetaminophen (APAP), found in Tylenol, a common household pain and fever reducer, can cause liver damage when more than the recommended dose is taken. At the turn of the millennia, 48% of acute liver failure cases were attributed to accidental overdoses of this drug.[9] A safe dosage does not exceed 7.5-10 g in 8 hours for those 12 years of age or older, and 90% of the molecule is then metabolized to sulfate and glucuronide conjugates which are excreted in urine.[21] Of the 10% that remains half is excreted unmetabolized. The remaining 5% is metabolized by the hepatic cytochrome P450 mixed function oxidase pathway to N-acetyl-p-benzoquinoneimine (NAPQI), a hepatotoxic metabolite. This molecule under normal conditions is neutralized by irreversible hepatic glutathione (GSH) conjugation followed by excretion in the urine. However, in the case of GSH saturation the metabolite covalently binds to lipids and proteins causing cellular damage.

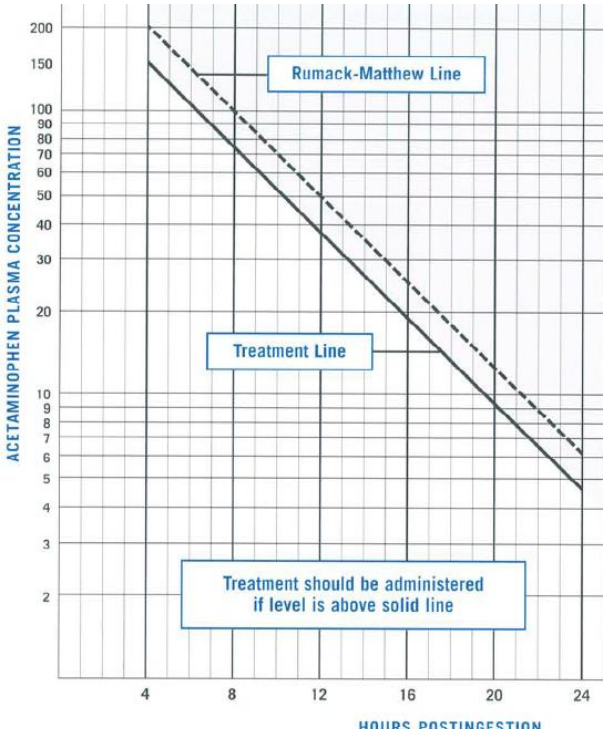

Figure 1. Single acute overdose nomogram: It plots concentration of APAP in blood plasma versus time after ingestion in order to estimate when there is risk of hepatotoxicity.[11] Treatment line is adapted from Rumack and Matthew.[16]

The standard treatment for the past 40 years has been to administer specific doses NAC either orally or, more recently, intravenously if the APAP levels in the patient's blood are above a specific point on the Rumack and Matthew nomogram (Figure 1).[8] NAC is extremely efficient when given to the patient within 8 hours of overdose despite plasma concentrations of APAP as high as 500 mg/mL at 4 hours.[18] On the other hand, treatment implemented after eight hours does

not prevent hepatotoxicity as effectively (Figure 2). In the case where the liver does fail, the only treatment then is a liver transplant. Even the use of activated charcoal, a method used to eliminate unbound toxins prior to absorption, does not prove to be effective in the instances where NAC fails to cure toxicity.[11] Therefore, patients who are not brought into the hospital within this short window of opportunity, eight hours, run a higher risk of liver failure and death.

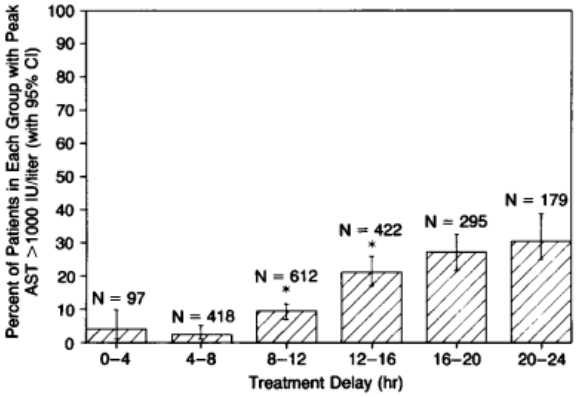

Figure 2. Delayed NAC treatment for patients with APAP plasma levels above treatment line and corresponding hepatotoxicity: According to interval between APAP ingestion and NAC treatment patients are grouped into four-hour increments. AST is aspartate aminotransferase, an indicator of liver damage.[18]

A study done by Smilkstein's group looked at 11,195 cases of suspected APAP overdose from 1976 and 1985. From the initial group 2,540 patients were treated with oral NAC.[18] Of the high risk patients (APAP levels of 500 mg/mL or higher) treated more than 16 hours after ingestion, 41% developed hepatotoxicity, which was an improvement over cases with no treatment administered leading to 58% with hepatotoxicity.[13] However, NAC has its drawbacks; due to its putrid odor and terrible taste, patient compliance can be difficult to obtain, which further delays the oral treatment that is very time sensitive.[8] In addition, the new premade intravenous solution has a fixed concentration of NAC in 200 mL so overdosing is more likely to occur since the 200 mL volume always contains the same. However, the treatment is supposed to be tailored to each patient's body weight.[11] Lastly, depending on a history of asthma or hypersensivity, rate of NAC infusion, and if NAC is given to a patient whose APAP levels are below the nomogram treatment line, then an anaphylactoid reaction can occur resulting in symptoms such as a rash, vomiting, or bronchospasm.[8]

NAC acts as an antidote by supplying biomaterial for cells to produce more GSH that binds to and clears out NAPQI safely from the body.[18] S-adenosyl-l-methionine (SAMe) is a molecule naturally in the body that is being studied as an alternative to NAC treatments for hepatotoxicity.[3] It is a methyl donor for polyamine synthesis, which scientists are hypothesizing provides protection by maintaining certain antioxidant enzymes. However, although SAMe shows some positive results so far, it does not outperform NAC. For these various reasons an alternative approach or a treatment that can be coupled with NAC to augment the recovery needs to be found.

