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Colchicine at a Low Dose Reduces Liver Inflammation in a Model of Immune-Mediated Hepatitis

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Abstract

Introduction: Colchicine is an anti-inflammatory drug with a limited effect on the systemic immune system and unwanted side effects. Chronobiology and variability-based dosing regimens may improve the efficacy of chronic drugs. This study investigates the effect of colchicine's low-dose and variability-based dosing regimens on Concanavalin-A (ConA)-induced hepatitis in mice.

Methods: ConA was injected intravenously into mice to induce immune hepatitis, a higher dose was used to simulate acute hepatitis, and repeated lower dosages simulated a chronic liver disease. Low-dose colchicine was orally administered to mice via gavage. The chronic model included a sub-group who received colchicine using a variability-based regimen. Studies were terminated after 16 hours and 11 days in the acute and chronic groups, respectively. Mice were tested for AST and ALT serum levels and liver pathology.

Results: Low-dose colchicine improved the well-being of mice in the chronic injury model, significantly decreased the ALT serum levels vs. vehicle-treated mice, and reduced necrosis and lymphocyte infiltration in the liver. Using a variability-based dosing regimen altered the drug's therapeutic effect. Acute colchicine treatment improved the general well-being of mice and showed a trend for a decrease in liver enzymes.

Conclusions: This study suggests that low doses of oral colchicine reduce liver injury in chronic liver damage cases. It was demonstrated by weight gain, overall well-being improvement, and healthier liver tissue appearance. These findings support the idea of testing oral low-dose colchicine in conditions that involve immune system dysfunction.

Keywords: Autoimmune Hepatitis; Colchicine; Concanavalin A; Variability; Microtubules

Abbreviations: ConA- Concanavalin-A; AST- Aspartate Transaminase; ALT- Alanine Transaminase; MTs- Microtubules; NF-kB- Nuclear Factor Kappa B; NLRP3- NLR Family Pyrin Domain Containing 3; TNFα- Tumor Necrosis Factor α; RhoA- Ras Homolog Gene Family Member A; IV- Intravenously; DIC- Disseminated Intravascular Coagulation; NKT- Natural Killer T.

Introduction

Colchicine exerts a limited systemic anti-inflammatory effect when administered in standard dosages. It treats gout, familial Mediterranean fever, pericarditis, and coronary syndrome [1-4]. Using the standard dosages of

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colchicine is associated with numerous side effects, primarily gastrointestinal, limiting its chronic use in many patients. Moreover, loss of response occurs in a substantial percentage of patients who use colchicine chronically. There is a need to find alternative dosing regimens that can improve the efficacy of colchicine without increasing its side effects [5-8]. Variable dosing regimens (chronotherapy), in which the dosage of a drug is adjusted based on the time of day, can improve the drug's effectiveness and reduce side effects by avoiding times when the drug is less effective or more toxic. Microtubules (MTs) are an essential component of the immune system's innate and adaptive arms, playing a role in the dynamics of inflammatory cells [5-7, 9]. Colchicine, a drug that disrupts the assembly of MTs assembly, plays a role in the dynamics of inflammatory cells by blocking the migration and recruitment of neutrophils, attenuates NF-kB induction and NLRP3 inflammasome formation, and suppresses the release of tumor necrosis factor α (TNFα) [5-8, 10-12]. In fibrotic states, colchicine inhibits the activation of the pro-fibrotic protein Ras homolog gene family member A (RhoA), preventing MTs disruption [8,13]. Colchicine, an MTs- depolymerizing agent, inhibits glomerular RhoA activation and attenuates glomerular sclerosis and interstitial fibrosis [8,13]. It explains why colchicine is effective in treating autoimmune and inflammatory conditions. The current study aims to investigate the potential benefits of low-dose colchicine and variable dosing regimens in the treatment of Concanavalin A (ConA) immune-mediated acute and chronic hepatitis.

Methods

Animals and Ethical Considerations

The experiment was conducted on male 84 type C57Bl/6 mice, 11-12 weeks old, weighing approximately 25 grams. The mice were held in the Animal Core of the Hadassah-Hebrew University Medical School. Mice were fed standard laboratory chow, given free access to water, and kept in a standard light-dark cycle. The experiment received ethical approval and oversight from the Hebrew University-Hadassah Institutional Committee for Care and Use of Laboratory Animals (Approval number OPRR-A01-5011).

