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

Dewi Ratna Sulistina,1 I Wayan Arsana Wiyasa,2 Windhu Purnomo3
1Doctoral Program of Public Health, Faculty of Public Health, Universitas Airlangga; 2Division of Fertility, Endocrinology and Reproduction, Obstetric and Gynecology Laboratory, Saiful Anwar General Hospital; Faculty of Medicine, Brawijaya University, Malang, East Java; 3Biostatistics and Population Department, Faculty of Public Health, Universitas Airlangga, Indonesia

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 Rhodamine 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 Rhodamine B in food processing.1,2

Rhodamine B is known to have toxic, carcinogenic and genotoxic effects.3 The effect of Rhodamine 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, cell injury to target cells (CNS, hypothalamus). Adenohypophysis). Increased ROS in the blood induces the cell apoptosis phase. Induction of apoptosis in hippocampal tissue is involved in various diseases, especially Alzheimer’s.6,13

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.14,15

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
(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 OilVIA 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 Rhodamin B on BAX expression with the Kruskal-Wallis test.

| Treatment | Mean ± SD | p-value |
|-----------|-----------|---------|
| Control   | 2.57±1.27a| 0.000   |
| 4.5 mg    | 6.43±2.5bc|         |
| 9 mg      | 11.57±3.21cd|       |
| 18 mg     | 18.71±1.11d|        |

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 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.

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

| Treatment | Mean ± SD | p-value |
|-----------|-----------|---------|
| Control   | 7 ±1.155c | 0.000   |
| 4.5 mg    | 4.571±2.07b|         |
| 9 mg      | 2.71±1.38a|         |
| 18 mg     | 2.286±0.756a|       |

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 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.

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 | -0.731 | 0.000 | Significant |

Results

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 Rhodamin administration and BCL-2 expression.

Discussion

Based on the results of testing the correlation between Rhodamin B and BCL-2 expression, while if you load the same letter, it means that there are significant differences, while if you load the same letter, it means that there is a significant relationship between Rhodamin B and BCL-2 expression. 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 a significant difference in BCL-2 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. Mean while, 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.

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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