Recently MSC MV have been shown to increase the survival rate in a lethal model of acute kidney injury in SCID mice.[5] MV are composed of lipid bilayers with transmembrane proteins throughout and envelope cytosol with various transcriptional elements and proteins from the donor cell.[19] MV are released by various cell types which then elicit specific responses from adjacent and distant cells.[1, 14] Materials transported by MV can influence the behavior of nearby cells in one of four ways (Figure 3). Hematopoietic stem cells that received MV isolated from embryonic stem cells displayed up-regulation of early pluripotent and early hematopoietic stem cells markers.[15] The exact factors transported that assisted recovery in previous experiments have not been determined yet. However, positive results showing the capacity for MSC MV to reprogram cells and help in mouse recovery models justify the use of MV in the setting of liver injury.

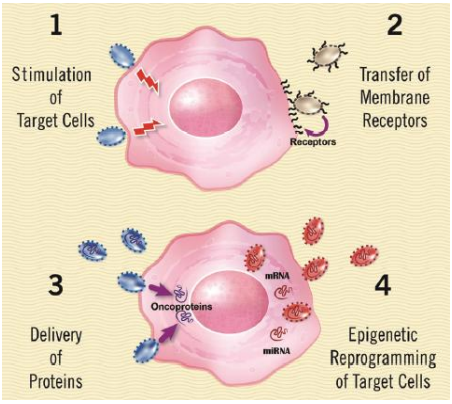

Figure 3. Diagram of MV-mediated intercellular communication: Signal by 1) stimulating target cell's surface expressed receptors, 2) depositing receptors from cell of origin onto target cell's membrane, 3) releasing transcription factors, oncogene products, and other proteins into target cell, or 4) shuttling mRNA and miRNA into target cell that produce epigenetic changes.[4]

The use of MV avoids the obstacle provided by

autologous stem cell therapy in which stem cells can unpredictably differentiate into the wrong type of cell or transform into a tumor after transplantation.[2, 20] Furthermore, Herrera's work suggests that liver regeneration is triggered by paracrine factors, and other labs have documented that MV contain ribonuclearproteins which direct intracellular traffic of RNA and other transcription factors.[6] MV have been shown to regulate function and repair damage in the lethal injury liver model of lipopolysaccharide and D-galactosamine in mice as well.[7] Thus, in light of NAC's method of protecting the liver by providing the cysteine required to restore the GSH levels,[10] it could be possible for MV to be injected in conjunction with NAC treatment in order to boost the therapeutic effects as MV could potentially increase the liver's ability to regenerate itself. This may allow for more effective treatment APAP poisoning.

Materials and Methods

Experimental Animals

All studies were approved by the Institutional Animal Care and Use Committee at Rhode Island Hospital. Six- to eight-week-old male C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME, USA) were used. Mice were acclimated one week prior to the experiment and fed ad libitum. Animals were housed 5 per cage and maintained on a 12 hour light/dark cycle. Mice were sacrificed by CO_2 followed by cervical dislocation.

Acetaminophen Injury

Group	Time (T) (hrs) 0	T4	T24	T28	T48
0-1	PBS	Takedown (TD)	–	–	–
0-2	APAP	TD	–	–	–
1	PBS	SHAM	–	–	–
2	PBS	MV	TD	–	–
3	APAP	SHAM	TD	–	–
4	APAP	MV	TD	–	–
5	PBS	SHAM	–	SHAM	TD
6	PBS	MV	–	MV	TD
7	APAP	SHAM	–	SHAM	TD
8	APAP	MV	–	MV	TD

Table 1. Group classifications by time based injections of control or experimental treatment.

Mice were fasted overnight, and food was returned to the mice 4 hrs after APAP administration. Each experimental group has five animals. Half of the mice (30) received intraperitoneal injections of 500 mg/kg, a lethal dosage, of APAP dissolved in warm PBS and 0.8% ethanol at Time (T) 0. The other 30 received PBS of equal volume. Group 0-1 and 0-2 were euthanized 4-5 hours after APAP administration. Blood, 2 mg liver sections for RNA, and liver for histology were collected. Group 2, 4, 6, and 8 received a 20μL intravenous (IV) injection of 85 μg murine-derived MV (musMV) in PBS 4 hours after APAP administration. Group 1, 3, 5, and 6 received a 20 μL IV injection of MV-free media dissolved in PBS (SHAM) of equivalent volume. At 24 hours Group 1, 2, 3, and 4 were euthanized and blood and livers were harvested. Group 6 and 8 received another dosage of MV treatment, and Group 5 and 7 SHAM injections 28 hours post APAP administration. At 48 hours mice were euthanized and

blood and livers were harvested for analysis. Experiment was repeated with 15 μg human-derived microvesicles (huMV).