Study Design

Two models were designed to study chronic and acute hepatitis. In the chronic hepatitis experiment, thirty mice were divided into three groups of ten and given ConA injections on days one and five. In the acute hepatitis experiment, twenty mice were divided into two groups of ten and given a single injection of ConA, and the experiment was terminated 16 hours later. Both experiments used ConA injected intravenously (iv) as the inductive agent for immune-mediated hepatitis

Induction of Immune-Mediated Hepatitis

The ConA (MP Biomedicals, Ohio, and USA) was prepared as described [14]. In brief, ConA was dissolved in 50 mmol/L Tris (pH = 7), 150 mmol/L sodium chloride, and 4 mmol/L calcium chloride. Colchicine from 0.5 mg tablets was dissolved in induced by intravenous tail injection of ConA (10 mg/kg in a volume of 120 ul) on days one and five. The experiment was terminated on the morning of day 11. Acute hepatitis was induced by an intravenous injection of high-dose ConA (20 104 mg/kg in a volume of 120 ul). The experiment was terminated 16 hours after the ConA administration.

Experimental and Control Groups

Table 1 describes the five groups of mice studied. Table 2 shows the times of administration in the variability-based regimen. The acute group was administered low-dose colchicine (0.02 mg/kg) or saline (0.02 mg/kg) by gavage two hours before a single ConA injection. For the chronic hepatitis groups, low-dose colchicine (0.02 mg/kg) or saline was administered by gavage at 10:00 every morning of the 10-day experiment. In the chronic- chronotherapy group, a low dose (0.02 mg/kg) of colchicine or saline was administered two hours before ConA administration at alternating daily administration times. In both fixed-time and alternating-time administration of colchicine chronic hepatitis groups, the studies were terminated on the morning of day 11, meaning a total of 10 full days of exposure of the mice to the ConA. At termination, mice were anesthetized with ketamine and xylazine; blood and tissue samples were collected.

Clinical Evaluation

The general well-being of the mice was examined daily except for days six and seven. Body weights and degree of activity were monitored intermittently and on day 11 before the termination.

Laboratory Evaluation

At the end of the experiment, blood was collected from all groups to analyze alanine transaminase (ALT) and aspartate transaminase (AST) using a standard lab analyzer [14].

Pathology

At the end of the experiment, the liver was removed from all groups of mice and weighed. It was then dissected, fixed in formalin, and embedded in paraffin for histological analysis. The liver was cut into thin sections and stained with hematoxylin and eosin (Sigma). A blinded pathologist performed the analysis. Pictures were taken using an Olympus IX-70 microscope.

Statistical Analysis

Results were analyzed using descriptive statistics and nonparametric tests due to the small sample sizes. The

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Table 1: Treatment and control groups.

Study design	Administration mode	Treatment	Days	Time of treatment administration
Chronic liver damage	Control	Saline	10	10:00
	Fixed-time regimen	Colchicine	10	10:00
	Variability-based	Colchicine	10	Variable, see Table 2
Acute liver damage	Control	Saline	1	14:00
	Treatment	Colchicine	1	14:00

Table 2: Times of Administration in the variability-based regimen.

Day	Time
1	10:00
2	17:00
3	12:00
4	11:00
5	15:00
6	-
7	-
8	10:00
9	17:00
10	12:00

Table 3: Comparison of groups in the ConA-mediated chronic liver injury.

Group		End Weight – Baseline Weight	ALT	AST
Control	Mean	-0.91	69.04	163.46
	Median	-1	71	200
	Std. Deviation	0.43	19.51	105.98
	N	9	9	9
Treated	Mean	-0.58	42	79.54
	Median	-0.62	40.4	83
	Std. Deviation	0.57	10.04	51.07
	N	10	10	10
Variability-based	Mean	-1.02	77.04	92.25
	Median	-0.88	83.15	107.5
	Std. Deviation	0.51	16.56	37.09
	N	8	8	8
Total	Mean	-0.82	61.4	111.28
	Median	-0.89	58.1	107
	Std. Deviation	0.52	21.59	78.55
	N	27	27	27

acute hepatitis model was analyzed with a T-test and the Mann-Whitney test. The chronic model was analyzed using ANOVA, Mann-Whitney, and the Kruskal-Wallis test. Post-hoc analysis was done using the Bonferroni method. Since three groups were analyzed in the chronic model, significance was set as $p \leq 0.017$, and for the acute model, $p \leq 0.05$. Analysis was done using SPSS (IBM, USA) and Microsoft Excel (Microsoft, USA).

Results

Effect of Oral Administration of Low-Dose Colchicine in the ConA-mediated Chronic Liver Injury

Effect of treatment on body weight and well-being: Low-dose colchicine had a beneficial effect on the well-being of the mice. The colchicine-treated mice gained weight earlier than the control group, while the control group mice appeared more lethargic than the colchicine-treated mice and continued to lose weight for a more extended period than the treated mice. No difference was noted in these parameters between mice receiving regular colchicine dosing or the drug's variable dosing. Figure 1A and Table 3 summarize the differences between the groups.

