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Proof of The Formation of OH Radicals from Methyl Paraben and Its Effect on Cancer Formation

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Abstract: Methyl paraben is often used by the cosmetic industry such as facial moisturizer, anti-aging, hair dye, skin whitening, shaving gel, eye shadow and others because it is considered non-toxic. However, in fact, several studies have found that methyl paraben is able to accumulate in the body, resulting in the formation of radicals. Because of its ability to produce radicals, this paper tries to prove that methyl paraben used in the cosmetic industry must be considered immediately. Because we suspect that exposure to radicals from methyl paraben will be able to interact with DNA, causing DNA mutations that lead to cancer. In this summary, we prove that exposure to methyl paraben in mice is able to produce DNA mutation biomarker products with the formation of 8-Hydroxy-2'-Deoxyguanosine (8-OHdG), either by direct metabolism or by oxidative stress triggered by the presence of metal ions in the Fenton reaction such as Cu and Fe. The method used is direct in-vivo exposure to test animals and in-vitro to prove it by synthesizing the DNA damage biomarker product using LCMS.

Keywords: Cancer, DNA Mutation, Fenton reaction, Metyl paraben, 8-Hydroxy-2'-Deoxyguanosine.

Introduction

The entry of a xenobiotic in the human body can cause various kinds of risks, depending on the toxicity of the xenobiotic. For example, by consuming cigarettes, alcoholic beverages or consuming foods that contain carcinogens (Broedbaek et al. 2011). Many carcinogens have been identified and their use has been banned and even no longer circulated in the wider community. However, there are several compounds that require a deeper study of their carcinogenic effects in the body, such as methyl paraben (Budiawan and Widiastuti, 2015). Because Methyl paraben can trigger the formation of free radicals in the human body through the Fenton/Fenton-like reaction. Then these radical compounds can react with the base forming Deoxyribonucleic Acid (DNA) (Budiawan, et al. 2020), and result in a conformational change in the basic structure of the DNA (Charles and Darbre, 2013). This event produces a new compound called the product of DNA Adduct (Cooke, et al. 2008). This event is a reversible event, whereby the DNA in the body can regenerate and repair its structure. However, if this event occurs repeatedly or exposure to xenobiotics occurs continuously, it can cause permanent damage to the DNA structure and risk causing permanent mutation of cell DNA (carcinogenesis) in the living organism (Decker and Wenninger, 1987). This study will prove that methyl paraben when metabolized in the body will be bioactivated to form 8-OHdG.

Materials and Methods

Study area

This research was conducted at the Biology Laboratory of the University of Indonesia for

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sample testing using LCMS, and at the University of Indonesia Biochemistry Laboratory for sample preparation consisting of breeding test animals, weighing animals, treatment of exposure to methyl paraben

Procedures

This research was conducted in vitro and in vivo to determine the formation of the DNA adduct 8-hydroxy-2'deoxyguanosine due to methyl paraben and copper metal. Meanwhile, the instrument used to perform the analysis included LCMS. The in vivo samples used in this study were the blood and urine of rats that had been treated in the cages of the test animals. Other chemicals used in the study were 8-OhdG (Sigma-Aldrich), 2'deoxyguansine (sigma), methyl paraben, copper (I) chloride, K_2HPO_4, KH_2PO_4, NaOH, sodium acetate, aquabides, ammonium acetate, acetonitrile, acid chloride, methanol.

Formation of 8-OHdG from Methyl Paraben

A total of 100 L 2'-dG 6 ppm in 0.1 M phosphate buffer solution (pH 7.4 and 8.4) were incubated with 100 L 60 ppm methyl paraben solution and 100 L aquabides for 7 and 12 hours with an incubation temperature of 37º C. and 60º C.

Formation of 8-OHdG from Methyl Paraben, Copper metal (I) and H_2O_2

A total of 100 L 2'-dG 6 ppm in 0.1 M phosphate buffer solution (pH 7.4 and 8.4) were incubated with 100 L 60 ppm methyl paraben solution, 100 L copper (I) chloride solution 60 ppm, 100 L 60 ppm H_2O_2 solution and 100 L aquabides for 7 and 12 hours with incubation temperatures of 37º C and 60º C.

Test Animal Preparation

The test animals were kept in cages and consisted of 5 rats in each cage representing each treatment group. Each treatment was given an identifier to make it easy to distinguish between treatment groups. The test animals were acclimatized for approximately 2 weeks in order to adapt to the new cage environment. During the acclimatization process, the test animals were given feed in the form of pellets and given to drink boiled water. Animal cages are cleaned every 3 times a week with disinfectant and change the cage mat, lighting the room where the pet is conditioned for 12 hours of light and 12 hours of darkness with a room temperature of 27-28°C.