In 72-hour experiment, APAP was not dissolved in ethanol and microvesicles used were isolated without a 10,000 g ultracentrifugation (See below). 25 μg musMV and 25 μg huMV were used per mouse. Group breakdown was equivalent to Table 1, but MV were administered only once 4 hours post APAP administration and animals were sacrificed 72 hours post APAP administration.

Mesenchymal Stem Cell Harvest and Preparation

Whole bone marrow was obtained by flushing the femurs and tibias of mice with sterile 1X phosphate buffered solution (PBS) supplemented with 5% heat inactivated fetal bovine serum (HIFCS) and 1% Penicillin-Streptomycin (PS) (Invitrogen). The cell suspension was strained through a 40-μm filter to remove large particles. Cells were centrifuged at 1300 rpm for 10 minutes, 4°C, and resuspended in fresh PBS/5% HIFCS/1%PS. Bones were cut into chips using a scalpel, and fragments were seeded to allow for MSC to emerge from the compact bone.[22] Bone marrow-derived MSC ($1.5X10^6$) were cultured in DMEMglutamax without L-glutamine and ribonucleotides but supplemented with 15% FBS, and 1% PS (final concentration, 50 ng/ml) (Invitrogen), and compact bone-derived MSC were cultured in DMEMglutamax plus L-glutamine and ribonucleotides and supplemented with 15% FBS, and 1% PS. Cells were counted and percent viability determined using Trypan Blue stain (Gibco). Cells were passaged 4-5 before lineage depletion.

For human MSC, cells were purchased from Texas A&M Institute for Regenerative Medicine and passaged 2-3 times before harvesting the MV in procedure described below.

Immunodepletion

One Dynabeads M-450 sheep anti-rat IgG (Dynal; Lake Success, NY, USA) per cell wase used (0.5ug antibody per 10^6 MSC). Cells were incubated with antibody cocktail containing CD11b, CD34, and CD45 for 15 minutes on ice. Then cells were incubated with the Dynabeads for 20 minutes at 4°C while gently rocked. Incubated cells were washed using Dynal magnetic particle concentrator to harvest immunodepleted cells. They are replated in depleted fetal bovine serum (FBS) medium.

Isolation of Microvesicles

One week after immunodepletion of MSC, media was collected. MV for the 48-hour experiment was isolated using differential centrifugation of 350g, 10,000g, and 100,000g. For the 72-hour experiment differential centrifugation of 350g and 100,000g was performed (the 10,000g step was omitted). MV-free media was collected and stored for use as the SHAM control by collecting supernatant of 100,000g centrifugation. Both were stored in 1% DMSO at -20°C. Total MV concentration was determined by a bicinchoninic acid (BCA) assay and Nanodrop spectrophotometer.

ALT-AST Analysis

Blood was collected by cardiac puncture, allowed to coagulate at room temperature, centrifuged, and the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured in the serum (Rhode Island Hospital Chemistry Labs, Providence, RI, USA).

Liver Histology

After a portion was placed in RNAlater (Invitrogen) for PCR analysis, the liver was preserved in formalin for histological sections.

qPCR Analysis

Liver RNA was isolated using the RNeasy Midi Kit (Qiagen, Valencia, CA, USA). RNA quantity and quality was measured using a Nanodrop ND/1000 spectrophotometer (Thermo Scientific, Foster City, CA, USA). All qPCR equipment and reagents were purchased from Applied Biosystems. Complementary DNA (cDNA) was produced from RNA via the High Capacity cDNA Transcription Kit. Reactions were performed using a 9800 Fast Thermal Cycler; one 10-minute cycle, 25°C; one 120-minute cycle, 37°C; one 5-minute cycle, 85°C. Gene expression was analyzed using the 7900HT Fast Real-Time PCR System. qPCR reactions included 20X assay mix, 2X TaqMan PCR Master Mix, and cDNA (.25-1X); one 10-minute cycle, 95°C; forty 15-second cycles, 95°C; one 1-minute cycle, 60°C. Protocol used for Fluorescent PCR Array Cards (SABiosciences) was carried out according to manufacturer's established guidelines. Genes included in the card were Abcb1a (Mm00440761_m1), Abcc2 (Mm00496899_m1), Abcc3 (Mm00551550_m1), Avpr1a (Mm00444092_m1), B2m (Mm00437762_m1), Car3 (Mm01281795_m1), Casp3 (Mm01195085_m1), Cd36 (Mm01135198_m1), Cd68 (Mm03047340_m1), Cdc14b (Mm00553630_m1), 18S (Hs99999901_s1), Cdkn1a (Mm04205640_g1), Cyp1a1 (Mm00487218_m1), Cyp1a2 (Mm00487224_m1), Gclc (Mm00802655_m1), Gsr (Mm00439154_m1), Hmox1 (Mm00516005_m1), L2hgdh (Mm00778242_s1), Mcm10 (Mm00712529_m1), Nqo1 (Mm01253561_m1), Ppara (Mm00440939_m1), Psme3 (Mm00839833_m1), Serpine1 (Mm00435860_m1), and Thrsp (Mm01273967_m1).

