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Padlianah, Mutmainnah Arif, and Ika Yustisia

Blood chemistry profiles of DMBA-induced mammary tumor in female Sprague Dawley rats

Padlianah[1], Mutmainnah Arif[2], Ika Yustisia[3,a)]

[1]*Master Program of Biomedical Science on Biochemistry and Molecular Biology Sekolah Pascasarjana Universitas Hasanuddin Makassar*
[2]*Master Program of Biomedical Science on Pharmacology Sekolah Pascasarjana Universitas Hasanuddin Makassar*
[3]*Departement of Biochemistry Faculty of Medicine Universitas Hasanuddin Makassar*

[a)]Corresponding author: ikayustisia@gmail.com

Abstract. Although aerobic glycolysis (Warburg effect) in cancers had been accepted as a significant characteristic of cancer, changes in metabolic state in each stage of tumorigenesis remain to be explored. In this preliminary study, we analyzed the changes of several blood chemistry parameters intending to describe the general metabolic state of the early and advanced stages of tumorigenesis in rat mammary tumor models. The mammary tumor was induced in female Sprague Dawley (SD) rats by subcutaneous injection of 7, 12-dimethylbenz[a]anthracene (DMBA). The rats were palpated on the breast every week after DMBA induction to detect tumor development. Blood chemistry examinations including blood glucose, triglyceride, cholesterol, HDL-cholesterol, urea, and creatinine, were performed at 8-weeks and 12-weeks after DMBA induction. Rats were fasted 12-14 hours before retro-orbital blood collection. The spectrophotometer measured all the parameters. The palpable mammary tumor was firstly detected at 7-weeks on 1 of 4 DMBA induced rats. At three weeks later all rats had palpable tumors that also confirmed by ultrasound imaging. The blood chemistry measurements revealed that DMBA-induced rats had a significantly higher level of triglyceride ($p=0.001$) and creatinine ($p=0.018$); and glucose ($p=0.007$) at 8-weeks and 10-weeks after DMBA induction compared to the non-induced rats, respectively while not in the other parameters. The stages of tumor development may have a different profile of blood chemistry parameters. Further research should be performed to elucidate whether the profile could describe the metabolic reprogramming during tumorigenesis.

Keywords: Mammary tumor, blood chemistry, tumor metabolism, DMBA, Sprague Dawley

INTRODUCTION

Cancer remains a major health problem around the world including in Indonesia [1]. The high incidence and mortality rates are still high mainly due to the limited method of early diagnosis and the unsatisfactory conventional treatment for cancer. The main factors that cause these limitations are the mechanism and the course of cancer (carcinogenesis) which is very complicated. Comprehensive knowledge of carcinogenesis can be the basis for the development of reliable diagnostic techniques and more effective new treatment methods.

One important mechanism in cancer growth is the metabolic process. Metabolism is the main factor underlying the survival and unlimited proliferation ability of cancer cells. The ability of cancer cells to carry out metabolic changes to meet their anabolic and energetic needs is one of the hallmarks of cancer [2]. In contrast to normal cells, fundamental metabolic changes in cancer cells include: 1) increased glucose and glutamine uptake; 2) the use of opportunistic modes of obtaining nutrients; 3) the use of intermediates derived from the glycolysis pathway and the tricarboxylic acid cycle for biosynthesis and the production of NADPH; 4) increased demand for nitrogen; 5) impaired regulation of genes involved in regulating metabolism; and 6) metabolic interactions with tumor microenvironment [3].

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We suggest that the metabolic process that takes place during tumor growth to malignancy may be detected through changes in the metabolites circulating in the blood. Therefore, this preliminary study aims to analyze several blood chemistry examinations, including glucose, cholesterol, HDL-cholesterol, triglycerides, urea, and creatinine in Sprague Dawley rats induced by the carcinogen 7,12-Dimethylbenzathracene (DMBA).

MATERIALS AND METHODS

Animals

The study used eight white healthy female Ratus norvegicus from Sprague-Dawley strains weighing 125-175 grams aged eight-week-old. The animals were bred in Animal Laboratory of Entomology Laboratory, Faculty of Medicine Universitas Hasanuddin under ideal conditions of temperature, humidity, and light. They received appropriate pellets and filtered water.

