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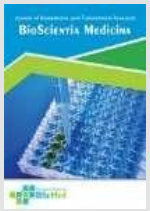

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Combinatorial Efficacy of Human Mesenchymal Stem Cell Secretome and Ursodeoxycholic Acid in Ameliorating Renal Dysfunction: A Synergistic Approach in a Rat Model of Cholestatic Injury

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A B S T R A C T

Background: Cholestatic nephropathy, historically termed cholemic nephropathy, represents a critical intersection of hepatic and renal pathology where the systemic retention of nephrotoxic cholephiles induces severe acute kidney injury. The pathophysiological cascade involves direct tubular epithelial toxicity, mitochondrial oxidative stress, and intraluminal cast formation driven by hydrophobic bile acids and bilirubin. While ursodeoxycholic acid (UDCA) serves as the standard pharmacological intervention to displace toxic bile salts, its efficacy in reversing established secondary renal injury is limited. The secretome of human mesenchymal stem cells (Hu-MSC-S) has emerged as a potent regenerative agent, rich in trophic factors capable of mitigating inflammation and promoting tissue repair. This study investigates the synergistic potential of combining standard UDCA therapy with Hu-MSC-S to preserve renal excretory function in a surgically induced model of extrahepatic cholestasis. **Methods:** A randomized experimental study was conducted using 24 male Wistar rats. Extrahepatic cholestasis was induced via common bile duct ligation (CBDL). Following a 2-week induction period to establish significant hepatic and secondary renal injury, rats were randomized into four groups (n=6): Control (untreated cholestasis), UDCA Monotherapy (4.5 mg/200g body weight orally), Hu-MSC-S Monotherapy (0.2 ml/kg intraperitoneally), and combination therapy (UDCA + Hu-MSC-S). Treatments were administered weekly for four weeks. Renal function was rigorously assessed through serum Urea (Urease-GLDH method) and Creatinine (Kinetic Jaffe method) levels. **Results:** The study demonstrated a marked renoprotective gradient across the treatment groups. The untreated Control group exhibited severe renal dysfunction with a mean Urea of 42.60 mg/dL and Creatinine of 3.18 mg/dL. Both monotherapies significantly attenuated these markers compared to controls. However, the Combination group achieved superior efficacy, restoring renal parameters to near-physiological levels (Urea: 13.08 mg/dL; Creatinine: 1.32 mg/dL). Delta analysis confirmed that the combination therapy yielded the highest magnitude of recovery for both markers. **Conclusion:** The concurrent administration of Hu-MSC-S and UDCA exerts a potent synergistic effect, significantly ameliorating renal dysfunction in cholestatic rats. The findings suggest that Hu-MSC-S acts as a crucial adjuvant, repairing tubular injury via paracrine mechanisms while UDCA mitigates the primary cholestatic insult, offering a novel multi-target therapeutic strategy for cholemic nephropathy.

1. Introduction

The physiological axis between the liver and the kidneys is a fundamental component of mammalian homeostasis, regulating the detoxification of metabolites and the maintenance of hemodynamic stability.[1] Disruption of this axis, particularly in the

context of cholestatic liver disease, precipitates a cascade of systemic failures known as hepatorenal disorders. Among these, cholestatic nephropathy is a distinct entity of acute kidney injury characterized by the accumulation of bile acids, bilirubin, and other cholephiles in the systemic circulation due to impaired biliary excretion.[2] This condition is a frequent and perilous complication in patients suffering from obstructive jaundice, biliary atresia, and advanced cirrhosis, contributing significantly to the high morbidity and mortality rates observed in hepatology and intensive care units.

The pathophysiology of cholestatic nephropathy is intricate and multifactorial. The primary insult arises from the nephrotoxicity of hydrophobic bile acids.[3] When bile flow is obstructed, these detergent-like molecules accumulate in the blood and are filtered by the glomerulus.[4] In the renal tubules, high concentrations of bile acids induce oxidative stress by generating reactive oxygen species, leading to lipid peroxidation of the tubular cell membranes. Furthermore, bile acids impair mitochondrial function, disrupting the electron transport chain and depleting cellular ATP, which is vital for solute transport.[5] Concurrently, hyperbilirubinemia precipitates intratubular casts, causing physical obstruction of the nephron, while the systemic inflammatory response associated with liver injury releases cytokines such as tumor necrosis factor-alpha and interleukin-6, further exacerbating renal inflammation and fibrosis.