RNA from mice receiving APAP or PBS only (taken down at T4) was analyzed via the array cards. Six hepatotoxicity genes displaying the greatest change in expression on the array

cards were used as the focus for analysis of liver RNA in the 48-hour and 72-hour experiments. In 96-well plates, the following genes were analyzed Alb (Mm00802090_m1), B2m (Mm00437762_m1), Cdc14b (Mm00553630_m1), Cdkn1a (Mm04205640_g1), Cyp1a1 (Mm00487218_m1), Nqo1 (Mm01253561_m1), Ppara (Mm00440939_m1), and Thrsp (Mm01273967_m1). Simultaneous duplicate reactions of the target and housekeeping genes were performed for each cDNA template analyzed. Duplicate cycle threshold (CT) values were obtained for each sample and averaged. The relative expression of each target gene was calculated via the $2^{-\Delta\Delta CT}$ method. The control group for all comparisons was mouse that received PBS and SHAM.

Statistical Analysis

Data were analyzed using the Student's t-test in cases where there were fewer than six measurements within the parent group. Results are considered statistically significant when $p \leq .05$. Data were presented as mean ± one standard error.

Results

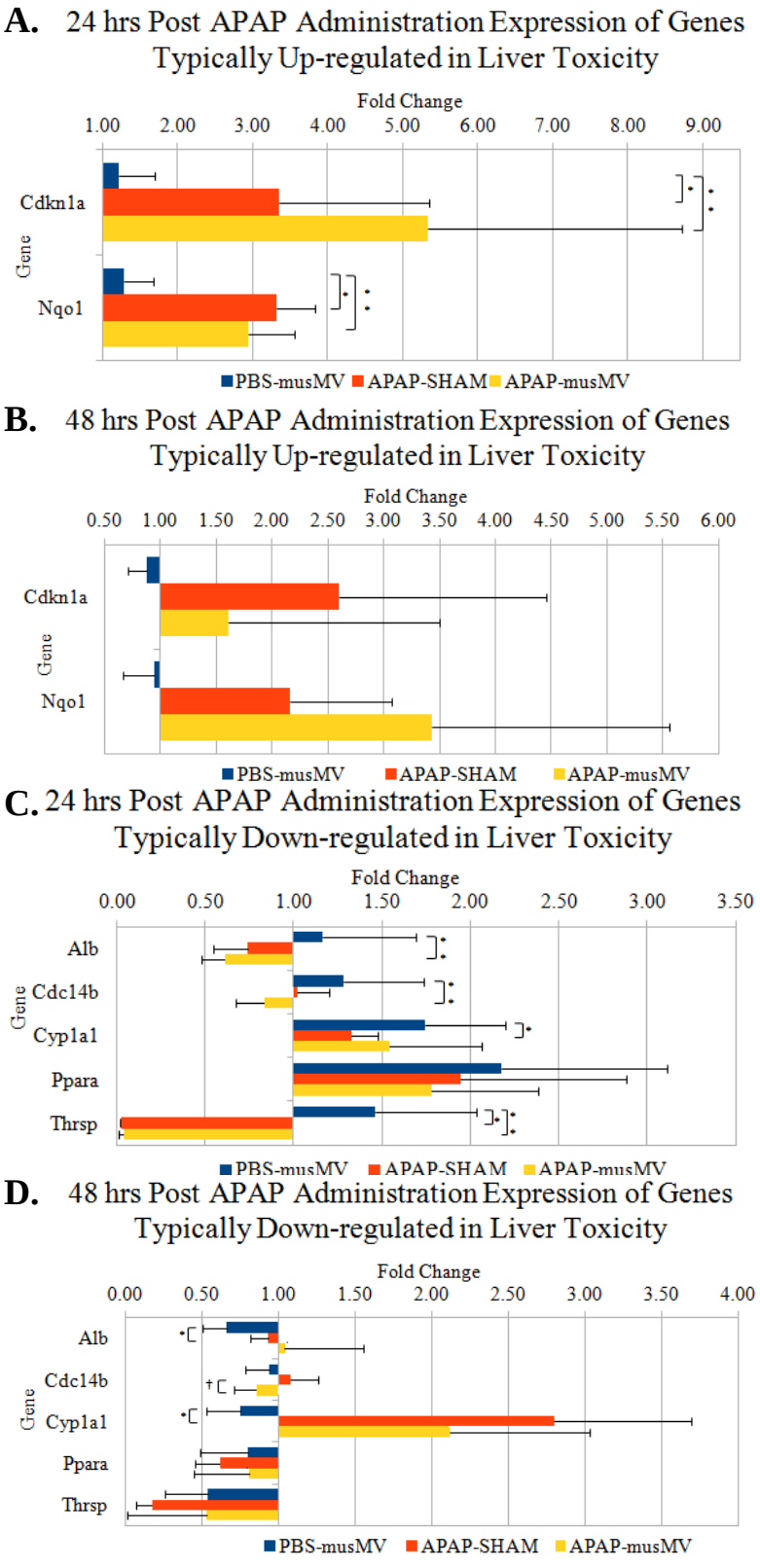

Figure 4. qRT-PCR of hepatotoxicity genes in 48-hour musMV experiment: Mean ± standard error, n = 5, $p \leq .05$, student's T test, *PBS-musMV vs. APAP-SHAM, **PBS-musMV vs. APAP-musMV, and †APAP-SHAM vs. APAP-musMV.

Compared to PBS-SHAM group, Cdkn1a and Nqo1, genes typically up-regulated in the setting of liver injury, are significantly up-regulated at 24 hours (Figure 4A), and Thrsp, a gene typically down-regulated, is significantly down at 24 hours in the APAP-SHAM and APAP-musMV groups (Figure 4C).[17] musMV treatment in APAP-injured mice normalized Cdkn1a and Thrsp expression 48 hours after APAP injury whereas expression of these genes after SHAM treatment remained abnormal (Figures 4B, D).

