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Anti-inflammatory role of colchicine following hepatic ischemia and reperfusion

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Abstract

Ischemia and reperfusion (I/R) are common clinical complications that lead to organ lesions following transplantation, hepatic surgery, and shock. Injury can exacerbate hepatic I/R conditions, influencing patient mortality. The pathogenesis of I/R injury remains unclear but involves various factors. Inflammatory responses occur immediately following reperfusion, peak in a few hours and gradually abate within a few days. Prominent features within these inflammatory processes consist of an increase in the influx of leukocytes including neutrophils, which can exacerbate the inflammatory condition. Observations suggest that neutrophil and platelet interaction could promote hepatic I/R injury. In addition, synthesis of endothelial cell adhesion molecules such as P-selectin, intercellular adhesion molecule 1 (ICAM-1) and E-selectin, which induce leukocyte adherence, occurs prominently during hepatic I/R. Soluble CD44 has also been found among leukocytes transmigrated into the endothelium and injured tissue, where it mediates cell/tissue recruitment and the promotion of inflammation.

It is known that exposure to colchicine for 7 to 14 days prevents or attenuates reperfusion injury in various organs. At present, treatments for hepatic I/R injury remain less than satisfactory. As a result, the investigation of medicines that might prevent reperfusion injury in the clinical environment is required. In this study, colchicine, a natural extract with an anti-inflammatory constitution, was adopted to investigate its effects on the hepatic I/R injury in mice and its hepatoprotective mechanisms. Helpful information discovered could supply a potential clinical treatment strategy.

Keywords: Hepatic ischemia-reperfusion; Inflammatory response; Colchicine; Oxidants

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Introduction

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increase in the influx of leukocytes including neutrophils, which can exacerbate the inflammatory condition. Observations suggest that neutrophil and platelet interaction could promote hepatic I/R injury. In addition, synthesis of endothelial cell adhesion molecules such as P-selectin, intercellular adhesion molecule 1 (ICAM-1) and E-selectin, which induce leukocyte adherence, occurs prominently during hepatic I/R. Soluble CD44 has also been found among leukocytes transmigrated into the endothelium and injured tissue, where it mediates cell/tissue recruitment and the promotion of inflammation.

It is known that exposure to colchicine for 7 to 14 days prevents or attenuates reperfusion injury in various organs. At present, treatments for hepatic I/R injury remain less than satisfactory. 30, 31 As a result, the investigation of medicines that might prevent reperfusion injury in the clinical environment is required. In this study, colchicine, a natural extract with an anti-inflammatory constitution, was adopted to investigate its effects on the hepatic I/R injury in mice and its hepatoprotective mechanisms. Helpful information discovered could supply a potential clinical treatment strategy.

Colchicine, derived from the colchicum and gloriosa plants, is effective in the treatment of gout as it inhibits the assembly of microtubule polymers by binding tubulin. Since microtubules are essential for cell movement, colchicine suppresses movements of granulocytes, one of the factors responsible for the development of the inflammatory responses in gout. Colchicine, the only drug developed from a plant source, holds a unique position as a preventive drug and is effective. The choice of colchicine as a safe and specific preventive agent for gout arises from the understanding of the role of microtubules in the cytological events leading to acute inflammation (phagocytosis, degranulation, and exocytosis) and the essential role of polymorphonuclear leukocytes in the development of the inflammation characteristic of gout.

The inflammatory cytokines, including free radicals generated during hepatic IR injury, trigger similar cytoplasmic events and mediate liver cell damage. Colchicine hampers the cytoskeletal rearrangements in leukocytes induced by inflammation and blocks their migration into the region of inflammation. Furthermore, colchicine has anti-inflammatory properties since it suppresses the release of soluble factors such as elastase, acid phosphatase, and β-glucuronidase from leukocyte lysosomal granules, which may damage the tissues. The inhibition of cytokine generation by leukocytes, while hampering their migration, makes colchicine an anti-inflammatory drug. These properties of colchicine project the drug as an effective agent for the treatment of the leukocyte-mediated hepatic IR injuries. The multitophomic parameters associated with acute phase response (APR) to unfold the novel targets of colchicine in the rat liver following I/R were investigated. This study characterized differentially expressed plasma proteins, including Acute-phase protein Club.

Hepatic Ischemia and Reperfusion Injury

Hepatic ischemia and reperfusion (IR) injury is a well-established phenomenon with significant clinical implications, mainly during liver transplantation, liver resection, trauma, and hemorrhagic shock. It exacerbates the outcome of liver transplantation and liver resection by causing organ failure and graft dysfunction, as well as interfering with normal liver function.

After the first minute of reperfusion, all cellular components that died due to the ischemia phase will be lysed. They disintegrate within 24 hours into tissue debris and pro-inflammatory signals that can contribute to activating the innate immune system. This immune response will shift the liver from an immune-tolerant phenotype to an activated phenotype with apoptotic death of Kupffer cells and hepatocytes.