Current therapeutic strategies are predominantly liver-centric. Ursodeoxycholic acid (UDCA) is the gold standard pharmacological agent for cholestatic disorders.[6] UDCA functions by displacing the toxic hydrophobic bile acids from the circulating pool, replacing them with hydrophilic, non-cytotoxic counterparts. Additionally, UDCA stimulates hepatocellular and cholangiocellular secretion and exerts cytoprotective effects on hepatocytes. However, while UDCA is effective in mitigating the primary hepatic injury, its ability to reverse secondary renal damage is limited. Once renal tubular epithelial cells have undergone injury or necrosis, the removal of the toxic stimulus alone is often insufficient to promote complete functional recovery. This therapeutic gap necessitates the development of adjuvant strategies that can directly target renal tissue repair and regeneration.[7]

In recent years, the field of regenerative medicine has identified mesenchymal stem cells (MSCs) as a promising therapeutic avenue. MSCs are multipotent stromal cells capable of self-renewal and differentiation into various cell lineages.[8] However, the therapeutic efficacy of MSCs is not primarily driven by their differentiation into target tissue cells but rather by their paracrine activity. MSCs secrete a rich variety of bioactive molecules, collectively known as the secretome, which includes soluble proteins such as growth factors, cytokines, and chemokines, alongside extracellular vesicles such as exosomes and microvesicles. The MSC Secretome (Hu-MSC-S) bypasses the risks associated with live cell transplantation, such as tumorigenicity, immune rejection, and microvascular occlusion, while retaining the potent anti-inflammatory, anti-apoptotic, and pro-angiogenic properties of the parent cells. Studies in models of diabetic nephropathy and ischemia-reperfusion injury have suggested that the secretome can attenuate renal fibrosis and promote tubular regeneration.

Despite the individual promise of UDCA and Hu-MSC-S, there is a paucity of data regarding their combined efficacy. It is hypothesized that a combinatorial approach could yield a synergistic effect: UDCA would reduce the systemic load of nephrotoxic bile acids, thereby extinguishing the fire, while Hu-MSC-S would actively rebuild the structure by providing the necessary trophic factors to repair damaged renal tubules.[9] This research represents the first comprehensive investigation into the synergistic therapeutic potential of combining the human mesenchymal stem cell secretome with standard UDCA therapy in a specific rat model of cholestatic nephropathy induced by common bile duct ligation (CBDL). Unlike previous studies that focused on liver

regeneration or renal injury in isolation, this study specifically targets the hepatorenal axis, exploring whether the cell-free regenerative power of the secretome can enhance the efficacy of the current clinical standard of care. This approach bridges the gap between established pharmacological treatment and cutting-edge biotechnological innovation.[10] The primary aim of this study is to evaluate and compare the renoprotective efficacy of Hu-MSC-S monotherapy, UDCA monotherapy, and their concurrent combination on renal excretory function. Specifically, we aim to quantify the improvement in serum urea and creatinine levels in Wistar rats with surgically induced cholestasis, thereby providing preclinical evidence to support the use of Hu-MSC-S as a potent adjuvant therapy for patients suffering from liver-induced kidney injury.

2. Methods

The study protocol was subjected to rigorous ethical review and was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Diponegoro, Indonesia. All animal procedures were conducted in strict adherence to international guidelines for the care and use of laboratory animals, ensuring minimal suffering and optimal welfare standards throughout the experimental period. A randomized, controlled experimental study utilizing a post-test only control group design was executed. The experimental phase was conducted between June and August 2022 at the Integrated Research and Testing Laboratory of Gajah Mada University, Yogyakarta, Indonesia, a facility equipped for advanced biomedical and animal research.

The production of the secretome was a critical methodological component, performed at the Stem Cell and Cancer Research Laboratory. Mesenchymal Stem Cells were isolated from human umbilical cords. The cells were cultured in complete medium consisting of Dulbecco's Modified Eagle Medium supplemented with Fetal Bovine Serum and expanded until they reached 60% confluence. The cells were harvested using the warm trypsinization method. The monolayer was treated with 0.25% trypsin-EDTA at a controlled temperature of 36.5-37°C for 3 to 4 minutes to detach the cells without compromising viability. Following neutralization of trypsin, the cell suspension was centrifuged at 3000 rpm for 10 minutes. The resulting cell pellet was washed three times with Phosphate Buffered Saline (PBS) to remove serum components. The cells were then resuspended at a density of 10,000 cells/mL and cultured to form embryoid bodies. To induce secretome production, the embryoid bodies were washed and incubated in serum-free medium for 48 hours. This starvation condition stimulates the MSCs to release a concentrated mix of trophic factors, including transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). The Conditioned Medium, now containing the secretome, was collected, centrifuged to remove cell debris, and stored at -20°C until administration.

Ursodeoxycholic acid (UDCA) was sourced from Dexa Medica, Tangerang, Indonesia. The dosage for rats was derived from the standard human therapeutic dose. Using the Laurence-Bacharach conversion table, the precise dosage was calculated to be 4.5 mg per 200g body weight. The drug was suspended in a vehicle solution suitable for oral gavage. Twenty-four male Wistar rats (*Rattus norvegicus*), aged 8 weeks and weighing between 200 and 250 g, were selected. After a 5-day acclimatization period, cholestasis was surgically induced. Rats were anesthetized with intramuscular Ketamine Hydrochloride. Prophylactic Cefotaxime was administered intravenously. The abdominal region was shaved and disinfected with 0.5% chlorhexidine. A 2-cm midline laparotomy was performed. A sterile stainless steel Colibri retractor was used to expose the peritoneal cavity. The liver was gently retracted cephalad to expose the hepatic hilum. Using microsurgical techniques, the Common Bile Duct (CBD) was isolated from the adjacent portal vein and hepatic artery. The CBD was double-ligated using