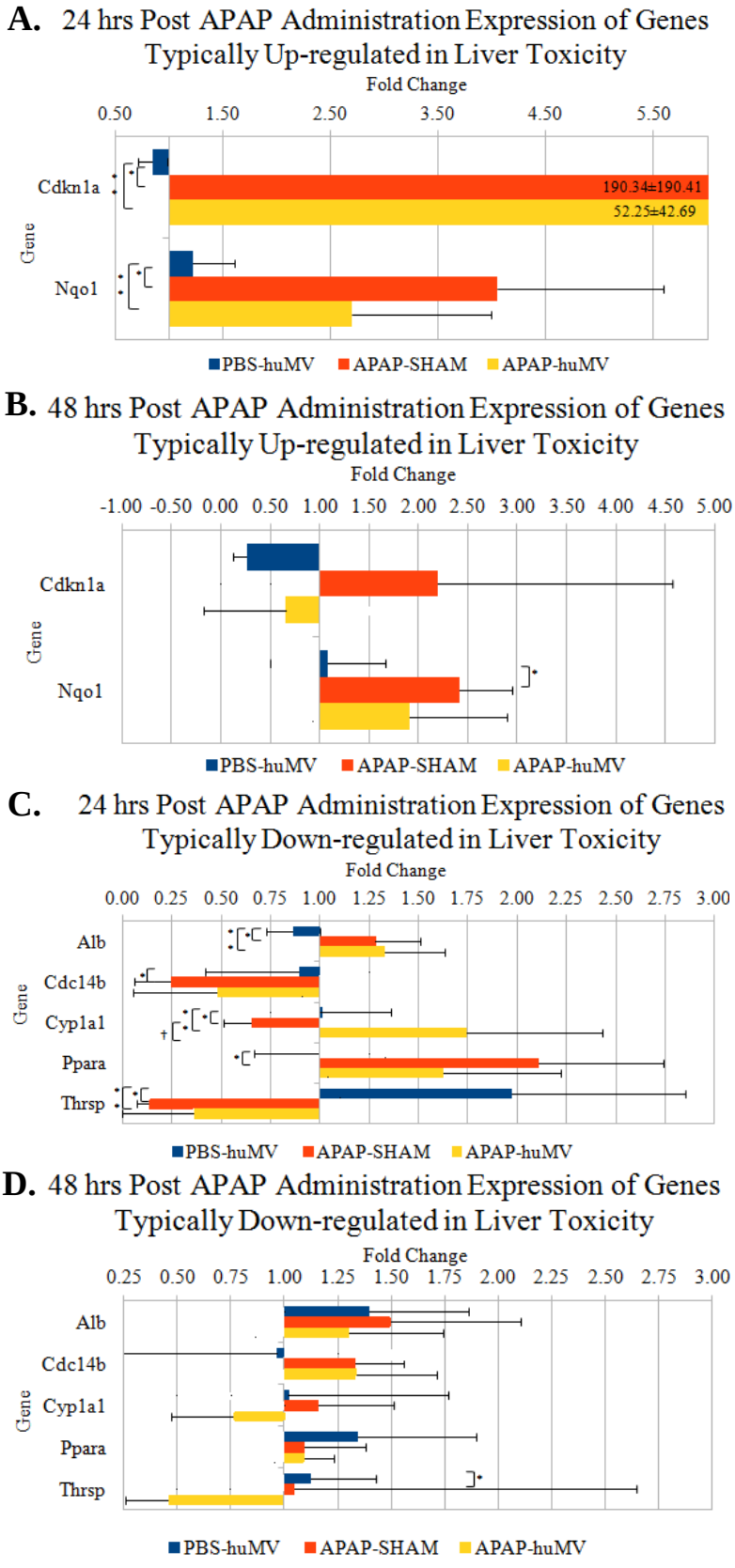

Figure 5. qRT-PCR of hepatotoxicity genes in 48-hour huMV experiment: Mean ± standard error, n = 5, $p \leq .05$, student's T test, *PBS-huMV vs. APAP-SHAM, **PBS-huMV vs. APAP-huMV, and †APAP-SHAM vs. APAP-huMV.

Compared to PBS-SHAM group, Cdkn1a and Nqo1, genes typically up-regulated in the setting of liver injury, are significantly up-regulated at 24 hours (Figure 5A), and Thrsp and Cdc14b, genes typically down-regulated, are significantly down-regulated at 24 hours in the APAP-SHAM and APAP-huMV groups (Figure 5C). huMV treatment resulted in a trend towards normalization of Cdkn1a expression (results not statistically-significant) in APAP-injured mice whereas expression of these genes after SHAM treatment remained abnormal (Figures 5B, D).

	PBS, no treatment	APAP, no treatment
Time	4 hrs	4 hrs
ALT (IU/L)	56±4.24	209±165.43
AST (IU/L)	159±25.46	655.3±613.70

	PBS-SHAM		PBS-musMV		APAP-PBS		APAP-musMV	
Time	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
ALT (IU/L)	160.00 ±71.73**	94.40 ±56.63**	230.00 ±113.52	213.00 ±283.32	557.40 ±782.83	501.60 ±217.43	886.00 ±973.28	690.00 ±700.31
AST (IU/L)	472.40 ±91.09	524.20 ±531.46	573.00 ±276.81	425.60 ±191.79	876.80 ±554.29	627.80 ±210.43	1103.20 ±876.80	815.60 ±559.50

Table 2. ALT/AST serum levels in blood from 48-hour musMV experiment: Mean ± standard error, n=2-5, $p \leq .05$, student's T-test, *AST 24 hrs vs. 48 hrs of same treatment group, **ALT 24 hrs vs. 48 hrs of same treatment group, and †PBS, no treatment vs. APAP, no treatment.

