Immunome Research

Prayitno et al., Immunome Res 2016, 12:1
http://dx.doi.org/10.4172/1745-7580.10000112

Research Article Open Access

Tumoricidal Activation of Macrophages using *Jatropha curcas* Leaf Extract: As a Proxy for the Treatment of Cancer

Prayitno A[1]*, Fitria MS[2] and Elmanda AY[3]

[1]*Department of Pathobiology, Faculty of Medicine, University of Sebelas Maret, Surakarta, Indonesia*

[2]*Department of Biology, Faculty of Mathematics and Natural Science, University of Sebelas Maret, Surakarta, Indonesia*

[3]*Departemen of Biologi, Faculty of Dentistry, University of Gajah Mada, Yogyakarta, Indonesia*

[*]**Corresponding author:** Prayitno A, Department of Pathobiology, Faculty of Medicine, University of Sebelas Maret, Surakarta, Indonesia, Tel: +62271664178; E-mail: drgadiprayitno@yahoo.com

Received date: March 04, 2016; **Accepted date:** April 28, 2016; **Published date:** May 09, 2016

Abstract

Background: *Jatropha curcas* (*J. curcas*) is a plant rich in methanolic. Macrophages can be activated to become tumoricidal through interactions with immunomodulators. The aim of this study was to investigate the effect of *J. curcas* leaf extracts on the phagocytic activity of macrophages.

Methods: Peritoneal-derived macrophages from male BALB/c mice were obtained using the method of Colligan et al. with some modifications. Latex was added as an antigen to cultures of BALB/c mice macrophages treated with two different concentrations of *J. curcas* extract and the cultures were observed every 30 minutes for 2 hours.

Results: Macrophage latex vacuoles were observed after treatment with 15 µg/mL *J. curcas* leaf extract, with a mean latex vacuole count of 7.2 vacuoles/cell and a phagocytic index (PI) of 369. Macrophage treatment with 250 µg/mL *J. curcas* leaf extract produced a mean latex vacuole count of 8.1 vacuoles/cell and a PI of 785.

Conclusion: Treatment with *J. curcas* leaf extracts increased the phagocytic activity of mice macrophages.

Key words:

Jatropha curcas; Immunomodulator; Macrophage; Tumoricidal

Introduction

Biological activities of the methanolic was under study. Leaf extracts that contains methanolic acid of *Jatropha curcas* showed the highest antioxidant activity. Other research in cytotoxicity assay results indicated the anticancer therapeutic property of the root extract against human colon adenocarcinoma (HT-29) cell line but its cytotoxic effect on human hepatocyte (Chang cell) was high [1].

Macrophages can be activated to become neoplasticidal by interaction with a substance filled immune modulators. Neoplasticidal macrophages can recognize and destroy neoplastic cells *in vitro* and *in vivo*. Although the exact mechanism by which macrophages discriminate between neoplastic and normal cells is unknown, it is independent of neoplastic cell characteristics such as immunogenicity, metastatic potential, and sensitivity to drugs. Moreover, macrophage destruction of neoplastic cells apparently is not associated with the development of neoplastic cell resistance. Macrophages are found in association with neoplasm in a definable pattern, suggesting that the most direct way to achieve macrophage-mediated neoplasm regression is *in situ* macrophage activation [2-4].

Method

Peritoneal-derived macrophages from male BALB/c mice (Hayati Science Lab. GMU Yogyakarta, Indonesia) were obtained using the method of Colligan et al. with some modifications [5]. Macrophages obtained by using a 10 ml syringe and hypodermic needle 25G. The results collected in sizes 25 ml centrifuge tube and stored on ice. Macrophage cultures performed in culture dishes with a diameter of 20 mm. Macrophages were distributed into each well of the plate so that the culture wells containing an average of 1000 cells. For ease of painting it on the basis of prior pitting the cover glass is placed before the cell is inserted. Further culture medium Roswell Park Memorial Institute (RPMI) 1640 (Sigma Chem. Co. St. Louis, USA) inserted into the wells as much as 10 ml. Medium replacement done once every 24 hours and incubated at room temperature.

Prior to treatment, the culture medium was removed and the macrophages were washed extensively with PBS-10F (7 nM phosphate buffer, pH 7.4 with 137 nM NaCl and 3 nM KCl). The cultures were then incubated with fractionated *R. communis* extract for 10 min at 37°C. The cells were washed again with PBS-10F to remove any remaining *R. communis* leaf extract. Latex was then added to the macrophage cultures as a test antigen, and the cultures were observed every 30 min over 2 hrs [6]. For time-lapse microscopy the samples were stained with Giemsa (Merck). Nikon phase contrast microscope images were collected with a 100X objective lens [7].

This research has been ratified by the feasibility of conduct to be done by Research and Ethical Committe Distric Hospital of Muwardi and Faculty of Medicine (University of Sebelas Maret) led by Dr. Hari Wuyoso (No. 188.4/14.666/2014 and 182/UN27.06/KS/2014).