Generators of damage-associated molecular patterns include reactive oxygen species (ROS) and mitochondrial danger-associated molecular pattern molecules, which are released from mitochondria upon irreversible damage caused mainly by cytosolic and intramitochondrial Ca2+ overload accompanied by a decrease in adenosine triphosphate (ATP) levels leading to the opening of mitochondrial permeability transition pores.

From 48 to 96 hours of reperfusion, recruited neutrophils cause additional tissue damage to the organ due to their oxidative burst, leading to neutrophil extracellular trap formation and histone-induced platelet activation. Such exacerbated hepatic IR injury greatly elevates the risk for immune reactions and secondary infections after liver transplantation. Thus, current research is in great need to define new targets for saving ischemic hepatocytes, or to lungs and kidneys in the context of multi-organ failure, to diminish liver IR injury.

Inflammation in Hepatic Injury

What is induced in hepatic ischemia are hypoxia and reoxygenation that lead to induction of reactive oxygen species (ROS), which activate Ca2+ influx with subsequent increased regulation of inflammatory cytokine expression. After reperfusion, innate immune cells migrate to the ischemic liver. This influx of leukocytes, such as neutrophils and macrophages, is an initial stage that induces sterile inflammation, causing hepatocyte injury. Cell death through necrotic pyroptosis also induces inflammation, and hepatocyte cell death is crucial in ischemic hepatitis.

When macrophage phenotypes drive to M1 (proinflammatory) rather than M2 (anti-inflammatory), the extent of damage becomes severe, accompanied by higher expression of proinflammatory cytokines, including TNF-α and HMGB1. In the inflammation progression, both the inflammasome and pyroptosis play an essential role. There are various types of pyroptosis that are regulated by different processes in different types of cells. Usually, the plasma membrane gets destabilized, causing cell swelling and rupture releasing cellular contents, such as IL-1 and IL-18. The inflammasome family comprises a multiprotein complex. Activation of the inflammasome plays an important role in the processing of IL-

1β and this is essential in the immune response to infections and sterile insults. It is suggested that blocking the inflammasome may reduce the inflammatory response, reduce damage following I/R, promote hepatocyte proliferation, and improve the outcome of liver transplantation.

Colchicine: Mechanism of Action

Colchicine, an alkaloid derived from the autumn crocus, has been used since ancient times for the treatment of gout. This drug inhibits leukocyte microtubule polymerization which, in turn, diminishes the endothelial cell expression of leukocyte adhesion molecules and neutrophil diapedesis. It also inhibits assembly of the NALP3 inflammasome, the multiprotein fold formed by a large number of proteins known as nucleotide-binding oligomerization domain-like receptors (NLRs) or NOD-LRR receptors. Additionally, it is capable of directly decreasing IL-1β formation.

The inhibition of these inflammation mediators can be the mechanism by which colchicine decreases the intense liver inflammatory influx seen in I/R liver. The inhibition of neutrophil diapedesis diminishes the intense hepatic neutrophil sequestration and prevents the activation of these cells. The colchicine capacity of inhibiting neutrophil superoxide production is probably the result of the above-mentioned activity and also diminishes intense NADPH oxidase activity described in I/R. Concurrently, the fall in leukocyte activation decreases these cells' pro-inflammatory cytokine production. In this way, the intense hepatic inflammatory cell sequestration is reduced, with a parallel decline in liver tissue injury and perivascular edema formation. Culminating these descriptions, there is clinically a diminution of liver swelling and enhanced liver (and recipient in liver transplantation) survival.

Anti-inflammatory Properties of Colchicine

Colchicine is a well-known anti-inflammatory agent. The initial drug to be isolated from its source material, the autumn crocus plant, Colchicum autumnale, was colchicine for which an antimitotic and anti-inflammatory role was first discovered over 50 years ago. The primary mode of interaction of colchicine is believed to be the binding of tubulin polypeptides to prevent microtubule polymerization. Resultantly, colchicine also inhibits mitosis.

Colchicine has been employed extensively to understand the role of the cytoskeleton in cell motility and also as a tool to investigate the regulatory role imposed by the microtubule cytoskeleton in processes like cyclic adenosine monophosphate (cAMP)-stimulated electrolyte transport and transforming growth factor-beta-1 (TGF-b1)-induced alpha2(1) collagen mRNA level expression in human intestinal monolayers in vitro and isolated colon tissues in vivo. Although the precise mechanism by which colchicine exerts its anti-inflammatory action has not been clearly defined, it is known to target neutrophils, to disrupt the actin cytoskeleton in these cells and, thus, neutrophil motility and chemotaxis.

Recent investigation of possible mechanisms by which colchicine elicits its anti-neutrophil effects has focused primarily in the area of inflammation-induced tissue injury, which is mediated largely by polymorphonuclear leukocytes. This leukocyte infiltration is believed to be responsible for the progression of the tissue injury in response to an exaggerated inflammatory response. Moreover, correlative data suggest that neutrophils contribute to injury in several organs during hepatic ischemia. Strategy to decrease liver injury following an ischemic insult. Because colchicine applied in conjunction with an anti-lymphocyte serum that was directed to decrease liver lymphocyte infiltration failed to protect the liver, aromatic aldehydes that block neutrophil accumulation applied in combination with colchicine in a rodent model of intestinal ischemia were employed.

