RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3569
Majalah Kedokteran Bandung. 2024;56(3):179–183

Majalah Kedokteran Bandung (MKB)

Received: September 2, 2023
Accepted: February 2, 2024
Available online: September 30, 2024

Correlation between Kupffer Cell Infiltration and Liver Parenchymal Cell Damages in Immunosuppressed Drugs-Induced Rats

Agni Febrina Pargaputri, Endah Wahjuningsih, Dwi Andriani
Department of Oral Biology, Universitas Hang Tuah, Surabaya, Indonesia

Abstract

The liver is the largest organ in the body, composed of both parenchymal and non-parenchymal cells. Chemical substances and various drugs can induce liver injury and involve Kupffer cells which are non-parenchymal cells that release biologically active substances, promoting pathological processes. This study aimed to evaluate the correlation between the number of Kupffer cells and liver parenchymal cell damages in immunosuppressed, drug-induced rats. The study was conducted from July to December 2019 at the Oral Biology Laboratory of the Faculty of Dentistry and the Biochemical Laboratory of the Faculty of Medicine at Universitas Hang Tuah Surabaya. Twelve healthy male Wistar rats were divided into two groups: Healthy (H) and Immunosuppressed Drug-Induced (ID) groups. Immunosuppression was induced using dexamethasone (0.5 mg/day/rat), administered orally for 14 days, combined with tetracycline (1%/day/rat). Liver samples from all rats were examined for Kupffer cell count and parenchymal cell damages were assessed using a light microscope with 400x magnification. Results revealed a significant difference in the number of Kupffer cells and liver parenchymal cell damages between the H and ID groups ($p<0.05$). Pearson correlation analysis indicated a significant correlation between Kupffer cell number and parenchymal cell damages ($p=0.000$). Continuous administration of immunosuppressive drugs may activate Kupffer cells, leading to damage of liver parenchymal cells. In conclusion, the infiltration of Kupffer cells is associated with liver parenchymal cell damages, mediated by various factors in the immunosuppressed drug-induced rat model.

Keywords: Immunosuppressed drugs, Kupffer cell, liver parenchymal cell

Introduction

The liver is the largest organ in the body which performs critical and interrelated functions.[1] Liver was made up mostly of parenchymal cells, called hepatocytes, which composed about 78 % of the liver volume, and approximately 6,3 % of non-parenchymal cells.[2] Major functions of the liver include blood filtration and retention; metabolism of carbohydrates, fats, proteins, hormones, and foreign substances; bile formation; storage of vitamins and iron; and the synthesis of coagulation factors.[1,3] Liver also arranges the flux and safety of the compounds that enter the circulation system.[2]

Immunosuppressant drugs are commonly prescribed for various chronic conditions such as rheumatological, neurological, and dermatological conditions, and also reduce the chances of the body rejecting the transplanted organ.[4,5] Immunosuppressants are also known to be the most prominent contributing factor causing harmful responses. Hepatotoxicity actuated by immunosuppressants is troublesome to assess since these drugs are in some cases utilized to treat liver illnesses.[6] The use of long-term and excessive immunosuppressant drugs is known to damage the liver parenchymal cells which will then degrade liver function.[3,9] Previous studies showed that the immunosuppressant drugs administration in 14 days given orally can influence liver albumin levels and serum aminotransferase enzyme levels in oral candidiasis rats model.[10,11] It indicated an impaired liver function and influenced hepatocyte function which crucial to perform drug metabolism.[1,11]

Kupffer cells also called reticuloendothelial cells, tissue macrophages which are distributed along the liver sinusoid, are one of the non-parenchymal cells that have a crucial role in hepatic and systemic homeostasis.[1,2,7] These cells