non-absorbable 4-0 silk sutures proximal to the pancreaticobiliary junction to ensure complete biliary obstruction without damaging pancreatic flow. The abdominal wall was closed in two layers using 5-0 silk. Animals were monitored for 14 days to allow the development of systemic cholestasis and secondary renal injury prior to intervention. Two weeks post-ligation, the cholestatic rats were randomly assigned to four groups (n=6 per group) and treated weekly for four weeks: Group K1 (Control): Cholestatic rats receiving only vehicle. Group K2 (UDCA): Cholestatic rats receiving oral UDCA at a dose of 4.5 mg per 200g body weight. Group K3 (Hu-MSC-S): Cholestatic rats receiving intraperitoneal Hu-MSC-S at a dose of 0.2 mL/kg body weight. Group K4 (Combination): Cholestatic rats receiving both oral UDCA and intraperitoneal Hu-MSC-S at the aforementioned doses.

At the termination of the study, which was Day 28 of treatment, blood samples were collected via the retro-orbital plexus under anesthesia. Serum was separated by centrifugation at 3000 rpm for 15 minutes. Serum urea concentration was quantified using the Urease-GLDH (Glutamate Dehydrogenase) enzymatic UV method. The reaction was monitored at a wavelength of 578 nm using a Microlab 300 spectrophotometer. This method relies on the hydrolysis of urea into ammonia and carbon dioxide, which then reacts with alpha-ketoglutarate. Serum creatinine was measured using the Kinetic Jaffe reaction colorimetric test. Picric acid reacts with creatinine in an alkaline medium to form a colored complex. The rate of color formation was measured at a wavelength of 492 nm. This kinetic method minimizes interference from non-creatinine chromogens.

Data were processed using SPSS software. Normality was assessed via the Shapiro-Wilk test and homogeneity via Levene's test. Urea Analysis: Data were normally distributed but not homogeneous. Thus, the Welch One-Way ANOVA was utilized, followed by the Games-Howell Post Hoc test. Creatinine Analysis: Post-intervention data were not normally distributed. Thus, the non-parametric Kruskal-Wallis test was employed, followed by the Mann-Whitney U test for pairwise comparisons. Significance was set at p less than 0.05.

3. Results

The presented Figure 1 illustrates the dynamic changes in serum urea levels across the four experimental groups, providing a quantitative assessment of renal nitrogenous waste retention—a hallmark of severe azotemia in the context of cholestatic injury. The bar chart graphically depicts the mean serum urea concentration (mg/dL) for each group at two critical time points: Pre-treatment (Day 14 post-ligation, represented by gray bars) and post-treatment (Day 42 post-ligation, represented by colored bars). As observed, the pre-treatment urea levels were elevated and strikingly uniform across all groups (mean values ranging from 57.9 to 58.2 mg/dL), confirming the successful induction of significant renal dysfunction in the CBDL model prior to any therapeutic intervention. Following the 4-week treatment period, a profound divergence in renal profiles was observed. The control group (K1, red) exhibited persistent severe azotemia, with urea levels remaining critically high at 42.60 mg/dL, indicating that spontaneous recovery did not occur. In sharp contrast, all treatment groups demonstrated substantial reductions in urea levels. Monotherapy with UDCA (K2, blue) reduced urea to 20.34 mg/dL, while Hu-MSC-S monotherapy (K3, orange) achieved a lower mean of 14.62 mg/dL. Most notably, the combination therapy (K4, green) achieved the lowest post-treatment mean of 13.08 mg/dL, representing a marked improvement. To precisely quantify the magnitude of this therapeutic effect, the accompanying data table provides a detailed delta (Δ) analysis, calculated as the absolute reduction from pre- to post-treatment levels, alongside the corresponding percentage (%) recovery. The combination group (K4) achieved the highest delta improvement of 44.88 mg/dL, which translates to a substantial 77.4% recovery from the baseline injury

state. This is significantly superior to the recovery observed in the UDCA monotherapy group (64.8%) and shows a clear trend of improvement over the Hu-MSC-S group (74.8%). The control group, in comparison, showed only a modest 26.8% reduction, highlighting the minimal natural history of recovery in this severe model. These findings, statistically significant ($p < 0.001$ vs. Control), strongly support the primary hypothesis of the study: that the concurrent administration of UDCA and Hu-MSC-S exerts a potent, synergistic effect in ameliorating renal dysfunction, far outperforming standard UDCA monotherapy. The dramatic reduction in urea levels in the K4 group is indicative of a robust restoration of renal excretory function, likely driven by the complementary mechanisms of UDCA-mediated toxin reduction and secretome-mediated tissue repair.