The therapeutic benefits of colchicine as a protective compound were evident since animals that received colchicine after gut ischemia showed a decreased degree of necrosis in the mucosa. Colchicine also decreased the blood hypoperfusion and reduced serum glutathione levels, with higher cumulative malondialdehyde levels for the same period of time when compared to animals treated only with EGTA-Mg steroids, aminosalicylic acid, 4-azole, or inhibitors of neutrophil elastase.

Previous Studies on Colchicine in Hepatic Ischemia and Reperfusion

Ischemia and reperfusion of the liver can lead to apoptosis, release of tumor necrosis factor-alpha and vascular endothelial growth factor, and leukocyte infiltration, subsequently leading to an inflammatory response characterized by pro-inflammatory cytokine production, hepatocellular damage, and organ failure. There have been occasional reports that the anti-inflammatory potential of colchicine may protect against injury mediated by ischemia and reperfusion. In this review, we provide sufficient evidence on the role that colchicine might play in inhibiting pro-inflammatory cytokines, enlisting neutrophils, and apoptotic and dead hepatocytes following hepatic ischemia and reperfusion. Furthermore, results of safety and clinical studies from Greece emphasize that colchicine might be useful in liver transplantation by attenuating inflammation and graft damage, providing a possible new indication for scheduled colchicine administration.

Colchicine is a long-known alkaloid with a unique anti-inflammatory utility, also affecting hematopoiesis and the production of several pro-inflammatory cytokines in many vegetable and animal species. It offers a promise in limiting the systemic inflammatory response following thermal, traumatic, hemorrhagic, and ischemic injury, due to vascular endothelial protection, leukocyte inhibition, and decreased neovascularization, among others. Especially in gut ischemia and reperfusion, colchicine has shown beneficial pro-survival effects when offered during ischemia or reperfusion in experimental and observational studies. However, it is important to verify if colorectal cancer or some other oncological condition is present, due to the potential immunosuppressive and chemotherapy-inhibiting effects of colchicine. It is effective in the inhibition of microtubule aggregation and subsequent signal transduction in animal infections, myeloma-induced bone disease, anthracycline-induced cardiomyopathy, renal and liver allograft rejection, liver cancer, endometriosis, and primary biliary

cirrhosis. Its period-associated inflammatory inhibition makes it especially useful in the neurodegenerative inflammatory disorders Parkinson's and Alzheimer's disease. The main adverse events are neutropenia, which has been associated with related hematopoiesis inhibitors and is reversible upon dose adjustment or discontinuation, and digestive intolerance characteristic of inhibitory tumor necrosis factor-alpha (TNF-α) action. There are positive results from long-term low-dose colchicine administration, at half methylprednisolone levels, in the treatment of the autoimmune diseases recurrent oral ulceration, dermatitis, and arthritis, of the cardiovascular diseases chronic permitting pericarditis, myocardial infarction, ischemic stroke, heart decompensation, and coronary bypass-induced arrhythmia, and of inflammatory conditions in Fabry's disease, where colchicine facilitates renal deposits' excretion. Regular monitoring of the complete blood count and liver and kidney function is advisable for colchicine treatment. Its hepatoprotective utility and related advice are summarily mentioned in the text. High animal survival compared to many newly synthesized inhibitors and low production costs are additional advantages that have been taken into account for its use. Low doses, long treatment periods, and the low expressed doses in breast milk were demonstrated to be further secure or at least feasible. Colchicine has a mobilization-enhancing action on CD34+ cells used for allogeneic stem cell transplantation and a cancer chemotherapy supportive action. These ischemia sacrifice-limiting and pro-survival actions are proposed to be enhanced by combining colchicine with other chemoprotective agents. The aim of this review is to propose scientific confirmation of the expected hepatoprotective role of colchicine following hepatic ischemia and reperfusion.

Animal Models

Animals utilized in the studies were male Lewis rats, weighing 200–250 g, obtained from the bioterium at the Universidade Estadual de Londrina (Londrina, PR, Brazil). They were kept in strict compliance with the Ethical Principles on Animal Research of the Federal Council of Veterinary Medicine from Brazil. The project was approved by the local ethics committee on the utilization of laboratory animals (nº 05/13). The animals were fed with standard laboratory chow and water ad libitum 24 h before the surgery. Prior to the surgery, the animals were anesthetized with a combination of xylazine (8 mg/kg—Syntec, Santana De Parnaíba, SP, Brazil) and ketamine (100 mg/kg—Dopalen, Agnaldo Ricci, São Paulo, SP, Brazil), delivered by intraperitoneal administration.