Formation of 8-OHdG on Methyl Paraben. Exposure Group

The rats were grouped and prepared as a methyl paraben exposure group and then fed pellets, drinking and disonde with a solution of methyl paraben 250 mg/Kg BW as much as 10 mL/kg BW orally (OECD Guidelines, 2008). Methyl paraben solution was given for 28 days and probed every day.

Formation of 8-OHdG on Copper Metal Exposure Group (I)

The rats were grouped and prepared as a metal exposure group of copper (I) then given pellet feed, drinking and disonde with a solution of copper (I) chloride 4 mg/Kg BW as much as 10 mL/kg BW orally (OECD Guidelines, 2008). Methyl paraben solution was given for 28 days and probed every day.

Formation of 8-OHdG on Methyl Paraben and Copper (I) Metal Exposure Group

The rats were grouped and prepared as a copper metal (I) methyl paraben exposure group then given pellet feed, drinking and disonde with a 250 mg/Kg BW methyl paraben solution and a copper (I) chloride solution with a volume of 5 mL/Kg BW orally (OECD). Guidelines, 2008). Methyl paraben solution was given for 28 days and probed every day.

Results and Discussion

The type of exposure in mice used in this study is sub-chronic exposure, which is exposure to small concentrations over a not too long period of time. Methyl paraben exposure of 250 mg/Kg BW was in accordance with previous studies (Diakowska, et al. 2007), while exposure to copper (I) metal was at a dose of 4 mg/Kg BW.

Based on observations, the body weight of rats exposed to xenobiotics tends to decrease. The body weight of the control group mice increased from 155.7 grams to 251.8 grams at the end of exposure. However, in the Cu (I) exposure group, the body weight of rats decreased from 229.5 grams to 190.95 grams (Wibowo, et al. 2017). This suggests that sub-chronic Cu(I) exposure affects rat body weight (Elder, 1984). The peak of the methyl paraben exposure chromatogram can be seen as below (Figure 1).

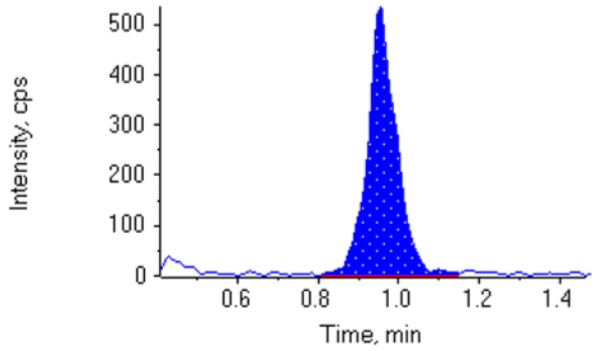

Figure 1. 8-OHdG Kromatogramfrom Methyl Paraben

Exposure to methyl paraben has been shown to cause oxidative damage to DNA. Previous research has shown that exposure to methyl paraben in various organisms can cause oxidative stress. As in embryos (Gambling and McArdle, 2004), Drosophila melanogaster (Jomova and Valko, 2011) and Rattusnorvegicus (Kala et al. 2015). Even Khanna et al (2014) stated that there was a significant positive correlation between MP levels and oxidative stress biomarkers such as MDA and DNA adduct 8-OHdG in the urine of pregnant women and infants. Methyl paraben itself can undergo autoxidation process by producing radicals that can trigger oxidative damage to DNA (Kuş et al. 2013). However, as is known, methyl paraben is hydrolyzed rapidly by the esterase enzyme. Only about 2.3-3.3% methyl paraben is not metabolized (Rizkita et al. 2020) and allows autoxidation reactions to occur in cells.

Metals such as copper can induce oxidative stress. Cu has been shown to modify low-density lipoprotein (LDL) and promote atherogenesis by increasing the transformation of macrophages into foam cells and by developing vasoconstrictor and prothrombotic properties (Rizkita, 2021). Excess Cu can cause peroxidative damage to membrane lipids through the reaction of lipid radicals and oxygen to form peroxide radicals, and cause peroxidation of hepatocyte lysosomal membranes (Kvasnicova et al. 2003).

Conclusions

Based on this research, the formation of 8-OHdG occurs due to metabolic interactions in the body that make methyl parabenbioactivated to form by-products in the form of hydroxy radicals that attach to DNA Guanine.

Conflict of Interest: The authors declare that there are no conflicts of interest concerning the publication of this article.

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