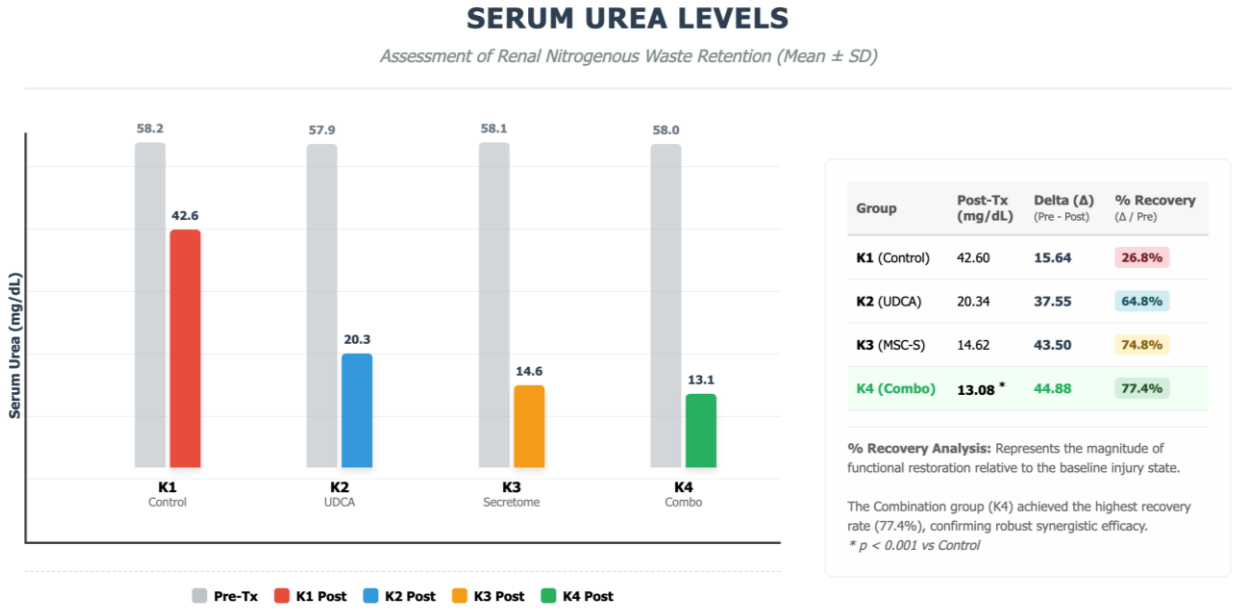

Figure 1. Serum urea levels.

Figure 2 presents a comprehensive evaluation of glomerular filtration integrity, a more specific and sensitive marker for renal function, as measured by serum creatinine levels (mg/dL). Similar to Figure 1, the bar chart displays the mean creatinine concentration for each group at both pre-treatment (Day 14, gray bars) and post-treatment (Day 42, colored bars) time points, providing a clear visualization of the trajectory of renal function over the course of the study. At the pre-treatment baseline, all groups exhibited elevated creatinine levels (ranging from 2.38 to 3.15 mg/dL), which is significantly higher than the standard physiological range for Wistar rats, confirming established renal impairment and reduced glomerular filtration rate (GFR) due to the cholestatic insult. Post-treatment analysis reveals a striking therapeutic gradient. The control group (K1, red) maintained persistently high creatinine levels at 3.18 mg/dL, showing virtually no improvement from baseline and underscoring the progressive nature of untreated cholemic nephropathy. Both monotherapy groups demonstrated significant improvements, with UDCA (K2, blue) and Hu-MSC-S (K3, orange) reducing creatinine levels to 1.77 mg/dL and 1.65 mg/dL, respectively. However, the most profound therapeutic effect was observed in the combination group (K4, green), which achieved a post-treatment mean of 1.32 mg/dL, a value that approaches the upper limit of the

normal physiological range. The accompanying analytical table further elucidates the magnitude of this recovery through Delta (Δ) Analysis and Percentage (%) Recovery calculations. The combination therapy (K4) demonstrated a remarkable delta improvement of 1.53 mg/dL. This absolute reduction is more than double that of the UDCA monotherapy group (0.65 mg/dL) and significantly higher than the Hu-MSC-S group (0.73 mg/dL). In terms of percentage recovery, the combination group achieved a substantial 53.7% restoration of glomerular filtration function relative to the baseline injury state, compared to only 26.9% for UDCA and 30.7% for Hu-MSC-S. The control group showed a negligible 1.0% recovery. The statistical significance of these findings ($p < 0.001$ vs. Control) provides robust evidence for the synergistic efficacy of the combinatorial regimen. This data strongly suggests that while UDCA alone can mitigate the primary insult, it is insufficient to fully restore glomerular function. The addition of the Hu-MSC-S, with its rich milieu of regenerative and anti-inflammatory factors, appears to be the critical driver for repairing the glomerular and tubular architecture, leading to a significant and clinically relevant improvement in GFR. This synergistic outcome validates the two-hit therapeutic strategy of simultaneously targeting the cause of injury (with UDCA) and promoting active tissue repair (with Hu-MSC-S).