Results

An increase in the phagocytic activity of cultured macrophages was detected following treatment with *J. curcas* leaf extract [Figure 1 (A and B) and Table 1]. Macrophages with latex-containing vacuoles were observed to form after treatment with 15 µg/mL *J. curcas* leaf extract. The mean latex vacuole (i.e. mean phagocyte) count per cell was 7.2 and the phagocytic index (PI) was 369. After treatment with 250 µg/mL *J. curcas* leaf extract, the mean latex vacuole count per cell was 8.1 with a PI of 785.

Concentration of extract	Macrophages ingested (%)	Mean phagocyte	Phagocytic Index (PI)
0 µg/ml	55	7.2	396
15 µg/ml	60	6.9	414
30 µg/ml	63	6.5	409
60 µg/ml	70	5.6	392
120 µg/ml	85	7.6	646
150 µg/ml	85	7.3	620
200 µg/ml	87	8.2	713
250 µg/ml	97	8.1	785

Table 1: Phagocytic index after treatment with many concentration of *J.curcas* extract. We can look the macrophage with latex vacuole after treatment with 250 µg/ml *J.curcas* extract. The macrophages ingested was 97%, mean latex vacuole (mean phagocyte) was 8.1 and the PI was 785.

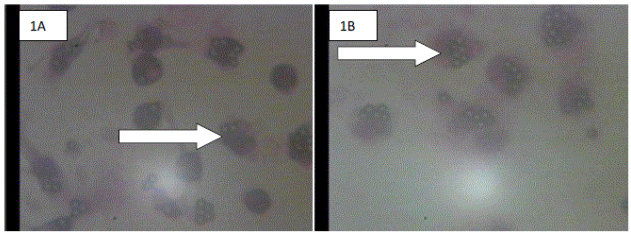

Figure 1 (A,B): We can look the macrophage with latex vacuole after treatment with 15 µ/ml *J. curcas* extract. The macrophages ingested was 55%, mean latex vacuole (mean phagocyte) was 7.2 and the PI was 369; We can look the macrophage with latex vacuole after treatment with 250 µg/ml *J. curcas* leaf extract. The macrophages ingested was 97%, mean latex vacuole (mean phagocyte) was 8.1 and the PI was 785.

Discussion

Terpenoid compounds are the major metabolites found in the Euphorbiaceae family. Among the terpenes, diterpenoids have dominated research in Jatropha species with respect to their novel chemical structures and medicinal values [8]. Recently, Oskoueian et al. [9] reported that extract of root and latex of J. curcas plant which contained phenolics, flavonoid and saponins showed notable antioxidant, anticancer and anti-inflammatory activities. These compounds have been reported to be involved in the biological activities of the plants [10]. Ricinus communis is a same species of plant that can vary greatly in growth patterns and appearance. This variability has been enhanced by breeders who have selectively bred a range of cultivars for different leaf and flower colors. *Ricinus communis* is the only plant whose seeds are rich in a hydroxy fatty acid, namely ricinoleic acid. This compound has known pharmacological activities (e.g., anti- microbial, anti-fungal, antioxidant and anti-Inflammatory) [11-13].

Tumor cells express tumor-specific antigens (TSA) which the immune system recognizes as foreign. Phagocytes identify these tumor cells and initiate phagocytosis via the nonspecific Fc-receptor. Macrophages are known to express receptors for the phagocytic process, namely IgG-R and Complement-R, which are also involved as intermediaries in the macrophage-mediated phagocytosis of tumor cells [14,15].

The triggering of phagocytosis by macrophages can induce inflammatory activity, which, if sufficiently severe, can affect the clinical outcome. Modulation of the environment of the inflammatory response using molecules or cells can enhance the healing process. The IL-1β and M1 macrophages were observed to be very important in the healing process following surgery through modulation of the inflammation environment [2].

The relationship between macrophages and tumor cells has been widely investigated. Macrophage activity was shown to improve significantly following intravenous delivery of liposomes containing immunomodulators. However, other studies have indicated that the activation of macrophages would be more useful if the immunomodulators were supplied in the form of an adjuvant as this would provide minimal side effects [16].

Research regarding the activity of macrophages has shown that the extension of pseudopodia is triggered by the cytokines IL-4 and IL-13, which also protect the type-1 macrophages from the influence of LPS and IFN-γ [17].

Sorafenib, which is used to fix macrophage polarization, triggers the interaction between tumor cells and natural killer (NK) cells. Sorafenib therefore affects the function of macrophages and NK cells in terms of the destruction of tumor cells. Sorafenib triggers the activity of inflammatory tumor cells connected with the activity of macrophages and triggers NK cells to fight tumor cells by expressing cytokines and NF-κB. The sorafenib compound can therefore be used in the clinic as an alternative polarization between macrophages and tumor cells [18].