Experimental Design

The animals were housed in three treatment groups. Rats were acclimatized for 14 days and were monitored the weight every week. The first group was induced of DMBA subcutaneously in the lower nipple area with a single dose of 25 mg DMBA dissolved in 0.5 mL sunflower oil and 0.5 mL 0.9% sodium chloride. The second group as a normal control given 0,5 ml sunflower oil and 0,5 ml 0,9 % sodium chloride and the last group as a negative control. The observation was made on changes in body weight, cancer incidence number and type of cancer every day for 14 weeks. The mammary tumors were measured and weighed, and the findings recorded. After 14 weeks, all the animals are terminated and dissected for removal of tumor tissue. The tumor tissues were made histopathology preparation and examined using a light microscope. Histological findings were not reported here. Animal blood was taken for metabolic analysis, to determine the levels of glucose, cholesterol, HDL-cholesterol, triglycerides, urea, and creatinine by using the colorimetric method. The wavelength of each parameter are (Glu= 510 nm; Tg.= 505 nm; Chol= 505 nm; HDL Chol= 505 nm; Ur= 578 nm; Cr=546 nm) (Adapted from Química Clínica Aplicada S.A).

RESULTS

Tumor development and rat growth

Palpation on mammary gland area was performed to detect the tumor development. Dense and hard tumor mass in the breast area was firstly detected at the seventh week in 1 of 4 rats receiving DMBA injections. Three weeks later all mice that received DMBA injections had a tumor mass that should be suspected of originating from the breast. Ultrasound examination was performed to confirm the formation of the tumor. The results showed heterogeneous echo images with calcification and irregular margins in the abdominal area which indicated malignancy. Further confirmation by histopathological examination will be carried out yet we could not report the results in this report because we are still waiting for the final stages of the carcinogenesis.

Until the week eight, there was no difference in rat growth ($p = 0.582$) which was assessed from the average weight gain of 17.50 ± 5.44 g and 21.50 ± 4.21 g, in DMBA-induced and control rats, respectively. Entering the week nine the rats that received DMBA began to experience weight loss and became increasingly evident at week 10 with an average weight loss of 10.75 ± 6.21 g. The weight of the control rats was constant when they entered the week 8 and decreased slightly at week 10.

Blood chemistry profiles at week eight after DMBA induction

There was a significantly higher triglyceride level ($p = 0.001$) in DMBA-induced rats compared to control rats (Sunf_NaCl0.9%) while glucose ($p = 0.796$), cholesterol ($p = 0538$), and HDL- cholesterol ($p = 0.081$) levels were not significantly different. Creatinine level was also significantly higher in DMBA-induced rats ($p = 0.018$) compared to Sunf_NaCl0.9% rats but not at urea levels.

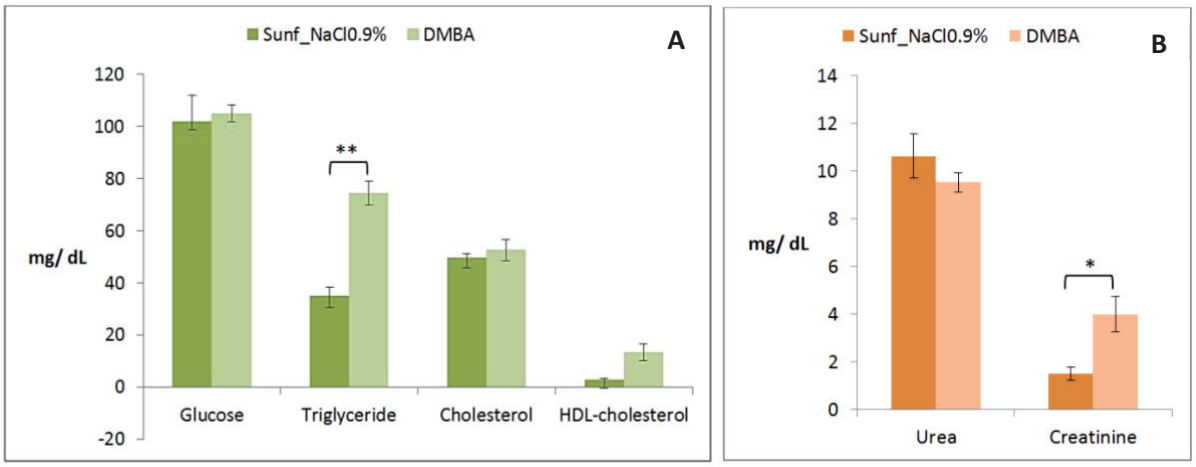

FIGURE 1. Blood chemistry profiles at week eight after DMBA induction. Blood glucose, triglyceride, cholesterol, and HDL-cholesterol levels (A); blood urea and creatinine levels (B) were measured using spetrophotometer. *p<0.05; **p≤0.001.