	PBS, no treatment	APAP, no treatment
Time	4 hrs	4 hrs
ALT (IU/L)	42.5±3.54†	156±37.24†
AST (IU/L)	204.5±13.44†	912±402.57†

	PBS-SHAM		PBS-huMV		APAP-PBS		APAP-huMV	
Time	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
ALT (IU/L)	286.00 ±84.85	213.00 ±270.99	316.00 ±0.00	124.80 ±93.63	508.25 ±302.57	453.80 ±256.48	599.40 ±406.30	464.60 ±511.24
AST (IU/L)	904.75 ±618.58*	364.00 ±337.68*	976.00 ±221.03*	301.80 ±134.19*	1090.20 ±559.15	795.20 ±457.50	1170.00 ±440.34*	362.20 ±267.88*

Table 3. ALT/AST serum levels in blood from 48-hour huMV experiment: Mean ± standard error, n=1-5, $p \leq .05$, student's T test, *AST 24 hrs vs. 48 hrs of same treatment group, **ALT 24 hrs vs. 48 hrs of same treatment group, and †PBS, no treatment vs. APAP, no treatment.

ALT) and AST levels of animals four hours after PBS injection were within normal limits (normal values respectably are ALT 17-77 IU/L and AST 54-298 IU/L);[12] however, both levels were above the normal range 24 and 48 hours after SHAM or MV treatment in PBS-injected mice (Tables 2, 3). musMV treatment did not significantly affect ALT and AST levels 24 or 48 hours after APAP injury. huMV treatment significantly reduced the AST levels of APAP injured mice at 48 hours.