Thiram is widely used as a dithiocarbamate pesticide and fungicide, but can also be used as a suppressor of inflammation; studies have shown that thiram can reduce the inflammation resulting from contact dermatitis. Explain when thiram interact with the agency and the intensity of the inflammation will decline.

Conclusion

J. curcas leaf extracts can increase the phagocytic activity of tumoricidal macrophages.

Acknowledgements

We thank the Higher Education Competitive Research Project Ministry of Education and Culture of the Republic of Indonesia for Grand Featured Research Universities 2013 funding. LPPT of Gajah Mada University. Special thanks to Prof. Rafik Karsidi, as rector of Sebelas Maret University, Surakarta, Indonesia, Prof. Darsono, as Chairman of the Institute for Research and Community Service, and Prof Zainal Arifin Adnan, as Dean of the Faculty of Medicine of Sebelas Maret University. Special thanks also to Prof. Wihaskoro Sosroseno for inspiration for this research.

Author Contributions

AP carried out the study design, developed the laboratory protocols, analyzed and interpreted the data, and drafted the manuscript. MSF prepared the *R. communis* extracts and cell cultures, and carried out the experiments. Both authors read and approved the final manuscript. EAY

References

1. Oskoueian E, Abdullah N, Saad WZ, Omar AR, Ahmad S, et al. (2011) Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from Jatropha curcas Linn. Journal of Medicinal Plants Research. 5: 49-57.
2. Mosser DM (2003) The many faces of macrophage activation. J Leukoc Biol 73: 209-212.
3. Whitworth PW, Pak CC, Esgro J, Kleinerman ES, Fidler IJ (1990) Macrophages and cancer. Cancer Metastasis Rev 8: 319-351.
4. Schmall A, Al-Tamari HM, Herold S, Kampschulte M, Weigert A, et al. (2015) Macrophage and cancer cell cross-talk via CCR2 and CX3CR1 is a fundamental mechanism driving lung cancer. Am J Respir Crit Care Med 191: 437-447.
5. Coligan JE, Kruisbeek AM, Marquilies DA, Shevach EM, Strober W, et al. (1994) Current Protocols in Immunology. USA: John Wiley & Sons. 14.0.1-14.1.6.
6. Karp G (1996) Cell and Molecular Biology. Concepts and Experiments, John Wiley & Sons. Inc. New York -Chichester-Brisbane-Toronto–Singapore 649-693.
7. Yvonne Clayton (1988) Pocket Picture Guides. Medical Mycology. JB. Lippincott Company. London-New York. 1988. p.28, 33, 41, 56, 62.
8. Devappa RK, Makkar HPS, Becker K (2011) Jatropha Diterpenes: a Review. J Am Oil Chem Soc 88: 301–322.
9. Balasundram N, Sundram K, Samman Sc (2006) Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem 99: 191-203.
10. Prayitno A, Asnar E, Astirin OP, Artanti AN, Fitria MS, et al. (2013) PKC Is a target to modulate the expression of receptor mediated endocytosis (RME) mice macrophages BALB/c for optimizing the phagocytosis toward Candida albicans. J Immune Base Ther Vaccine 2: 44-48.
11. Lord MJ, Jolliffe NA, Marsden CJ, Pateman CS, Smith DC, et al. (2003) Ricin Mechanisms of cytotoxicity. Toxicol Rev 22: 53-64.
12. Rana M, Dhamija H, Prashar B, Sharma S (2012) Ricinus communis L.A review. Int J Pharm Tech Res 4: 1706-1711.
13. Asano K, Nabeyama A, Miyake Y, Qiu CH, Kurita A, et al. (2011) CD169-positive macrophages dominate antitumor immunity by cross presenting dead cell-associated antigens. Immunity 34: 85-95.
14. Manning CN, Martel C, Sakiyama-Elbert SE, Silva MJ, Shah S, et al. (2015) Adipose-derived mesenchymal stromal cells modulate tendon fibroblast responses to macrophage-induced inflammationin vitro. J Stem Cell Res Ther 6: 74.
15. Nardin A, Abastado JP (2008) Macrophages and cancer. Front Biosci 13: 3494-3505.
16. McWhorter FY, Wang T, Nguyen P, Chung T, Liu WF (2013) Modulation of macrophage phenotype by cell shape. Proc Natl Acad Sci USA 110: 17253-17258.
17. Sprinzl MF, Reisinger F, Puschnik A, Ringelhan M, Ackermann K, et al. (2013) Sorafenib perpetuates cellular anticancer effector functions by modulating the crosstalk between macrophages and natural killer cells. Hepatology 57: 2358-2368.
18. Kurpios-Piec D, WoÅºniak K, Kowalewski C, Gajewska B, Rahden-StaroÅ I (2015) Thiram modulates pro-inflammatory mediators in RAW 264.7 murine macrophage cells. Immunopharmacol Immunotoxicol 37: 90-102.