Blood chemistry profiles at week ten after DMBA induction

At week ten, more parameters are expected to show a significant difference, but in reality, the only glucose level of DMBA rats was significantly higher (p = 0.007) compared to Sunf_NaCl0.9% rats. Triglyceride (p = 0.873), cholesterol (0.341), HDL-cholesterol (0.146), urea (0.218), and creatinine (0.279) levels did not show any significant differences between the two groups.

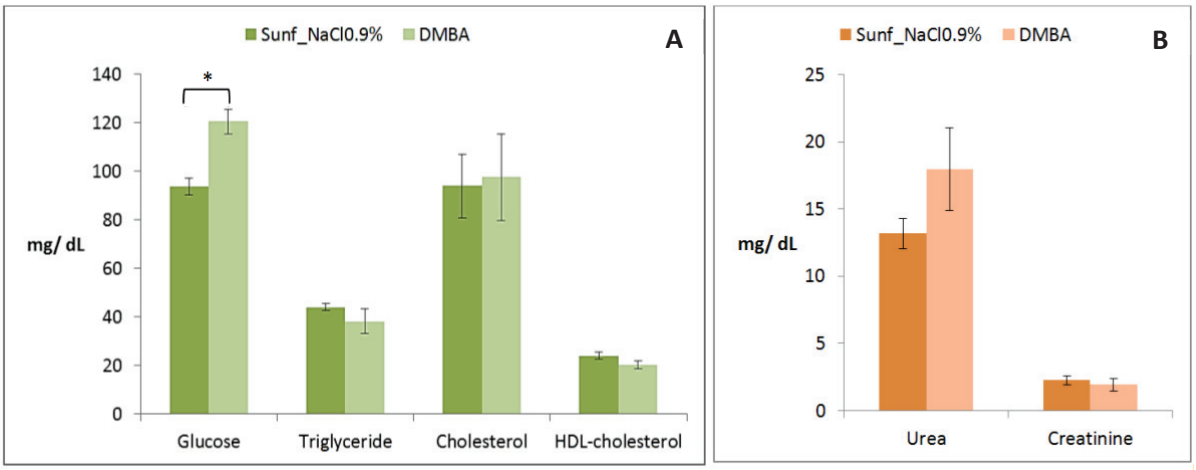

FIGURE 2. Blood chemistry profiles at week ten after DMBA induction. Blood glucose, triglyceride, cholesterol, and HDL-cholesterol levels (A); blood urea and creatinine levels (B) were measured using spetrophotometer. *p<0.05.

DISCUSSION

Metabolic changes in cancer have long been known to have a significant role in the development of cancer. However, how the intratumoral metabolic activities are reflected in blood chemistry profiles still showed controversial results.

The results of this study revealed DMBA-induced rats had a significantly higher triglyceride concentration than the control group even though the concentration values among the two groups were still within the normal limits of rat blood triglycerides. Higher triglyceride concentrations at week eight after DMBA induction may be related to the development of cancer. Previous research showed tumor cells expressed malic enzyme which was a lipogenic enzyme in which its activity correlates with *de novo* fatty acid synthesis [4-6]. Besides, lipoprotein

lipase (LPL) may regulate the clearance of blood from tissue to tissue and its activity in white adipose tissue is reduced in patients with cancer thus contributing to hypertriglyceridemia [7-8].

Different conditions occur in the week ten after DMBA induction. DMBA rats showed a higher fasting blood glucose level (120.45 ± 5.01 mg / dL) compared to the control group. The value was considered to be above the normal value of fasting blood glucose levels in rats (71.10 ± 23.25 mg / dL) [9]. This elevated levels of blood glucose was likely to indicate an increase in glucose requirements for cancer development. In this respect, hyperglycemia could provide a high glucose fuel source for cancer cells supporting rapid proliferation. Indeed, in vitro studies with cancer cell lines indicate that high concentrations of glucose levels regulate enhanced expression of genes associated with promoting cancer cell proliferation, invasion, and migration [10-12].Very dramatic weight loss at week ten accompanied by increased glucose levels might be a sign of an increase in the gluconeogenesis pathway to meet glucose requirements for cancer progression. Comprehensive research needs to be carried out to support this notion.

CONCLUSION

Changes in triglyceride and blood glucose levels at different stages in DMBA-induced rats may determine the role of triglyceride and glucose metabolism in the development of cancer. More comprehensive research needs to be conducted at the more various stages of cancer development and an adequate number of samples assessing the activity of metabolic pathways and their specific metabolites.

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