Effect of Treatment on Liver Enzymes: The average ALT and AST at experiment termination were lower in the colchicine-treated mice group, with the ALT being statistically significant (Figure 1B).

Effect of Treatment on Liver Pathology: Low-dose colchicine alleviated liver damage compared to mice in the control group that demonstrated necrosis that was visible in gross pathology (Figure 1C; Figure 2).

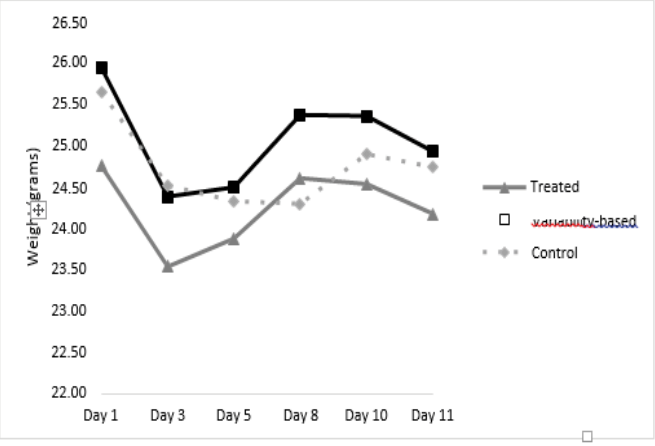

Figure 1A: The body weight of mice in the Con-A-mediated chronic liver injury study group. The body weight of mice in the three groups was followed every 48 hours throughout the study.

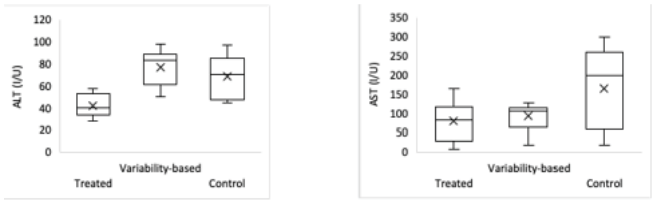

Figure 1B: Liver enzyme levels of mice in the Con-A-mediated chronic liver injury study group. AST and ALT serum levels were measured at the end of the study in the three groups. Using Kruskal-Wallis analysis, the p-value of ALT was 0.001, and AST was 0.256. Using Mann-Whitney analysis, the p-value of ALT was 0.003, and AST was 0.102.

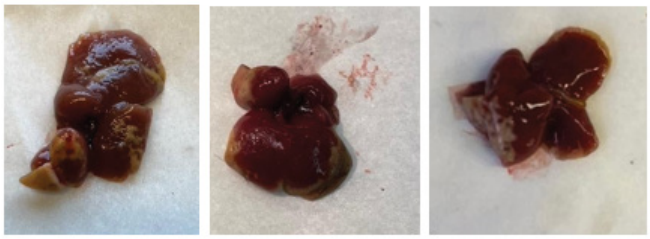

Figure 1C: Gross pathology of livers of control group mice in ConA mediated chronic liver injury showing marked necrosis in these mice.

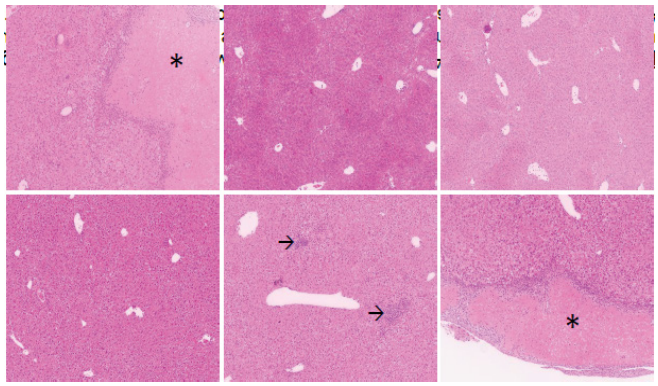

Figure 2: Liver histology of mice in the Con-A-mediated chronic liver injury study group**.** Histological sections of livers from mice treated with colchicine after ConA administration in the chronic model (A) revealed markedly attenuated damage compared with sections of control group livers (B, C, D), in which hepatocyte necrosis was present (* marks the necrotic section) and more prominent lymphocytic infiltrate (→). Livers of mice treated with colchicine after ConA administration in the acute model (E) were similar in appearance to control group livers (F), showing minimal lymphocytic infiltrate and no necrosis.