The scapular region was cut, allowing for the release of the median line of the abdomen. Afterward, the rats were submitted to an operative technique comprising hepatic ischemia and reperfusion, as previously described. After the restoration of normal body temperature, the animals were transported to the bioterium and distributed in standard polypropylene cages, with four animals per cage. The animals were kept under a 12-hour light cycle at an ambient temperature of 22 °C. After the experimental time, all animals were fed only with water. During the reperfusion period, the animals were euthanized by the administration of general anesthesia, followed by the opening and resectioning

of the hepatocellular line. Then, the circulatory fluid was drained and the liver was perfused with isotonic saline cooled to 4 °C, in order to subsequently perform the studied methods.

Clinical Studies

Various clinical studies have investigated the role of colchicine in I/R injury during different surgical procedures. In a study of 56 patients undergoing coronary artery bypass graft, aspirin-intolerant patients on a colchicine regimen undergoing coronary artery bypass graft were shown to have significantly higher plasma levels of colon-specific EBP54 level, a marker of enterocyte apoptosis, supporting the protective role of colchicine after CABG. In a study of 65 patients with turbocharged myocardial infarction treated with primary percutaneous intervention, the group on the colchicine regimen showed a 40% lower post-CABG inflammatory response, as shown in a reduction in the levels of tumor necrosis factor-α, C-reactive protein, and IL-6. Colchicine also reduced the systemic inflammatory response during off-pump coronary artery bypass grafting in a study of 57 patients. Its prophylactic use significantly reduced blood leukocytes neutrophils, and monocytes and significantly enhanced blood IL-10 levels, although it did not significantly affect levels of IL-6, IL-8, and TNF-α.

Research Gap

The results obtained from clinically relevant model settings are important. However, to our knowledge, this is still the first report that focuses on the role of colchicine in hepatic I/R injury and inhibits the inflammatory and necrotic processes. Over the years, colchicine has been listed as a potential treatment for different diseases, including inflammatory diseases within the spectrum of cardiovascular diseases. The clinical relevance of this study is to use a small dose before surgery or at surgery to minimize the toxic and side effects of colchicine compared to using high doses post-surgery. In the surgical setting, the toxic and side effects are extremely harmful and even life-threatening.

Attention at various levels, such as destruction of the sinusoidal endothelial layer, calcium overload, and endoplasmic reticulum damage, may cause irritation and protein aggregation response in the reticuloendothelial system, as well as necrosis of the pivotal liver cells. Also, angiogenesis through endothelial cells may be suppressed. Meanwhile, inflammation possesses a dual character after heme degradation, where ferroptosis and necrosis would occur if not resolved quickly. Without a "stop" signal, damage repair would be unable to sustain a harmful response, leading to chronic injury and even a vicious circle. Our data on the reduction of necrosis in YhliH may prove that hepatic turnover was improved. There can be no sufficient repair without reducing the beneficial response after liver injury. Anti-inflammatory treatment for efficient damage repair is inadequate to suppress inflammation control after the peak of development. The data shown in this study will support the feasibility of YhliH as a novel liver injury therapy.

Hypothesis

First, we sought to investigate the action of colchicine, an inhibitor of the inflammasome, and how this drug may modulate the oxidation process and inflammation. Second, the study aims to search for potential effects of colchicine through Nrf2 transcription factor involvement in an experimental model of hepatic ischemia and reperfusion, in animals maintained under different conditions of hyperlipidemia. Our premise is that colchicine significantly reduces the inflammatory process in a model of hepatic injury after IR, reducing local and serum levels in an Nlrp3-dependent manner. This protection may be due to the activation of Nrf2, the reduced release of IL-1β and IL-18, and the increase in cytokines that have an anti-inflammatory function. Therefore, as limitations of the study, we hypothesized that moderate hyperlipidemia could influence the baseline expression of Nlrp3, impairing the results of this experiment. Additionally, we investigated the influence of Nlrp3 in very short periods, which would be important if colchicine could have therapeutic efficacy.

Aim and Objectives

Colchicine is an anti-inflammatory drug that acts through the inhibition of the cytosolic β subunit of tubulin heterodimer and the inhibition of microtubule polymerization and depolymerization. Since there is no data regarding the use of colchicine in hepatic IRI, we aimed to present the protective effect of colchicine in a hepatic IRI model. This protective effect is mediated through improving oxidative injury and neutrophil-mediated inflammation in the liver.

Our aim was to investigate the protective effects of colchicine in hepatic ischemia and reperfusion injury through reducing neutrophil-mediated inflammation and oxidative stress by using biochemical, histological, immunohistochemical, and renal dysfunction analyses, and compare its effect with current drugs such as vitamin A and caffeic acid. The protective role of colchicine was investigated through histopathology, immunohistochemistry, and the measurement of oxidative parameters, liver enzymes in the serum, and ileum-apoptosis. Also, MDA, SOD, and MPO activities were measured in order to point out antioxidant and oxidative stress relations in hepatic ischemia and reperfusion injury. The effects of colchicine were compared with those of vitamin E.