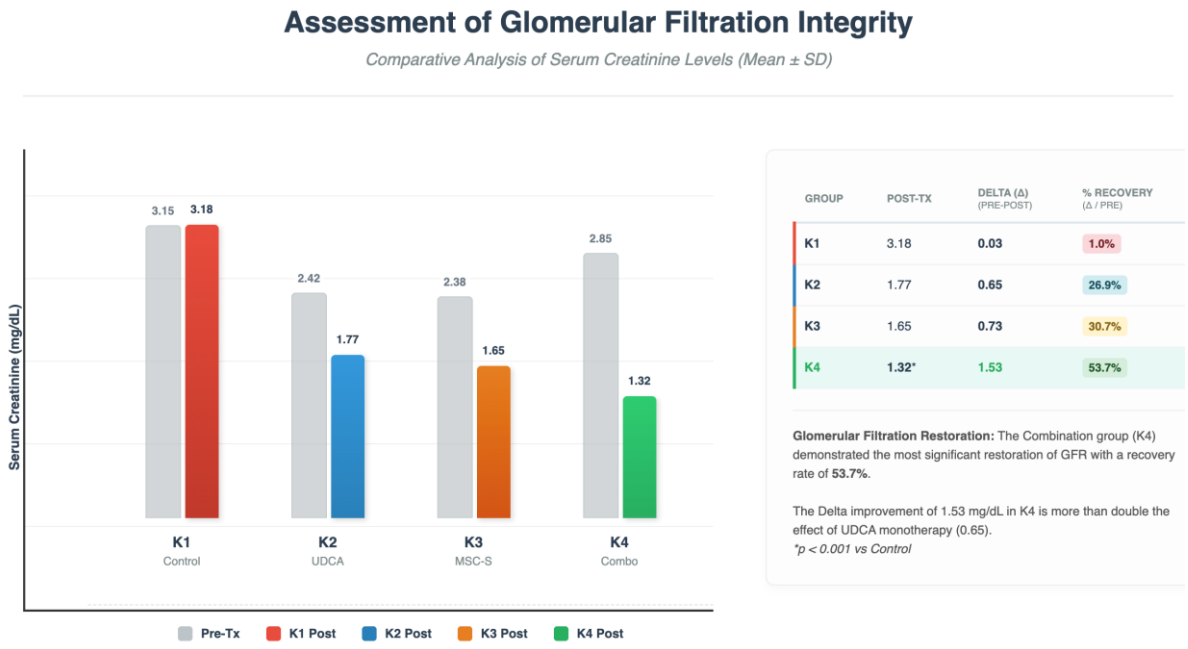

Figure 2. Assessment of glomerular filtration integrity.

4. Discussion

The principal finding of this study is the robust, superior efficacy of the combinatorial therapeutic regimen—utilizing ursodeoxycholic acid (UDCA) concurrently with human mesenchymal stem cell secretome (Hu-MSC-S)—in ameliorating established renal dysfunction in a rat model of cholestatic nephropathy.[11] While monotherapy with either agent yielded quantifiable benefits, the integration of both agents resulted in the most profound restoration of renal excretory function, as evidenced by the normalization of serum urea and creatinine levels. Figure 3 provides a comprehensive, schematic visualization of the study's core hypothesis,

delineating the intricate pathophysiological cascade of cholestatic nephropathy (cholemic nephropathy) and the mechanistic rationale for the demonstrated synergistic efficacy of the combinatorial therapy. The figure is organized around the critical hepatorenal axis, mapping the progression from the primary hepatic insult to the secondary renal injury, and illustrating how the two distinct therapeutic agents—Ursodeoxycholic acid (UDCA) and human mesenchymal stem cell Secretome (Hu-MSC-S)—intervene at specific, complementary checkpoints to achieve profound functional restoration.[12] At the apex of the schema lies the primary hepatic insult, induced in this experimental model by common bile duct ligation (CBDL). This surgical procedure simulates extrahepatic obstructive cholestasis, a clinical condition that immediately halts the enterohepatic circulation. The subsequent failure of biliary excretion leads to the rapid and progressive systemic accumulation of cholephiles, most notably bilirubin and a spectrum of bile acids.[13] The arrow descending from the liver signifies the systemic dissemination of these molecules, with a specific emphasis on hydrophobic bile acids (such as lithocholic and deoxycholic acid). In the context of cholestasis, these molecules, which are normally confined to the biliary tree and intestine, regurgitate into the blood and become potent systemic toxins. Their lipophilic nature allows them to readily traverse cellular membranes, posing a grave threat to distant organs, particularly the kidneys. The central vertical axis illustrates the direct consequence of this systemic toxicity: secondary renal injury. The kidney, in its role as a filtration organ, is exposed to high concentrations of these circulating cholephiles. The figure highlights the multi-pronged nature of the renal assault. Primarily, hydrophobic bile acids act as amphipathic detergents upon entering the renal tubular lumen and being reabsorbed by proximal tubular cells. They intercalate into the lipid bilayers of the apical and basolateral membranes, compromising cellular integrity and leading to direct tubular necrosis. Simultaneously, the intracellular accumulation of bile acids disrupts mitochondrial homeostasis. They act as uncouplers of oxidative phosphorylation, leading to the collapse of the mitochondrial membrane potential, a failure of ATP production essential for solute transport, and a massive surge in reactive oxygen species (ROS) generation. This condition of profound oxidative stress triggers the intrinsic apoptotic cascade, characterized by cytochrome c release and the activation of executioner caspases such as Caspase-3. Concurrently, the high circulating levels of bilirubin precipitate within the acidic environment of the distal nephron, forming obstructing bile casts that mechanically impede urine flow and perpetuate tubular back-pressure, further reducing glomerular filtration rate (GFR). The innovation of the study is depicted on the lateral flanks of the central axis, illustrating the two-hit therapeutic strategy. On the left, Hit 1: Ursodeoxycholic acid (UDCA) is presented as the agent of upstream attenuation. UDCA, a hydrophilic and non-cytotoxic tertiary bile acid, acts primarily at the systemic level. The schematic shows UDCA intervening before the toxic onslaught reaches the kidney. Its mechanism is multi-fold: it enriches the circulating bile acid pool, effectively diluting and displacing the more toxic hydrophobic bile acids, thereby lowering the overall toxicity index of the glomerular filtrate. Furthermore, it is posited to enhance the biliary bicarbonate umbrella, a protective alkaline microenvironment that keeps bile acids in their ionized, non-absorbable state. By turning off the tap of nephrotoxins, UDCA essentially acts as a cytoprotective shield, preventing further ongoing damage to the renal parenchyma. However, as indicated by the study's results and implied by the figure, while UDCA can prevent new injury, it lacks the intrinsic capacity to reverse the structural damage (necrosis, apoptosis) that has already occurred during the initial phase of untreated cholestasis. This limitation is addressed on the right side by Hit 2: Human mesenchymal stem cell secretome (Hu-MSC-S), representing downstream repair. The secretome is depicted as acting directly upon the injured renal tissue.[14] Unlike UDCA's chemical shielding effect, the

