Rhodamin-B increases Hippocampus cell apoptosis in *Rattus norvegicus*-oxidative stress related to Parkinson, Alzheimer, cancer, hyperactive, anterograde amnesia diseases

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Abstract

Rhodamine B is a textile dye compounds containing chlorine (Cl^-), alkylating (CH3-CH3), Poly Aromatic Hydrocarbons (PAH) which activate the enzyme cytochrome P-450 as well as the structure of quinone which is very redox that leads to the formation of Reactive Oxygen Species (ROS). ROS increases induce apoptosis of the intrinsic pathway. The imbalance ratio between BAX and BCL-2 stimulates apoptosis in Hippocampus tissue. "The selected design was "the post test only control group" using twenty-eight Wistar female Rattus norvegicus mouse age of 10-12 weeks. There was a significant difference (p-value <0.05) of total BCL-2 expression between the control group to the treatment group. Correlation coefficient of 0.945 indicates that the level of the relationship/ correlation is very strong category. Increasing doses of Rhodamine B was given, accompanied by the decrease in the expression of BCL-2. Correlation coefficient of -0.731 indicates that the level of the relationship/ correlation belongs strong category. It is concluded that Rhodamin B has been verified as capable to increase the expression of BAX and to reduce the expression of BCL-2 in hippocampus tissue on Rattus norvegicus.

Introduction

Although the ban on the use of Rhodamine B dyes has been regulated in the Minister of Health Regulation No. 722/Menkes/Per/VI/88 and Permenkes RI No. 239/Menkes/Per/V/85 but the wider community still uses Rhodamin B in food processing.[1,2]

Rhodamin B is known to have toxic, carcinogenic and genotoxic effects.[3] The effect of Rhodamin B if it is consumed for a long time is that it will continuously cause irritation in the respiratory, eye disorders, bladder cancer, liver damage, heart, lymph, kidney, pancreas, central nervous system and brain damage.[4,5] Rhodamine B oral exposure in male and female rats for 30 days caused structural damage to liver histology, renal macroscopic changes, histopathology of the kidney proximal tubules of male mice, low radioactivity uptake in the brain, inhibited growth, diarrhea, death, lymphatic liver cancer, bladder dilation, liver poisoning, loss of body weight, body cell volume, total serum protein, discoloration, degradation of hair and skin become red and rough, abnormal behavioral changes (aggressive, cannibal), intrauterine death, impaired growth and internal abnormalities of the fetus, cell damage to liver and kidneys in pregnant rats Rattus norvegicus.[6-13]

Rhodamin B has a quinon structure which is a very redox active molecule causing the formation of ROS (reactive oxygen species) which leads to oxidative stress and cell injury to target cells (CNS, hypothalamus, Adenohypophisis). Increased ROS in the blood induces the cell apoptosis phase. Induction of apoptosis in hippocampal tissue is involved in various diseases, especially Alzheimer's.[14,15]

Hippocampus is a small organ located in the medial temporal lobe of the brain and is an important part of the limbic system which is the area that regulates emotions, memory, especially long-term memory and plays an important role in spatial navigation. Damage to the hippocampus can cause memory loss and difficulties in building new memories. In Alzheimer's disease, hippocampus is one of the first areas of the brain that is affected causing confusion and memory loss so that it is often seen in the early stages of this disease.[15]

In this study, researchers focused on the effects of Rhodamine B on the expression of BAX (Bcl-2 Antagonist X) and BCL-2 (B-cell lymphoma-2) in the hippocampal tissue in Rattus norvegicus.

Materials and Methods

Rattus norvegicus Wistar strain female mouse, healthy, aged 10-12 weeks found in the experimental animal raising unit (UPHP) with the consideration that mice are experimental mammals (laboratory animals) as a research model before being treated in humans.

The experimental animals were all adapted first at room temperature of 22-25 ° C for 11 days at the UB Pharmacology Laboratory, Faculty of Medicine. Before being given treatment, rats were synchronized with the estrous cycle with the whitten method for 5 days. Then, the rats were grouped into the control group (given standard feed ad libitum), group I

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Key words: rhodamine B, BAX, BCL-2, hippocampus tissue.

Contributions: the authors contributed equally.

Conflict of interests: the authors declare no potential conflict of interest.

Funding: none.

Clinical trials: This research has been approved by research ethics committee of Faculty of Medicine Brawijaya University, Malang, Indonesia.

Conference presentation: part of this paper was presented at the 3rd International Symposium of Public Health, 2018 October 31-November 1, Universitas Airlangga, Surabaya, Indonesia.

Acknowledgment: the authors would like to thank all technicians in Laboratory of Pharmacology, Pathology and Biomedical Science for helping this study.

Dedication: the article is dedicated to Doctoral Program of Public Health, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia

Received for publication: 28 July 2019.
Revision received: 9 September 2019.
Accepted for publication: 15 October 2019.

Journal of Public Health in Africa 2019; 10(s1):1175
doi:10.4081/jphia.2019.1175

(given standard feed and Rhodamin B dose 150 ppm (4.5 mg/ 200 gBW), group II (given standard food and Rhodamin B dose 300 ppm (9 mg/ 200 gBW), group III (given standard food and Rhodamin B 600 ppm dose (18 mg/ 200 gBW). Rhodamine B was given per sonde for 36 days.