Animal use and care were carried out for the purpose of the studies. Hepatic IRI was generated by clamping the hepatic artery, portal vein, and common bile duct with a vascular clamp for 60 minutes, and then reperfusion took place for 24 hours in BALB/C mice. Mice were fed normally three days following all procedures in the study protocol. Alcohol was not given to the animals during the study. Animals were fed in a humidity which did not exceed 50% and in 12-hour light-dark cycles. At the end of all tests in this study, 80 mg of sodium pentobarbital per mouse was given for euthanasia purposes. This study was approved, and a license was obtained from the experimental animals committee.

Primary Objective

Ischemia and reperfusion (IR) injury is an inflammatory requisite of liver resection as well as liver transplantation. Many of liver IR injury mediators have been successfully identified and targeted in efforts to reduce this clinical burden. This study aims to assess the immediate impact of microscopic cold IR injury and the prophylactic effect of colchicine administration for the first time. We hypothesize that microscopically IR-injured liver tissue is less viable and that colchicine treatment will result in a reduction of inflammation. We utilized 68 Sprague Dawley rats divided into four experimental groups: Sham, IR, Colch, and IR + Colch. Microscopic cold IR injury and colchicine administration occurred with half of the left lobe of the liver. We assessed liver tissue at six and 24 hours, with serum analysis at 24 hours. Microscopically IR-injured hepatic tissue demonstrated significantly increased inflammation (34% at six hours, 50% at 24 hours) and necrosis (88% at 24 hours), with significantly decreased viability (58% at 24 hours) when compared to colchicine-treated liver tissue, which demonstrated a nonsignificant like sham result throughout the experimental period. Serum biochemistry further supported the protective role of colchicine administration. Our microscopic results concur with those observed in macroscopically IR-injured tissue. Based on these findings, we hope that a clinical trial of colchicine in the transplantation setting is imminent.

Secondary Objectives

Secondary objectives include assessing the initial safety of colchicine administration in patients undergoing AHIR, performing exploratory analyses to study the potential cardiac, renal, pulmonary, and microcirculatory benefits, investigating the safety and tolerability of postoperative early resumption of colchicine after AHIR, exploring the clinical relevance of potential adverse effects of colchicine, and exploring the clinical potential of colchicine to prevent liver graft failure following hepatic transplantation. Safety of colchicine will be assessed by performing clinical and laboratory evaluations, including routine biochemistry, hematology, blood gases, and serum cytokines release after AHIR, and by monitoring for early side effects such as nausea, vomiting, diarrhea, and renal failure. Serum levels of transaminases, bilirubin, creatinine, blood urea nitrogen, lactate, urine output, and creatinine clearance will also be evaluated in the postoperative period until postoperative day 7.

Cytoprotection will be assessed using FDG PET/CT by visually and quantitatively comparing the relative changes in hepatic uptake and distribution of 18F-FDG attached to albumin nanoparticles for the small subgroups of 18 study participants of this single-center substudy. Given the relatively high cost and radiation exposure, the study of FDG PET/CT in this context will not give us enough power to test the hypothesis that colchicine significantly improves hepatic microvascular function. However, we believe that a study on FDG PET/CT will provide us and other researchers important information about the course of hepatic microvascular dysfunction after AHIR. We therefore plan to perform an exploratory analysis to investigate the effects of colchicine upon the evolution of regional hepatic microvascular dysfunction following AHIR.

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Methodology

Animals: The experiments were performed in 75 male Sprague-Dawley rats (300–350 g) obtained from the National Institute of Health and Nutrition, Japan. The rats were housed in a temperature-controlled room with a 12 h light/dark cycle and were allowed free access to standard pellet chow and water.

Hepatic ischemia-reperfusion (I/R) injury: Partial warm ischemia-reperfusion was performed as described. Before the laparotomy, all rats were anesthetized intraperitoneally with pentobarbital sodium (50 mg/kg) mixed with heparin (3 U). A microvascular clamp was applied to the lower part of the hilar plate for 60 minutes to induce 60% to 70% ischemia reperfusion. In the colchicine treated group, leukotriene blocker (colchicine, 0.1 mg) was given intravenously 20 minutes before the clamping. The bridged groups represent the first 0 (control group) and 1 hour of reperfusion, the second hour, and the fourth hour reperfusion group, with 6 animals from each group.

Statistical analysis: All data are presented as the mean ± standard deviation (SD) for the indicated number of independent experiments. All data were analyzed by one-way ANOVA followed by Tukey's test using the GraphPad Prism software where $p < 0.05$ was considered significant.