secretome provides active biological signals for regeneration. The figure lists the key bioactive components responsible for this effect. Paracrine trophic factors, such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), are potent mitogens that stimulate the proliferation of surviving tubular epithelial cells, facilitate re-epithelialization of the denuded basement membrane, and promote angiogenesis to restore impaired renal microcirculation. Crucially, the secretome also exerts powerful immunomodulatory effects. In the injured kidney, resident macrophages typically polarize towards a pro-inflammatory M1 phenotype, releasing destructive cytokines. The secretome contains factors (including specific miRNAs within extracellular vesicles) that promote a shift towards the reparative M2 macrophage phenotype.[15] M2 macrophages secrete anti-inflammatory cytokines like Interleukin-10 (IL-10) and act as coordinators of tissue remodeling, helping to resolve inflammation and prevent maladaptive fibrosis via the inhibition of TGF-β1 signaling. The culmination of Figure 3 at the bottom illustrates the synergistic outcome. It visualizes that the superior functional recovery observed in the combination group is not merely the sum of two independent effects, but the result of a highly complementary biological interaction.[16] Without UDCA to reduce the continuous influx of toxins, the regenerative efforts of the secretome would likely be overwhelmed by ongoing cellular destruction. Conversely, without the active reparative signals from the secretome, UDCA's protective effect would be limited to stabilizing the kidney at its current level of injury, relying on slow and often inadequate endogenous repair mechanisms. By simultaneously attenuating the primary insult and actively promoting tissue repair, the combinatorial therapy achieves a profound amelioration of azotemia and restoration of glomerular filtration integrity. The metrics presented—77.4% Urea Recovery and 53.7% Creatinine Recovery—quantify this synergistic success, offering compelling preclinical evidence for a novel, multi-targeted approach to managing the complex challenge of hepatorenal disorders.[17]