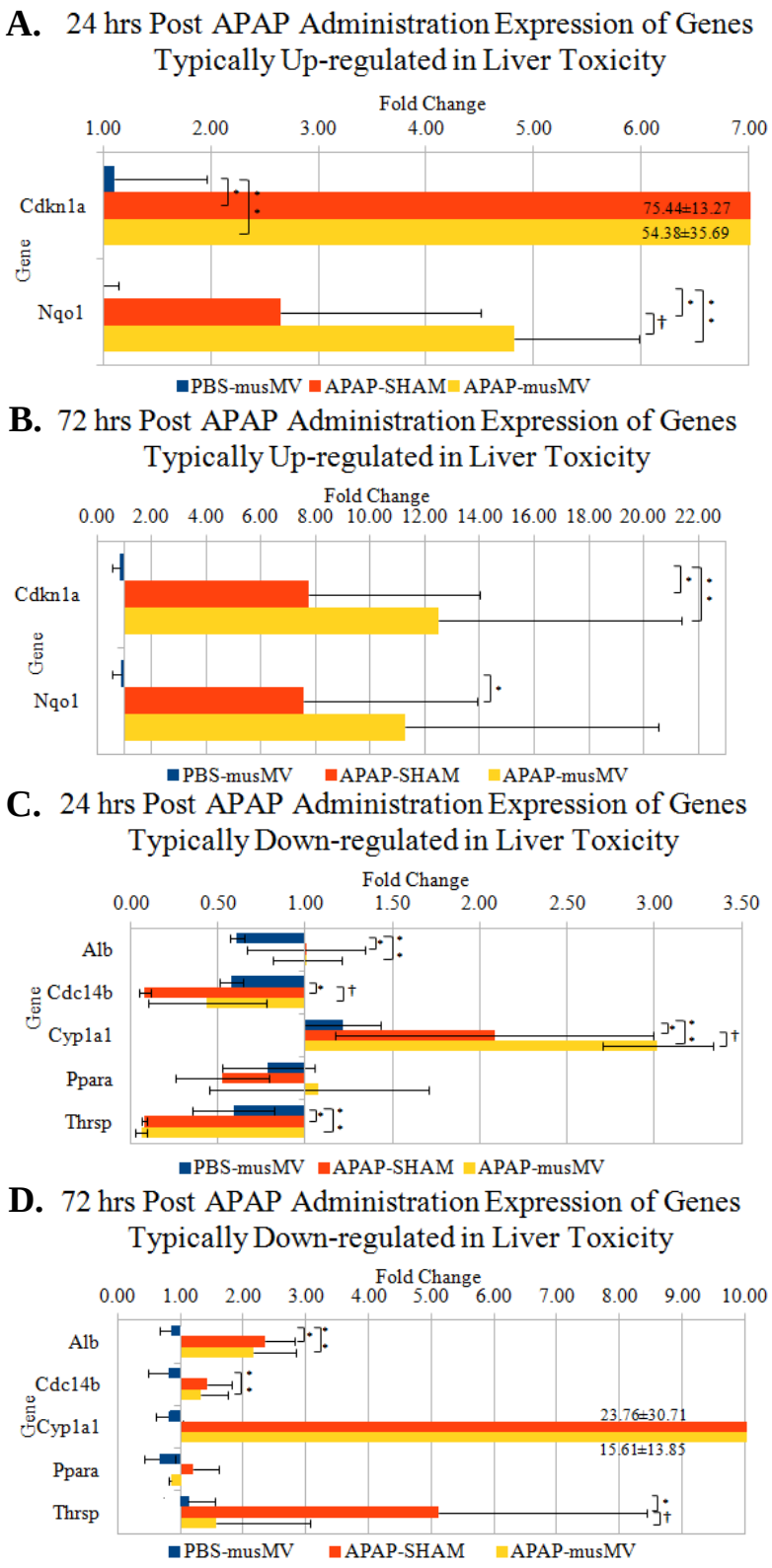

Figure 6. qRT-PCR of hepatotoxicity genes in 72-hour musMV experiment: Mean ± standard error, n = 5, $p \leq .05$, student's T test, *PBS-musMV vs. APAP-SHAM, **PBS-musMV vs. APAP-musMV, and †APAP-SHAM vs. APAP-musMV.

Compared to PBS-SHAM group, Cdkn1a and Nqo1, genes typically up-regulated in the setting of liver injury, are significantly up-regulated at 24 hours (Figure 6A), and Thrsp and Cdc14b, genes typically down-regulated, are significantly down-regulated at 24 hours in APAP-SHAM and APAP-huMV groups (Figure 6C). musMV treatment in APAP-injured mice significantly normalized Thrsp expression 72 hours after APAP injury (Figures 6B, D).

	PBS, no treatment	APAP, no treatment
Time	4 hrs	4 hrs
ALT (IU/L)	31.5±6.36	74.67±38.44
AST (IU/L)	128.50±60.10	188.00±50.27

	PBS-SHAM		PBS-musMV		APAP-SHAM		APAP-musMV	
Time	24 hrs	72 hrs	24 hrs	72 hrs	24 hrs	72 hrs	24 hrs	72 hrs
ALT (IU/L)	168.00 ±192.57	42.00 ±17.04	68.67 ±9.45**	29.75 ±5.32**	13724.25 ±3405.01**	1010.80 ±1495.02**	6834.00 ±4296.23**	1102.80 ±1194.00**
AST (IU/L)	177.40 ±118.70	81.20 ±23.18	103.67 ±27.61	79.20 ±22.33	8812.50 ±2372.75*	692.20 ±999.98*	5189.00 ±4050.67*	475 ±437.10*

Table 4. ALT/AST serum levels in blood from 72-hour musMV experiment: Mean ± standard error, n=3-5, $p \leq .05$, student's T test, *AST 24 hrs vs. 48 hrs of same treatment group, **ALT 24 hrs vs. 48 hrs of same treatment group, and †PBS, no treatment vs. APAP, no treatment.

musMV treatment significantly reduced the ALT and AST levels of APAP injured mice at 48 hours. However, SHAM treatment also significantly reduced their levels (Table 4) .

Results of histology (Figure 7) are in the process of being analyzed; necrosis and cell proliferation will be quantified. Qualitatively livers at 24 and 72 hours post APAP administration treated with MV had a similar amount of necrosis as SHAM treated livers (Figure 7C, D, G, H). Histologic analysis of MV-treated mice showed areas of lymphocytic infiltration, likely representing chronic inflammation (Figure 7I). This can often be seen when particulate matter is injected intravenously, such as with intravenous drug abusers (personal correspondence with Dr. Murray Resnick, Department of Surgical Pathology at Rhode Island Hospital).