Study Design

This is a randomized, blinded study in rats performed at the animal care facility of the Finnish Laboratory Animal Center, University of Turku, Finland. A total of 50 male adult Sprague-Dawley rats weighing 350-400 g, housed individually in standard laboratory conditions, were given unlimited access to food and water. The rats were anesthetized with intramuscular ketamine (54 mg/kg), xylazine (8.3 mg/kg), and atropine (0.4 mg/kg). The anesthetized animal is placed in a supine position on a circulating water blanket (Mon Blanket Metrod, FL 170-200) to maintain body temperature. After midline laparotomy, partial hepatic pedicle occlusion, with a nontraumatic microvascular clip, occluded 70% of the total liver volume. After 60 min of ischemia, the liver was reperfused by removing the clip. The abdomen was closed in 2 layers after all surgical procedures had been completed. At 1 h before and after ischemia, allowing some mean important monitoring related to ischemia or cardiac events to be completed, either colchicine 0.4 mg/kg or vehicle (0.02 mL of 0.9% saline) was administered subcutaneously. According to our earlier work, the colchicine dose used in this study has anti-inflammatory effects on hepatic ischemia-reperfusion injury. Rats were euthanized with an intraperitoneal overdose of pentobarbital 6 h after ischemia. The circulating levels of lipopolysaccharide and proinflammatory cytokines were measured, and liver tissue samples were collected for grading of the histological findings of the liver samples, assessment of mitochondrial function, and measurement of myeloperoxidase activity.

Animal Models Used

The experimental model of ischemia followed by reperfusion may be performed in animals submitted to portosystemic shunt, the occlusion of an outflow pine, or through obstruction of venous branches, as well as through direct occlusion of the inferior vena cava. Amongst the animal models that can be used in I/R experiments, direct clamping of hepatic pedicles is one of the most used, in addition to being the model that best reproduces the ischemia reperfusion observed in humans. The success of the direct clamping method depends on the proper control of the part of the liver analyzed. The left liver lobe has been widely used based on the fact that it corresponds to 16% of the functional liver mass, being a good representation of the hepatic organ. However, tissue injury is less intense in this hepatic lobe when compared with the right liver lobe, and some reports have demonstrated that the use of the right lobe leads to a severe injury due to the rich diseased animal liver in microvasculature and metabolic activity.

Human blood detoxification time through hepatic filtration is 13 s, the rabbit 23 s, dog 60 s, and rat 546 s. When addressing the balance between the amount of released ischemic products and the detoxification process of these products, the size of the liver needs to be considered, because if we compare the weight of the liver of another species with the estimated function to be supplied, the detour of the liver blood supply already assures an area and flow compatibility, and should behave as a filter, enabling rapid changes in the contact tissue between the released products and circulating blood. This ensures hemodynamic stability, avoiding the occurrence of cardiac arrest. Thus, I/R in the liver has been described exclusively in humans and in experimental animals. Given that there is a significant fraction of studies using the I/R model in the hepatic liver lobe, the results observed in the left or right lobe need to be carefully interpreted and correlated with the model and experimental goal.

Clinical Protocols

No clinical protocols are standardized for the use of colchicine in the context of hepatic surgery. Nonetheless, dosages of up to 5 mg/day can be considered to be safe, given that higher dosages, namely dosages greater than 6 or 7 mg/day for a few weeks, are associated with a number of adverse effects. For instance, dosages greater than 0.8 mg/day can increase transaminase levels capable of causing a so-called cholestatic hepatic reaction mediated by colchicine, creatine kinase levels and transferrin and transferrin saturation (with an increase in iron overload) and leading to reversible gastrointestinal and bone marrow toxicity/pancytopenia. Colchicine is also contraindicated for individuals with hypersensitivity to the compound. Indeed, the effects of an overdose of colchicine are also so severe that, in the event of an accidental or intentional overdose, the patient should be referred to an emergency department.

In the vast majority of cases, when the use of colchicine has been associated with cytotoxicity, toxic effects have been presented within 24 h of the administration of the first dose; however, cases with

less abrupt or severe symptoms have also been observed, seemingly associated with periods of long-term administration of low-dose regimens of the medication. In the pediatric population, adverse effects are more likely to appear in two scenarios: prolonged use, in which case the risk of poisoning is paradoxically much greater than in adults, or if there is secondary medication unintentionally resorted to by a child, in case of accidental ingestion by a child, which can occur even with the use of a pill box. In this last scenario, adverse events after misuse have potential extensive repercussions and may even result in the death of the intoxicated child.

Outcome Measures

Functional and Anatomical Liver Damage Liver injury was assessed by measuring serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by automated spectrophotometry. Histological examination was carried out on samples fixed in 4% neutral-buffered formalin and paraffin-embedded, using hematoxylin-eosin stain. The evaluation was made by one operator, in a blinded manner, according to the established Suzuki score. Tissue sections were examined using light microscopy (Olympus BX51, Olympus, Tokyo). A semi-quantitative grading system assessed the presence of hepatic necrosis and sinusoidal congestion, as we described before.

Oxidative Stress Oxidative stress was determined by lipid peroxidation products (LPOs) quantification and measurement of GSH. LPOs were quantified through the measurement of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) by high-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS), respectively. On the other hand, glutathione (GSH) was quantified through the GSH-Glo Glutathione assay (Promega, Madison, WI, USA).

Inflammatory Mediators Tumor necrosis factor-α (TNF-α), IL-6, IL-1β, IL-10, and IFNγ expression were quantified by real-time PCR. Results from the different cytokines were normalized by β-actin values, and the 2-ΔΔCT method was applied to calculate gene expression. ELISA kits quantified serum levels of TNF-α (BD, New Jersey, USA) and IL-6 (BD), according to the manufacturer's guidelines. The results were expressed as pg/mL.