Corresponding Author:
Agni Febrina Pargaputri
Department of Oral Biology, Universitas Hang Tuah, Jl. Arief Rahman Hakim No. 150, Surabaya, Indonesia
Email: agni.febrina@hangtuah.ac.id

are essential in the pathogenesis of liver disease. They have a role in modulating inflammation, arranging the process of tissue repair, angiogenesis, and fibrosis.[7] Immunosuppressant drugs act as chemical substances that may induce cell damage and injury in the liver.[11] These drugs are digested and absorbed, which then enters the liver with the bloodstream.[12] Chemical substances and several drugs are known to involve Kupffer cells to release biologically active substances that promote pathological processes and induce liver injury.[6] Active Kupffer cells are the main source of inflammatory mediators in injured and necrosis hepatocellular, which can exhibit an increase in cytotoxicity and chemotaxis.[7] The release of inflammatory mediators, growth factors, and reactive oxygen species as the results of toxic agents can activate Kupffer cells, which subsequently have a navel role in liver response to toxicity.[13] Reactive oxygen species (ROS) produced by Kupffer cells are also linked to the induction of inflammation in the liver.[14,15] Persistent inflammation can lead to a reduction in hepatocytes and subsequent tissue damage. Kupffer cells, which function as key custodians in the liver, play a significant role in the initiation and regulation of cirrhosis and liver fibrosis. They contribute to chronic liver inflammation by inducing hepatic stellate cell myofibroblastic transformation, which is associated with the production of ROS, cytokines, and growth factors. The production and regulation of metalloproteinases by Kupffer cells further disrupt homeostatic mechanisms in the deposition of the extracellular matrix, contributing to liver fibrosis.[15]

Several in vitro studies have been developed to assess hepatotoxicity in drug-induced liver injury using primary human hepatocytes and hepatocyte-like cells derived from induced pluripotent stem cells.[16] Research involving experimental animal models and initial clinical trials in humans suggests that targeting Kupffer cells could represent a promising therapeutic approach for treating both acute and chronic liver diseases.[11] Understanding the harmful effects of Kupffer cells in drug-induced liver injury may encourage the expansion of therapeutic strategies to enhance clinical outcomes in liver injury. To date, there has been no research specifically addressing the relationship between the number of Kupffer cells and liver parenchymal cell damage induced by immunosuppressive drugs. Therefore, this study aims to investigate the correlation between Kupffer cell infiltration and liver parenchymal cell damage in immunosuppressive drug-induced rat models.

Methods

The present study used a true experimental design with a post-test-only control group. Research was conducted from July to December 2019 at the Oral Biology Laboratory of the Faculty of Dentistry and the Biochemical Laboratory of the Faculty of Medicine at Universitas Hang Tuah Surabaya. The subjects were healthy male Wistar rats (*Rattus norvegicus*), aged 6 months and weighing between 180 and 200 grams. Sample size calculations were performed using the Higgins formula (1991), which determined that 6 rats per group were needed. Sampling was done using simple random sampling. All rats were acclimated for seven days before the study commenced and were then randomly assigned to two groups: the Healthy (H) group and the Immunosuppressed Drug-Induced (ID) group.
The induction of immunosuppressed drugs was conducted on day 8 using dexamethasone 0.5 mg/day/rat orally for 14 days, added with tetracycline 1 %/day/rat to prevent bacterial infection.[17,18] The dose of dexamethasone was reduced to 0.05mg/day/rat and 0.1%/day/rat for tetracycline on day 11.[17]

At the end of the study, all rats were euthanized. The rats were administered ketamine (10 mg/kg body weight) and diazepam (5 mg/kg body weight) for euthanasia, and their livers were subsequently extracted. Histopathological preparations were prepared using Hematoxylin and Eosin (HE) staining.[19,3] The number of Kupffer cells and liver parenchymal cell damage were observed using a 400x magnification light microscope with 5 fields of view.[3] Kupffer cells are depicted as amoeboid-shaped cells with oval nuclei and elongated cytoplasmic processes, attached or close to the sinusoid.[20] The examination of liver parenchymal cell damage was carried out by counting cells that show features of karyolysis, karyorrhexis, and pycnotic.[3]

The normality of the data was assessed using the Shapiro-Wilk test, and homogeneity was evaluated with the Levene test. The difference in the number of Kupffer cells and liver parenchymal cell damage between the two groups was analyzed using an independent sample t-test, with significance set at $p<0.05$. The correlation between Kupffer cell count and liver parenchymal cell damage was determined using

Pearson's correlation test, with significance set at $p<0.01$.