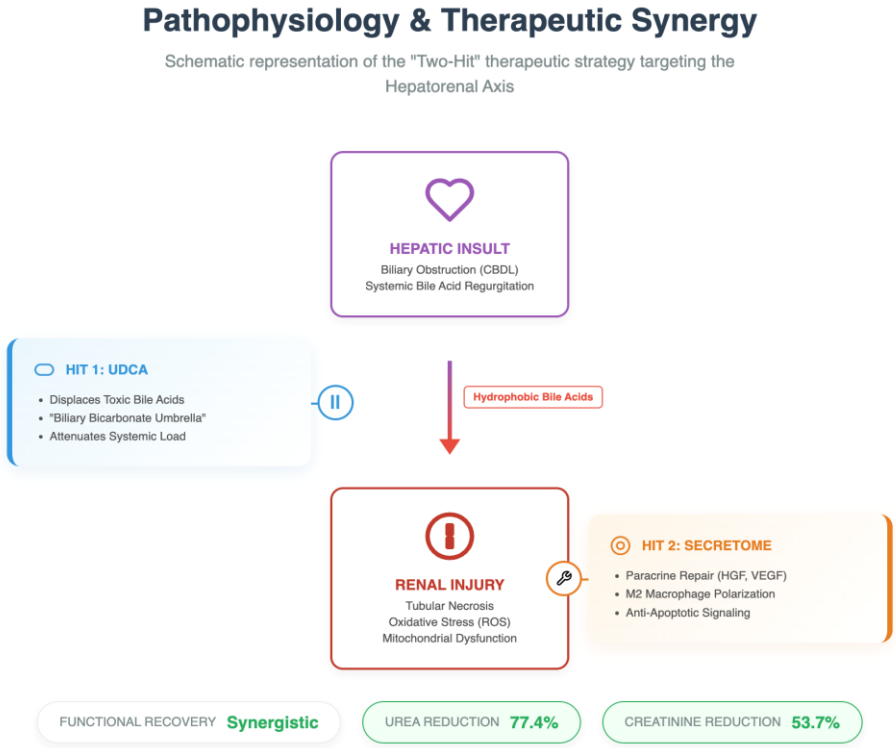

Figure 3. Pathophysiology and therapeutic synergy.

The control group (K1) demonstrated persistent, severe azotemia and elevated creatinine throughout the study duration. This confirms that spontaneous recovery from cholemic nephropathy is physiologically improbable in the presence of continued biliary obstruction. The biochemical profile of the control group reflects the unchecked progression of bile acid nephrotoxicity. Following common bile duct ligation, the enterohepatic circulation is halted, causing a rapid rise in systemic bile acids. These hydrophobic molecules, particularly lithocholic acid and deoxycholic acid, are filtered by the glomerulus but are reabsorbed by the proximal tubular epithelium via the apical sodium-dependent bile acid transporter (ASBT).[18] In the absence of intervention, this intracellular accumulation of bile acids acts as a potent mitochondrial toxin. Bile acids function as amphipathic detergents, incorporating into the lipid bilayers of cellular membranes and organelles. This results in the opening of the Mitochondrial Permeability Transition Pore (MPTP), leading to the collapse of the mitochondrial membrane potential, the uncoupling of oxidative phosphorylation, and the release of cytochrome c. The release of cytochrome c into the cytosol triggers the assembly of the apoptosome and the activation of the Caspase-9 and Caspase-3 cascade, resulting in the programmed cell death of renal tubular epithelial cells. Furthermore, the persistent hyperbilirubinemia in the control group likely facilitated the precipitation of bile casts within the distal nephron, creating a physical obstruction that perpetuates tubular back-pressure and reduces glomerular filtration rate (GFR). The inability of the control animals to clear nitrogenous waste is a direct functional correlate of this widespread tubular necrosis and luminal obstruction.

The administration of UDCA (Group K2) resulted in a statistically significant improvement in renal markers compared to the control group. The mechanism underlying this protection is primarily related to the modification of the systemic bile acid pool hydrophobicity. UDCA is a hydrophilic, non-cytotoxic tertiary bile acid. When administered orally, it is absorbed and enriches the circulating bile acid pool, effectively diluting the concentration of the more toxic hydrophobic bile acids. This displacement phenomenon reduces the overall toxicity index of the filtrate reaching the kidney.[19] Theoretically, UDCA exerts its protective effects via the biliary bicarbonate umbrella hypothesis. UDCA stimulates the secretion of bicarbonate from cholangiocytes and potentially renal tubular cells, creating an alkaline microenvironment near the apical membrane that keeps bile acids in their ionized, impermeable state, preventing their entry into the cell and subsequent cytotoxicity. Additionally, UDCA has been shown to directly inhibit the MPTP opening, thereby preserving mitochondrial integrity even in the presence of other stressors. However, despite these protective mechanisms, UDCA monotherapy failed to fully normalize creatinine levels (1.77 mg/dL vs 1.32 mg/dL in the combination group). This limitation suggests that while UDCA is effective at preventing further injury by reducing the toxic load, it lacks intrinsic regenerative capacity to repair nephrons that have already sustained structural damage during the initial two weeks of untreated cholestasis.

The Hu-MSC-S monotherapy group (K3) demonstrated a remarkable reduction in renal injury markers, numerically surpassing the efficacy of UDCA alone. This finding is significant as it implies a direct renoprotective effect independent of bile acid metabolism. The efficacy of the secretome is attributed to its rich composition of trophic factors and extracellular vesicles (EVs). Specifically, the secretome is known to contain high concentrations of hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF). HGF is a potent mitogen for renal tubular epithelial cells. In the context of AKI, HGF binding to its receptor (c-Met) activates downstream signaling pathways such as PI3K/Akt and MAPK/ERK, which promote cell survival, proliferation, and migration, essential processes for re-epithelialization of the denuded basement membrane. Furthermore, HGF acts as a natural antagonist to Transforming Growth Factor-beta 1 (TGF-β1), the