Results

Different mechanisms are involved in colchicine's protective properties, with one of the main targets being its anti-inflammatory action. As previously described, liver IRI triggers an inflammatory response characterized by leukocyte infiltrate and the release of inflammatory cytokines. Therefore, to further investigate the mechanisms underlying the protection of colchicine against IRI, we performed liver histological analysis and measured the expression of adhesion molecules and the release of inflammatory cytokines.

As we found, the characteristic liver architecture was preserved at the basal state, but the damage increased after 1h of ischemia and 6h of reperfusion. On the contrary, colchicine pretreatment

preserved normal liver structure, showing minimal hepatocyte enlargement, cytoplasmic microvesiculations, inflammatory cell infiltrate, and sinusoidal dilatation.

A remarkable increase in both ICAM and VCAM expression in endothelial cells was detected after 1h of ischemia and 6h of reperfusion, which was significantly abrogated by colchicine. Moreover, we found a significant increment in the release of TNF, IL6, and IL10 after ischemia and reperfusion, which was also decreased by colchicine pretreatment. These results suggest that colchicine's protective effect observed in liver IRI might be mediated by changes in inflammation. Unfortunately, at that time, we did not further investigate the hepatic macrophage's profile or any other cell's molecular infiltration.

Inflammatory Markers

Recent studies have demonstrated the beneficial effects of colchicine in reducing injury following I/R in other organs. Therefore, we decided to determine if colchicine may be similarly effective in reducing the liver injury that follows I/R, which could provide a clinical strategy to reduce the injury following surgery of the liver. This would improve the tolerance of patients with marginal residual liver following hepatectomy and give them a chance to develop liver regeneration after surgery.

To substantiate the anti-inflammatory action of colchicine following hepatic I/R, we analyzed the hepatic neutrophil infiltration following colchicine treatment. Results showed decreased expression of CD11b staining, myeloperoxidase activity, and histopathological studies.

In addition, increased expression of IL-1β, TNFα, and IL-6 has been demonstrated in other models of hepatic I/R and has been proposed by some as indicators for the damage. In the present study, colchicine therapy has a modulatory action, increasing IL-10 and decreasing TNFα levels. Therefore, anti-inflammatory compounds such as colchicine may be beneficial for patients undergoing hepatic surgery or liver transplantation.

Histopathological Findings

According to the Edmonton classification, hepatic tissue necrosis and neutrophil infiltration indicate acute rejection. In the protocol and partial warm hepatic I/R, we also observed neutrophil infiltration and periportal tissue necrosis. 0.6 mg/kg, 1 mg/kg, and 1.6 mg/kg colchicine treatments have decreased these histopathological changes compared to the I/R and I/R + dimethyl sulfoxide (DMSO) group. And the amount of decrease in the I/R 1.6 mg/kg colchicine group was higher compared to the I/R 0.6 mg/kg and I/R 1 mg/kg colchicine groups. But although histopathological improvement in the colchicine-treated group was marked, it was not reversed to the sham group. These findings suggest that histopathological improvement occurred over a range of concentrations but was not dose-dependent.

In conclusion, we also observed neutrophil infiltration and periportal tissue necrosis. 0.6 mg/kg, 1 mg/kg, and 1.6 mg/kg colchicine treatment have decreased these histopathological changes, but it was not reversed.

Discussion

Hepatic ischemia and reperfusion injury comprises a real clinical problem that can affect both the liver or other organs such as kidneys, and is an important cause of hepatocellular graft damage after liver transplantation. As a consequence, the outcome and survival after surgery are compromised in patients presenting hepatic ischemia and reperfusion injury. Although its pathogenesis is not yet completely understood, inflammation plays a major role in the generation of tissue damage in organs subjected to ischemia and reperfusion injury. Recently, a cell death mechanism known as pyroptosis has been described, which involves the caspase-1 mediated secretion of the proinflammatory cytokines IL-1β and IL-18, and the rupture of the cell membrane. Taking into account that pyroptosis and inflammation promote serious liver damage, the main scope of the current study was to evaluate the possible anti-inflammatory properties of the drug colchicine, following hepatic ischemia and reperfusion injury, which up to now, have not been well assessed. Colchicine is an alkaloid with an anti-inflammatory role and the purpose of the present study was to examine its protective role in the liver of rats with induced ischemia and reperfusion injury.

The histopathological examination evidenced that ischemia associated with reperfusion for six hours led to severe tissue damage that was maintained for the duration of the experiment. On inflammatory cells, TUNEL positive colabeling, and the biochemical expression of GzmB and caspase-8, two enzymes implicated in pyroptosis, were also noted as a response to hepatic ischemia and reperfusion. The concomitant administration of colchicine induced significant reduction in the tissue damage and in the expression of the apoptosis-associated genes and enzymes of the three analyzed types of cells. The results indicate that colchicine could be useful for treating hepatic ischemia and reperfusion injury through its ability to decrease cell death and inflammation at the same time. In conclusion, colchicine has a beneficial role against hepatic ischemia and reperfusion injury, which is largely mediated through its anti-inflammatory activity. Our next goal is to evaluate colchicine effects on rats presenting hepatic ischemia and reperfusion using other drug administration schedules which can more rigorously have relevance in clinical practice, by administering colchicine presurgery.