This research was approved by the Research Ethics Committee of the Faculty of Dentistry, Universitas Hang Tuah Surabaya (No: EC/010/KEPK-FKGUHT/VII/2019).

Results

The Shapiro Wilk test showed the data were normally distributed and the Levene statistical test showed homogeneous data ($p>0,05$). The number of Kupffer cells in ID group increased compared to H group, as well as the number of liver parenchymal cell damage (Table 1). The number of Kupffer cells and liver parenchymal cell damage was significantly different in H group when compared with ID group (Table 1).

The analysis of the relationship between the number of Kupffer cells and liver parenchymal cell damage was conducted using a Pearson correlation test, which revealed a strong positive correlation with a correlation coefficient (r) of 0.943. This result was statistically significant, with a p-value of 0.000 ($p<0.01$).

Discussion

This study revealed an increase in the number of Kupffer cells and liver parenchymal cell damage following the administration of immunosuppressive drugs. These findings suggest that prolonged use of immunosuppressive drugs may lead to the activation of Kupffer cells, resulting in subsequent damage to liver parenchymal cells. In contrast, previous research indicated a reduction in Kupffer cell numbers following acetaminophen induction.[21] In acetaminophen-liver injury, Kupffer cells play protective and pro-repairing roles by recovering hepatocyte proliferation and preventing necrosis.[22] However, several prior studies revealed the elevation of activation Kupffer cells due to an increase of endotoxin and administration of carbon tetrachloride (CCl4) in animal models.[23] The microenvironment shapes the unique immunosuppressive features and functions of Kupffer cells.[23] An early hepatocellular injury can occur in reaction to the enactment of adaptive and innate immune responses in drug-induced liver injury.[24] The complexity of the immune system can arise because the liver is an unique immunological organ that houses multiple innate and adaptive immunity which are complementary and interrelated.[23] The reaction of the innate and adaptive immune system could be a key component leading to liver damage caused by chemical agents.[6] The maintenance of the equilibrium between host defense against pathogenic agents and harmless antigen resistance becomes a crucial role of innate immune cells within the liver including Kupffer cells. In any case, when exposure to certain agents, or infections is present, the equilibrium can be upset, leading to the activation of Kupffer cells.[24]

Corticosteroids as immunosuppressed drugs used in this study may act as chemical substances that induce the activation of Kupffer cells. The excessive amount of drugs that enter the liver, will activate the Kupffer cells to execute its function in getting rid of foreign chemicals and particles from portal circulation system into the liver efficiently.[20] Thereby, Kupffer cells reside in liver sinusoids also have a role in phagocytic function, enabling them to get rid of bacteria, viruses, and cellular debris derived from the arterial and portal circulation.[25,26] Kupffer cells also act as early defense against particle and immunoreactive substances that enter the gastrointestinal tract through portal circulation.[26] Besides phagocytic function, Kupffer cells are able to release cytokines inflammatory, oxygen radicals, and proteases, which may be involved in the development of liver injury.[20]

The correlation analysis in this study revealed a significant and strong association between the number of Kupffer cells and liver parenchymal cell damage. This finding suggests

Table 1 The Kupffer Cells and Liver Parenchymal Cell Damage Number in Each Group

Variable	H Group	ID Group
Kupffer cells	3.8 ± 1.1^{a}	7.96 ± 6.2^{b}
Liver parenchymal cell damage	16.2 ± 1.9^{a}	146.6 ± 25.9^{b}

*a,b: The different superscript letters on the same line showed significant differences at level $p<0.05$