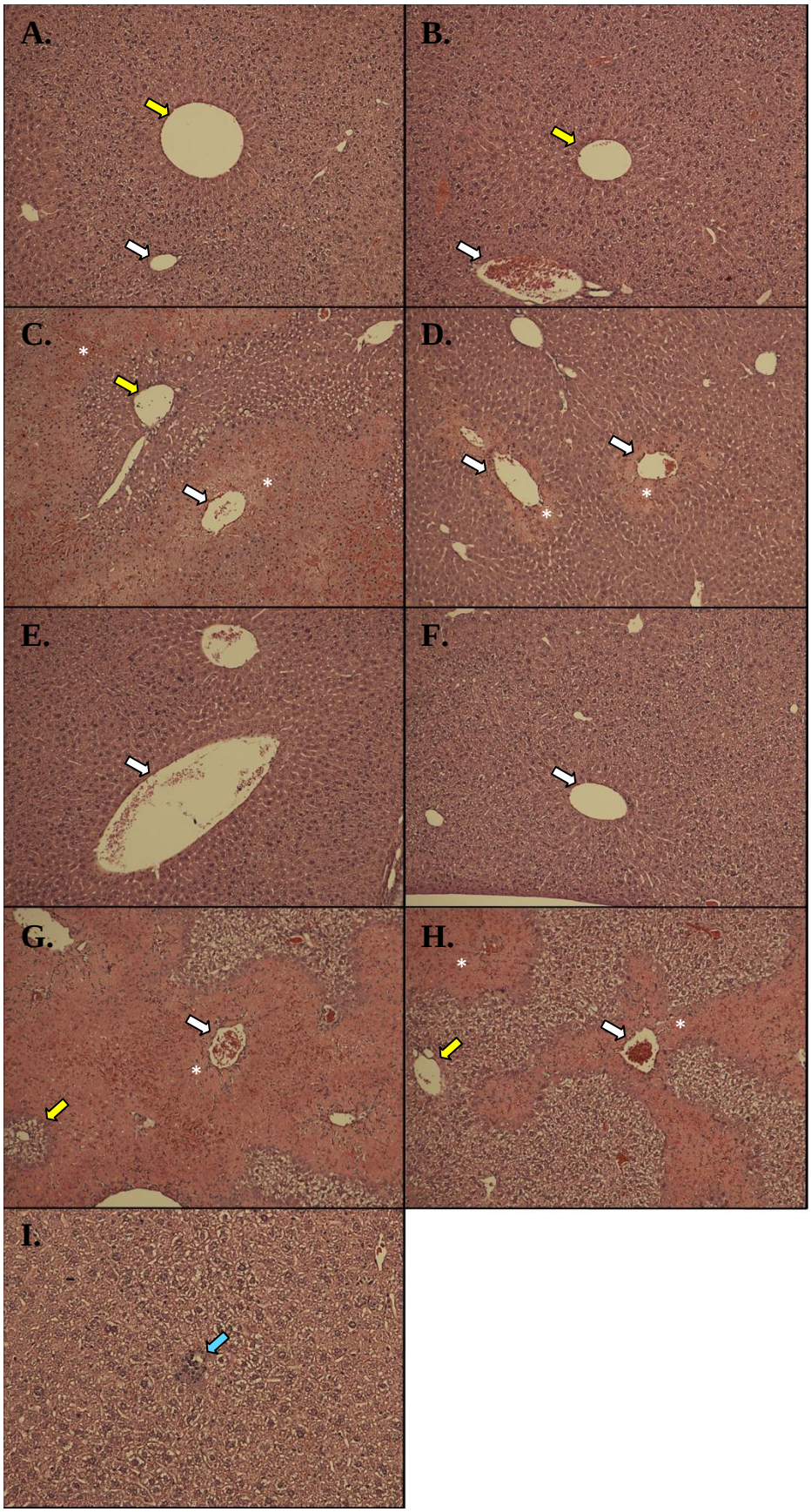

Figure 7. HE stained 10X light microscopy from 72-hour musMV experiment: At 24 hrs liver treated with (A) PBS-SHAM, (B) PBS-MV, (C) APAP-SHAM, and (D) APAP-MV. At 72 hrs liver treated with (E) PBS-SHAM, (F) PBS-MV, (G) APAP-SHAM, and (H) APAP-MV. (I) Presence of granuloma (blue arrow) and lymphocytes in MV treated livers. Liver parenchyma around the central vein (white arrow) has extensive necrosis (asterix) after APAP treatment. Liver parenchyma around the portal vein (yellow arrow) is relatively spared.

Discussion

In APAP-injured mice treated with huMV or musMV, Cdkn1a expression (a regulatory gene in cell proliferation) normalized after 48 hours, suggesting a decrease in apoptosis and increase in cell proliferation.[7] This may represent increased regeneration in response to MV treatment. musMV treatment in APAP-injured mice also normalized Thrsp expression (a regulatory gene in liver's production of lipids) after 48 and 72 hours.[7] This may represent improved liver function in response to MV treatment. ALT and AST levels, often used clinically as an evaluation of hepatocellular injury, were inconclusive in all three experiments in that MV treatment did not result in improvement of these levels. Only huMV treatment resulted in a significant reduction in AST levels of APAP injured mice at 48 hours; however, the same trend was noted in SHAM-treated, APAP-injured mice. Signs of damage at the cellular level were minimal in histology at 24 hours but progressed substantially by 48 hours and onward. We have yet to determine if MV treatment has any impact on the histology of APAP-injured livers.

In addition to quantifying the degree of necrosis (direct measurement of the surface area of necrosis) and regeneration (staining for proliferation with Ki-67) of histology obtained so far, the next step is to perform a survival study. This would allow us to measure the effectiveness of MV treatment in terms of mortality rate. If a trend towards better recovery is determined, we will investigate the combined use of MV with NAC and test the enhancement of NAC effects. It is possible that MV infusion may lead to greater hepatocyte GSH production than with NAC treatment alone, resulting in better treatment for patients arriving in the hospital later than 8 hours post APAP ingestion.

Due to technical difficulties with the ALT and AST assays, some of the values (Table 2, 3, 4) only represent an $n<5$. The MV utilized in the 72-hour experiment were obtained without an additional 10,000 g centrifugation as this is a technique used by Camussi et al. who reported

MV-assisted recovery in the setting of acute kidney injury.[5] In the 48-hour experiment, ethanol was used as a diluent for APAP as this was recommended by the manufacturer. However, after completing the experiment we realized that even 0.8% ethanol could potentially induce liver damage, so in the 72-hour experiment, APAP was only dissolved in PBS. Furthermore, we had previously attempted the 48- hour experiment using the median lethal APAP dosage (LD_{50}), 250 mg/kg, but there was an insignificant difference between PBS controls and APAP-injured mice suggesting that no injury was sustained. The need for quality controls arose due to the lack of injury during the first run of the experiment with LD_{50} and unusual histology of PBS controls.

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I would like to thank the Quesenberry lab for all the support and guidance I have received over the past year and a half. I have come to understand well many cellular and molecular techniques due to their patience and hands-on teaching. The group immediately made me feel welcomed when I joined the team, and they have made my lab experience a lot of fun. Besides doing research to fulfill my concentration's requirement, I wanted to know what research was actually like first-hand in order to decide if this was the career path I wanted. Thanks to this wonderful experience, I am sure that research is something I love and can enjoy doing for the rest of my life.

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