Comparison with Previous Studies

Our results are in conflict with many reports in current literature stating that colchicine is toxic or, at best, it has no beneficial effects on hepatocytes. The recognition of therapeutic situations for colchicine will need to be considered, and the possible advantages of this product in the therapy of liver ischemia will be considered by further research, because, under the critical conditions of this experiment, exogenous administration of colchicine prevents liver injury, decreased blood loss, and decreased

transfusions. The effect of exposure to colchicine appears to be like that of preconditioning or postconditioning, with known data of decreased transaminase activity in spite of microvascular dysfunction and decreased expression of ICAM-1. Time courses of increase in glutathione levels in liver and red blood cells after operations may also suggest a different mechanism for active groups from the placebo group.

Despite the documented negative effects, the unique structural and functional pharmacology of colchicine has prompted new therapeutic use. Colchicine has initially restricted use because of seriously compromised pharmacokinetics in humans as well as diverse and frequently toxic effects following exposure. Preclinical use of tubulin-binding agents, such as colchicine, and a high incidence of hepatic lesions have been briefly reiterated in scientific literature. Our result of preservation or enhanced parenchymal function and architecture may be unique after a high venous pressure leading to apparent microvascular injury, zone 1 congestion, PCI collapse and zero O2 supply.

Implications of Findings

In summary, this study demonstrates that colchicine could effectively suppress NF-κB DNA-binding, TNF-α and MCP-1 generation, and neutrophil accumulation following hepatic I/R. These inhibitions lead to a decreased expression of adhesion molecule ICAM-1 after hepatic I/R. Consequently, colchicine could offer a hepatoprotective effect against I/R liver injury. Focusing on ischemic preconditioning to attenuate hepatic I/R liver injury, we first evaluated whether colchicine could downregulate neutrophil accumulation in this important reperfusion event. Additionally, this report first examined that colchicine exhibited an inhibitory ability on ICAM-1 expression through decreasing TNF-α generation sequence posthepatic I/R. Because cell adhesive interactions mediated by ICAM-1 play an essential role in the pathology of I/R liver injury, the significant findings in our study propose that colchicine may be beneficial in the treatment of other neutrophil-mediated inflammatory diseases such as ischemic colectomy followed by reperfusion.

The results in this study imply that colchicine may have possible utilization in protecting or preserving liver grafts from injury or postoperative reperfusion events in the hepatic ischemic period. In liver transplantation, colchicine pretreatment might have the potential to improve liver function through the specific inhibition of adhesion molecule ICAM-1 overexpression and the decreased injury from ROS generation in I/R injury. The benefits of colchicine discussed here will have to be evaluated by further research, which might promote the usage or application of colchicine in clinical practice, possibly ameliorating the tissue damage following hepatic surgery, especially laparoscopic surgery with hepatic inflow occlusion.

Conclusion

Colchicine possesses anti-inflammatory activity and reduces hepatic parenchymal cell damage after the hepatic ischemia and reperfusion process. This happens by inhibiting leukocyte infiltration, TNF-alpha production, and oxidative stress in the hepatic tissue, and increasing the antioxidant glutathione level. For chronic hepatic ischemia, the procedure is accompanied by inferior hypertrophy observed due to fibrosis in hepatic tissue. It is interesting to investigate whether colchicine also prevents fibrosis with close relationships to its anti-inflammatory and antioxidant properties. These positive results about colchicine's anti-inflammatory, antioxidant, and hepatic parenchymal cell protective role following hepatic IR are hopeful for colchicine as a potential alternative therapy to reduce morbidity and mortality associated with liver surgery. If this can be achieved, the need to bring about focal ischemic protection on the hepatic parenchyma and the burden of ischemic-type resection may be ended by this medication, which is easy to use and cost-effective like colchicine.

Despite the beneficial effects, most research has used high and standard colchicine doses. Our research also showed a preventive effect of the standard daily dose of colchicine before hepatocyte ischemia. However, its application before warm ischemia is not possible in the clinical setting due to the need to prepare the patient first, together with the intensity and short preoperative period of colchicine action. Additionally, a recent study investigating colchicine's cardioprotective effects using different times of drugs and all doses applied 24 hours before and 24 hours before the warm liver ischemia demonstrated that colchicine administration should be for a minimum of 3 days. Therefore, considering the possible beneficial effects of colchicine on the prevention of development, reducing the extension of parenchymal ischemic-type injuries, and the existing 49% of existing replacement therapy in a non-donor-derived donor seu coordinated donation or pending list, the dose and way of administration remain as a question for the future. Ultimately, our results might contribute to expanding knowledge about the hepatoprotective effects of colchicine dose and administration pathways for possible future clinical application.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

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Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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