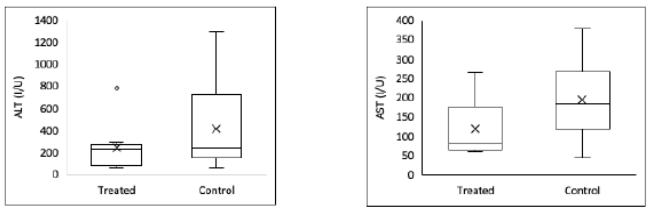

Figure 3: Liver enzyme levels of mice in the Con-A-mediated acute liver injury study group. AST and ALT serum levels were determined at the end of the study in treated and control groups. Using Mann-Whitney analysis, the p-value of AST was 0.094, and the ALT was 0.462.

Effect of Oral Administration of Low-Dose Colchicine in the ConA-mediated Acute Liver Injury

Effect of Treatment on Body Weight and Well-Being: Low-dose colchicine had a beneficial effect on the well-being of the mice. Only two colchicine-treated mice had bloody urine, and one died before termination. In the control group, five mice had bloody urine on the day of termination, and an additional mouse had bloody discharge from his eyes. The bleeding in these mice suggests severe hepatitis with possible disseminated intravascular coagulation (DIC). No significant effect was noted on body weight.

Effect of Treatment on Liver Enzymes: (Figure 3). Oral administration of low-dose colchicine alleviated acute liver injury, and ALT and AST were higher in the control group (p=0.094). There was no statistical significance, probably due to the small sample size.

Effect of Treatment on Liver Pathology: (Figure 2). The colchicine-treated groups showed a reduced lymphocytic infiltrate and no necrosis. As compared with controls.

Discussion

Our data show that oral administration of low-dose colchicine has a beneficial effect on immune-mediated hepatitis. In the chronic model, treatment reduced AST and ALT serum levels, improving liver appearance and pathology. The acute treatment regimen resulted in a clinical improvement and a trend toward improving liver enzyme levels. Colchicine, a plant alkaloid, when administered at a therapeutic dose, targets tubulin, alters MT dynamics and leads to cell cycle arrest and apoptosis [15]. Colchicine is negligibly absorbed in low-dose but retains anti-inflammatory properties by affecting the gut immune system (unpublished). Targeting the gut microtubules by oral low-dose colchicine may be a new gut-related systemic immune system regulatory switch [5-8]. Autoimmune hepatitis is characterized by chronic inflammation. It can present in various forms, from acute liver failure to chronic hepatitis [16]. The primary treatment approach for this condition involves using corticosteroids and azathioprine. However, around 15% of patients do not respond adequately to this treatment [17]. ConA induces immune-mediated hepatitis in mice by activating and recruiting T cells to the liver, predominantly CD4+ T cells and NKT cells. During the early stages of injury, necrosis and apoptosis of liver cells (hepatocytes) occur. Later, there is a significant amount of cell lysis and necrosis [14,18-31]. The ConA model is a helpful tool for studying hepatitis because it shares many similarities with immune-mediated hepatitis in humans, such as autoimmune hepatitis and distinct entities of drug toxicity leading to immune activation [32-34]. The results of the present study show that oral administration of low-dose colchicine prevented the effect in ConA-mediated immune-mediated hepatitis. Oral immunotherapy is a

method for redirecting the immune response and reducing inflammation by targeting the gut immune system. It is based on the concept of oral administration of adjuvants that target the gut immune system and, by that, reduce systemic inflammation. It has the advantage of reducing the unwanted side effect of systemic anti-inflammatory agents. Using oral immunotherapy was beneficial in multiple preclinical models [25, 35-46]. Recent studies showed its beneficial effects in clinical trials of patients with immune-mediated disorders [47-60]. Variability is inherent to biological systems and specifically to multiple immune functions [61-68]. Chronotherapy is a treatment method based on the premise that administering medications at different times of the circadian cycle minimizes side effects and therapeutic response [69]. Using variability-based therapeutic regimens is proposed to improve the response to medications [66, 70-87]. The use of a variability-based therapeutic regimen was tested in the study and was less effective than a fixed-dose regimen, supporting the premise that chronobiology plays a role in the anti-inflammatory effect of low-dose colchicine. Some potential limitations of the study include a small sample size and a lack of comparison with a high dose of colchicine as a control. Additionally, the study's results may have been different if the regimen based on variability had been adjusted to better align with the active period of the mice. In summary, this study suggests that low doses of colchicine administered orally can help reduce liver injury in cases of chronic liver damage. It was demonstrated by weight gain, overall well-being improvement, and healthier liver tissue appearance. These findings support the idea of testing oral low-dose colchicine in conditions that involve immune system dysfunction.

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