that the administration of immunosuppressive drugs may induce Kupffer cell activation, which in turn can contribute to liver parenchymal cell damage. The positive correlation indicates that an increase in Kupffer cell numbers is associated with a corresponding increase in parenchymal cell damage. The study implies that prolonged use of immunosuppressive drugs may lead to Kupffer cell activation, which can trigger the expression of pro-inflammatory cytokines and chemokines. If these inflammatory mediators are not adequately regulated, they may ultimately result in liver cell damage.[7,27] Cytokines such as interleukin-6 (IL-6), tumor necrosis factor α (TNFα), and chemokines are released by Kupffer cells, leading to acute inflammation.[24] Mitochondrial DNA from apoptotic parenchymal cells activate the STING/NF-kB signaling pathway in Kupffer cells and leads to amplification of inflammation. Several inflammation cells such as monocytes and neutrophils are recruited by Kupffer cells through CCL2 and CXCL1 which can worsen the inflammation.[23] Activated Kupffer cells in this study resulted in the release of proteolytic enzymes which are known to cause liver damage in mouse models.[28] The release of reactive oxygen species and proteases by Kupffer cells can induce necrotic cell damage by extravasating and adherence of neutrophils to parenchymal cells after chemotactic stimulation.[7] Reactive oxygen species induce inflammation through the enhancement of transcription factor NF-kB activation, which regulates the formation of cytokines and chemokines, which subsequently cause cell injury and contribute to the liver damage severity.[7,29]

A limitation of this study is the lack of comparison regarding the duration of immunosuppressive drug administration. Further research is needed to assess how varying lengths of drug exposure impact liver health, both positively and negatively. In conclusion, Kupffer cell infiltration is associated with liver parenchymal cell damage in drug-induced immunosuppression in rat models. Kupffer cells activate various mediators, cytokines, and reactive oxygen species, which can induce damage to liver parenchymal cells in these models.

References

1. Guyton and Hall. Textbook of medical physiology. 14th ed. Singapore: Elsevier; 2021. p. 871.
2. Mohammed K. Hassani. Liver structure, function and its interrelationships with other organs: a review. International Journal Dental and Medical Sciences Research. 2022;4(1):88–92. doi: 10.35629/5252-04018892
3. Pargaputri AF, Andriani D, Hartono MR, Widowati K. Effect of hyperbaric oxygen treatment on liver hepatocyte damage in oral candidiasis immunosuppressed rats. Odonto: Dental Journal. 2022;9(2):319–26. doi: 10.30659/odj.9.2.319-326
4. Yaseen H, Haroon K. Immunosuppressive drugs. Encyclopedia of Infection and Immunity. 2022:726–40. doi: 10.1016/B978-0-12-818731-9.00068-9
5. Fenella BP, Thura WH, Mina K, Phyo KM. Systematic review of immunosuppressant guidelines in the COVID-19 pandemic. Ther Adv Drug Saf. 2021;12:2042098620985687. doi: 10.1177/2042098620985687
6. Elena T, Judit C, Mercedes R, MA Isabel Lucena, Raúl JA. Hepatotoxicity induced by new immunosuppressants. Gastroenterol Hepatol. 2010;33(1):54–65. doi: 10.1016/j.gastrohep.2009.07.003.
7. Hiroko T, Shuhei N. Importance of kupffer cells in the development of acute liver injuries in mice. Int. J. Mol. Sci. 2014;15:7711–30. doi:10.3390/ijms15057711
8. Prasetiawan E, Sabri E, Ilyas S. Gambaran histologis hepar mencit (mus musculus l.) strain ddw setelah pemberian ekstrak n-heksan buah andaliman (Zanthoxylum acanthopodium Dc.) selama masa pra implantasi dan pasca implantasi. Saintia Biol. 2012;1(1):40–5.
9. Pargaputri AF, Andriani D. Serum albumin levels of oral candidiasis immunosuppressed rats treated with hyperbaric oxygen. International Journal of Integrated Health Sciences. 2020;8(1):8–13. doi: 10.15850/ijihs.v8n1.2086
10. Pargaputri AF, Andriani D. Hepatocellular liver function of immunosuppressed rats with oral candidiasis after hyperbaric oxygen treatment: Alanine transaminase and aspartate transaminase levels. Arch Orofac Sci. 2021;16(Supp.1):5–9. doi: 10.21315/aos2021.16.s1.2
11. Oliver K, Frank T. Liver macrophages in tissue homeostasis and disease. Nature Reviews Immunology. 2017;17:306–21. doi: 10.1038/nri.2017.11
12. Meurman JH, Siikala E, Richardson M, Rautemaa R. Non-Candida albicans Candida