After 36 days of exposure to Rhodamin B, all rats were anesthetized using inhaled chloroform, the mice were turned off and hippocampus tissue samples were taken by surgery on the brains of rats and put into 10% formalin solution and carried out the hippocampus tissue removal slice by observing the mouse brain anatomy for making slides.

Analysis of BAX and BCL-2 expression in hippocampus tissue using immunohistochemical staining was conducted and observed with a microscope. Brown hippocampus tissue shows BAX and BCL-2 expression, while if it is purple, it shows no expression of BAX and BCL-2. The calculation of BAX and BCL-2 expressions based on weak color intensity (1), medium (2), strong (3), very strong (4) using the help of OliVIA software. Data results are then processed using SPSS for Windows software.

This study has passed ethics at the Ethics Committee of the Medical Faculty of the University of Brawijaya.

Table 1. Comparison of the effects of Rhodamine B on BAX expression with the Kruskal-Wallis test.

Treatment	Mean ± SD	p-value
Control	2.57±1.27[a]	0.000
4,5 mg	6.43±2.5[1bc]	
9 mg	11.57±3.21[cd]	
18 mg	18.71±1.11[d]	

On average ± SD, if it contains different letters, it means that there are significant differences, while if you load the same letter, it means that there is no meaningful difference.

Table 2. Comparison of the effects of Rhodamine B on BCL-2 expression with Anova.

Treatment	Mean ± SD	p-value
Control	7 ±1.155[c]	0.000
4,5 mg	4.571±2.07[b]	
9 mg	2.714±1.38[a]	
18 mg	2.286±0.756[a]	

Results

Based on the results of testing the assumptions of normality and assumption of homogeneity, the assumptions of homogeneity are not fulfilled. Furthermore, testing was conducted to determine the effect of nonparametric administration of Rhodamin B on the expression of BAX using the Kruskal-Wallis test (Tables 1-3).

Discussion

Based on the results of BAX total expression data analysis in Table 1 using the Kruskal-Wallis test, the p-value of 0.0000 was smaller than α=0.05 (p<0.05). Thus, from this test, it can be concluded that there was a significant effect of Rhodamine B in increasing BAX expression. In other words, there are significant differences in the expression of BAX due to the different doses of Rhodamin B. The control group had the lowest average BAX expression. Significantly increased expression of BAX was shown by Rhodamine B at all doses. This was indicated by the average value of ± SD group given Rhodamin B containing different letters from the control group. Meanwhile, the highest average expression of BAX was shown by the group of rats exposed to Rhodamin B at a dose of 18 mg/ 200 gBW, which was 18.71 but not significantly different from the administration of 9 mg/ 200 gBW. This shows that the highest increase in BAX expression is shown in doses of 9 mg and 18 mg.

Based on the results of the analysis using ANOVA, it was obtained a p-value of 0,000, smaller than α=0.05 (p<0.05). So, from this test, it can be concluded that there is a significant effect of Rhodamin B on the decrease in BCL-2. In other words, there is a significant difference in BCL-2 due to different doses of Rhodamine B. In the comparison of all levels of the treatment group, a p-value of less than 0.05 was obtained except in the comparison between the dose of 9 mg/ 200 gBW with 18 mg/ 200 gBW. This shows that there is no difference in average BCL-2 between groups of rats given Rhodamin B at a dose of 9 mg/ 200 gBW at a dose of 18 mg/ 200 gBW. Or in other words, giving Rhodamin B with a dose of 9 mg/ 200 gBW and 18 mg/ 200 gBW gave the same effect on the decrease in BCL-2. Based on the results of the analysis, it can be seen that the dose of Rhodamine B which can significantly reduce BCL-2 to the lowest point is the dose of 18 mg/ 200 gBW but not significantly different from the dose of 9 mg/ 200 gBW. Rhodamin B dosage at all levels can significantly reduce BCL-2.

Based on the results of testing the correlation between Rhodamin B administration and BAX expression, a correlation coefficient of 0.945 with a p-value of 0.000 was obtained. At the 5% error level (α=0.05), it was shown that the p-value obtained was less than 0.05 (p<0.05). From this test, it can be seen that there is a significant relationship between Rhodamin B administration and BAX expression. On the results of testing the correlation between the administration of Rhodamin B with BCL-2 expression, a correlation coefficient was found at -0.731 with a p-value of 0.000. At the 5% error level (α=0.05), it was shown that the p-value obtained was less than 0.05 (p<0.05). From this test it can be seen that there is a significant relationship between Rhodamine administration and BCL-2 expression.

Table 3. Rhodamin B correlation test for BAX and BCL-2 expression.

Variable	Correlation coefficient	p-value	Description
Expression of BAX	0.945	0.000	Significant
Expression of BCL-2	-0731	0.000	Significant

Conclusions

Rhodamin B has been verified as capable to increase the expression of BAX, to reduce the expression of BCL-2 in hippocampus tissue on Rattus norvegicus.

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