master regulator of renal fibrosis. By inhibiting TGF-β1/Smad signaling, the secretome likely prevented the transition of epithelial cells into myofibroblasts (Epithelial-Mesenchymal Transition), thereby preserving renal architecture. VEGF, another critical component of the secretome, plays a vital role in maintaining the peritubular capillary network. Cholestasis is associated with profound renal vasoconstriction and ischemia. VEGF promotes angiogenesis and improves renal microcirculation, thereby reducing hypoxic injury to the metabolically active proximal tubules. Additionally, the secretome contains Insulin-like Growth Factor-1 (IGF-1), which has been shown to accelerate recovery from acute tubular necrosis by stimulating protein synthesis and inhibiting apoptosis. Crucially, the secretome also exerts immunomodulatory effects. Extracellular vesicles within the secretome carry miRNAs that can be internalized by renal macrophages. In the injured kidney, macrophages typically adopt a pro-inflammatory M1 phenotype, secreting nephrotoxic cytokines such as TNF-alpha and IL-1beta. The MSC secretome promotes the polarization of these cells toward an anti-inflammatory M2 phenotype. M2 macrophages secrete Interleukin-10 (IL-10) and trophic factors that resolve inflammation and facilitate tissue remodeling. This shift in the immune microenvironment allows for a transition from a state of active tissue destruction to one of repair and regeneration.[20]

The superior performance of the Combination group (K4) provides strong support for a two-hit therapeutic strategy. The synergy observed is likely not purely mathematical but biological, arising from the complementary mechanisms of the two agents. Upstream Attenuation (The First Hit): UDCA acts upstream at the level of the systemic circulation and the liver. By displacing toxic bile acids, UDCA effectively turns off the tap of nephrotoxins flowing to the kidney. This creates a permissive environment where ongoing cellular injury is minimized. downstream regeneration (The Second Hit): Concurrent with the reduction in toxic load, the Hu-MSC-S provides the active reparative signals directly to the renal tissue. Without the reduction in toxic load provided by UDCA, the regenerative efforts of the secretome might be overwhelmed by the continuous chemical assault. Conversely, without the regenerative signals of the secretome, UDCA would only prevent new injury, leaving the kidney to rely on slow, endogenous repair mechanisms to heal established necrosis. The combination of reducing the insult (UDCA) while simultaneously promoting repair (Secretome) accelerates functional recovery beyond what either agent could achieve in isolation. This is evidenced by the Delta Creatinine improvement in the combination group (1.53 mg/dL) being more than double that of the UDCA group (0.65 mg/dL), illustrating a potent biological amplification of therapeutic benefit.[17,18]

A unique aspect of this study is the administration of human-derived proteins (Hu-MSC-S) to immunocompetent rats. Typically, xenogeneic administration would be expected to elicit a neutralizing antibody response or immune rejection, potentially diminishing therapeutic efficacy over repeated dosing. However, the sustained efficacy observed over four weeks suggests a lack of significant rejection. This may be explained by the phenomenon of hepatic tolerance or high-zone tolerance. The liver is a tolerogenic organ; in states of severe liver injury and antigen overload (such as CBDL), the systemic immune response is often dampened or shifted toward anergy. Furthermore, the intraperitoneal route of administration directs the secretome into the portal circulation, exposing it first to the liver's resident immune cells (Kupffer cells), which may facilitate tolerogenic processing of the foreign antigens. This immune privilege allows the human factors to exert their biological effects across species barriers in this specific model of hepatorenal failure. The decision to administer the secretome intraperitoneally was strategic. In hepatorenal syndrome, renal failure is often functional, driven by hepatic failure. By administering the secretome into the peritoneal cavity, the bioactive factors are absorbed primarily into the

portal vein, delivering a high concentration first-pass bolus directly to the liver. This likely improves hepatic function (albumin synthesis, detoxification capacity) more effectively than systemic intravenous administration. Improvements in liver function translate secondarily to the kidney by improving effective arterial blood volume and reducing the hepatorenal reflex. Thus, the renoprotection observed in the Hu-MSC-S groups is likely a composite of direct renal uptake of systemic factors and indirect benefits derived from improved hepatic status.

It is important to acknowledge that this study focused on functional biochemical outcomes. While urea and creatinine are robust clinical markers of glomerular filtration and nitrogen retention, they do not provide structural visualization of the renal tissue. The term regeneration used in the context of this discussion refers to functional restoration, and while the biochemical data strongly imply tissue repair, histological confirmation via specific staining for necrosis or fibrosis was not included in this dataset. Additionally, the standardization of the secretome was based on cell culture volume rather than total protein quantification, which is a variable inherent to biological products. Finally, the use of a xenogeneic model without immunosuppression, while successful here, warrants careful consideration in future translational study designs.[19,20]

5. Conclusion

This study establishes that the combinatorial therapeutic regimen of human mesenchymal stem cell secretome and Ursodeoxycholic Acid exerts a potent ameliorative effect on renal dysfunction in a rat model of cholestatic injury. The findings demonstrate that this dual-modality approach significantly outperforms standard UDCA monotherapy in reducing serum urea and creatinine levels. The mechanism appears to follow a complementary two-hit paradigm: UDCA attenuates the primary toxic insult by modulating the bile acid pool, while the Hu-MSC-S actively promotes functional restoration through paracrine trophic signaling and immunomodulation. This synergy offers a promising translational framework for the development of novel adjuvant therapies for patients with refractory hepatorenal disorders, bridging the gap between conventional pharmacology and regenerative medicine.

6. References

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