yeasts of the oral cavity. In: Méndez-Vilas A (ed.), Communicating Current Research and Educational Topics and Trends in Applied Microbiology. 2017:719–31.
13. Ruth AR, Patricia EG, Cynthia J, Lisa MK, Ivan R, James EK. Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis. Toxicol Sci. 2007;96(1): 2–15. doi: 10.1093/toxsci/kfl173.
14. Elise Slevin, Leonardo Baiocchi, Nan Wu, Burcin Ekser, Keisaku Sato, Emily Lin, et al. Kupffer cells: inflammation pathways and cell-cell interactions in alcohol-associated liver disease. Am J Pathol. 2020;190(11):2185–93. doi: 10.1016/j.ajpath.2020.08.014.
15. Subhra KB and Alberto M. Kupffer Cells in Health and Disease. Macrophages: biology and role in the pathology of diseases. 2014:217–47.
16. M. Teresa Donato, Gloria Gallego-Ferrer, and Laia Tolosa. In vitro models for studying chronic drug-induced liver injury. Int. J. Mol. Sci. 2022;23(19):11428. doi: 10.3390/ijms231911428
17. Pargaputri AF, Andriani D. The effect of hyperbaric oxygen therapy to the amount of lymphocytes in oral candidiasis immunosuppressed model. Denta J Kedokt Gigi. 2018;12(2):36–44.
18. Andriani D, Pargaputri AF. Enhance of il-22 expression in oral candidasis immunosuppressed model with acanthus ilicifolius extract therapy. IOP Conf Ser Earth Environ Sci. 2019;217:012056.
19. Paramita DO, Endah W, Dwi A. Efektivitas suplementasi teripang emas (Stichopus hermanii) dalam mencegah terjadinya oral candidiasis pada tikus wistar. Denta 2018; 12(1):9–15. doi: 10.30649/denta.v12i1.155
20. Hajira Basit, Michael L. Tan, Daniel R. Webster. Histology, kupffer cell. Treasure Island (FL): StatPearls Publishing; 2022.
21. David BA, Rezende RM, Antunes MM, Santos MM, Freitas LM, Diniz AB, et al. Combination of mass cytometry and imaging analysis revealsorigin, location, and functional repopulation of liver myeloid cells in mice. Gastroenterology. 2016;151:1176–91. doi: 10.1053/j.gastro.2016.08.02415.
22. Kopec AK, Joshi N, Cline-Fedewa H, Wojcicki AV, Ray JL, Sullivan BP, et al. Fibrinogen drives repair after acetaminophen-induced liver injury via leukocyte alphambeta2 integrin-dependent upregulation of mmp12. J Hepatol. 2017;66:787–97. doi: https://doi.org/10.1053/j.gastro.2016.08.024.
23. Li W, Chang N, Li L. Heterogeneity and function of kupffer cells in liver injury. Front. Immunol. 2022;13:940867. doi: 10.3389/fimmu.2022.940867
24. Farah Tasnim, Xiaozhong Huang, Christopher ZWL, Florent Ginhoux, and Hanry Yu. Recent advances in models of immune-mediated drug-induced liver injury. Front. Toxicol. 3: 605392. doi: 10.3389/ftox.2021.605392
25. Takashi Sakai and Wen-Ling Lin. Kupffer cells as a target for immunotherapy. sakai t, lin w-l. kupffer cells as a target for immunotherapy. J Multidisciplinary Scientific Journal. 2022;5:532–7. doi: 10.3390/j5040036
26. Laura JD, Mark B, Hui T, Michele TP, Laura EN. Kupffer cells in the liver. Compr Physiol. 2013;3(2):785–97. doi: 10.1002/cphy.c120026
27. Mark B, Laura JD, Zhang-Xu L, Hui T, Laura EN. Macrophages and kupffer cells in drug-induced liver injury. Drug-Induced Liver Disease (Third Edition). 2013. Academic press. p: 147-155. doi: 10.1016/B978-0-12-387817-5.00009-1
28. Reham Hassan. Kupffer cells in hepatotoxicity. EXCLI Journal. 2020;19:1156-1157.
29. Kendran AAS, Arjana AA, Pradnyantar AASI. Aktivitas enzim alanine-aminotransferase dan aspartate aminotransferase pada tikus putih jantan yang diberi ekstrak buah pinang. Buletin Veteriner Udayana. 2017;9(2):132–8.