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Rhodamine B induces oxidative stress and cervical epithelial cell proliferation in the uterus

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ABSTRACT

This study aimed to investigate the effect of Rhodamine B exposure on oxidative stress and cervical epithelial cells proliferation in the uterus. Twenty eight female Wistar albino rats were divided into four groups ($n = 7$ each): one control (untreated) group; and three Rhodamine B groups at several doses (4.5, 9, 18 mg/200 g body weight/day) for 36 days. Colometric analysis of malondialdehyde (MDA) level as a marker of lipid peroxidation and histological analysis of the cervical epithelial cells proliferation was performed. The MDA levels and proliferation of epithelial cells were significantly higher in all Rhodamine B groups compared to control group ($P < 0.05$). The MDA levels were increased in a dose-dependent manner in the Rhodamine B groups. Moreover, the proliferation of epithelial cells was also increased by Rhodamine B in a dose-dependent manner. In conclusion, subchronic Rhodamine B administration induces lipid peroxidation and cervical epithelial cells proliferation in a dose dependent manner.

1. Introduction

Rhodamine B (RB) is a synthetic dye crystal with a green or red purple hue. The utilization of Rhodamine B dye includes for paper, textile dyes, dyes for histologic specimens, and cosmetics [1]. When exposed to light, Rhodamine B can form reactive oxygen compounds. There are two types of reaction that result in the formation of reactive oxygen compounds. The first reaction will increase the energy of Rhodamine B and transferred to biomolecules to form reactive oxygen compounds. The second reaction is the reaction of the energy transfer to molecular oxygen to form singlet oxygen [2–4]. When Rhodamine B enters our body together with food, it may trigger oxidative stress on ovarian follicles and decrease the number of primary, secondary, and de Graaf follicles [5].

Depending on the levels of reactive oxygen compounds (ROS), various transcription factors sensitive to change in redox status will be activated and coordinate intracellular biological response. Modest oxidative stress will induce Nrf2, a transcription factor implicated in the gene transactivation that encodes antioxidant enzyme activity. Moderate oxidative stress will trigger an inflammatory response through NF-κB and AP-1 activation. Severe oxidative stress will result in the disruption of mitochondrial pores and electron transfer disorders that will ultimately lead to apoptosis [6]. Cervix is part of a reproductive organ protecting the developing fetus, as well as the contents of the pregnant uterus, from the external vaginal environment. The cervix has remodeling properties as a response for birth [7]. To our knowledge, there is no study concerning the toxic effect of Rhodamine B on the uterine cervix as an important organ in delivery. Thus, our study was aimed to investigate whether the exposure of Rhodamine B induces oxidative stress and modulates the proliferation of cervical epithelial cells.

2. Material and methods

2.1. Animal

Twenty eight female Wistar albino rats, aged 8–12 weeks, weighing 160–250 g were used in this study. These animals were purchased from Gadjah Mada University, Yogyakarta, Indonesia. Subjects were divided into four groups ($n = 7$ each), including control (untreated group) and Rhodamine B groups to whom Rhodamine B will be injected at different doses (4.5, 9, and 18 mg/200 g body weight/day). The subjects were housed in a clean wire cage and maintained under standard research laboratory environment (temperature 25 ± 2 °C with 12/12 h of dark/light cycle). These animals were fed with a standard pellet diet and received water ad

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libitum as well as acclimatized to laboratoric conditions for one week prior to the experiment.

2.2. Tissue sampling

At the end of the intervention, subjects from all groups were anesthetized. The uterine cervical was collected, weighed, and latter rinsed with physiological saline. All samples were stored at −80 °C until the time of analysis.

2.3. Rhodamine B

Rhodamine B (Rhodamine B, Sigma–Aldrich, St. Louis, MO, USA) was dissolved with double distilled water and administered orally using oral gavage. Subjects in the treatment groups were administered with Rhodamine B for 36 days based on previous studies [5].

2.4. Malondialdehyde analysis

The BIOXYTECH MDA-586TM Spectrophotometric Assay for Malondialdehyde assay kit (Catalog No: 21044) was purchased from Oxis International, Inc. (Foster City, CA, United States). The analysis was performed based on the procedure details available in the assay kit.

2.5. Hematoxylin-eosin (HE) staining

The histopathological profile of the uterine cervical tissues was analyzed using HE staining based on the previous study [8]. An experienced pathologist was appointed to count the number of cervical epithelial cells in proliferation state. Observations were made using Olympus microscope and dot XC software on 10 slides (400× magnification on 10 fields of view). Proliferating cells are characterized by a larger cell nucleus and dark purple hue, and were expressed in percentage of cell (%).

2.6. Ethics

This experimental study has been approved by the research ethics committee of the Faculty of Medicine of Brawijaya University Malang, Indonesia. The study was conducted according to the guidelines issued by the Institutional Animal Care and Use Committee of Brawijaya University, Malang, Indonesia.

2.7. Statistical analysis

Data was presented as mean ± SD and differences between groups were analyzed with *t*-student and 1-way ANOVA tests using SPSS 15.0 statistical package. The post hoc test was used if the ANOVA result was significant. *P* value of <0.05 was considered statistically significant.

3. Results

Table 1 presents the MDA levels in the cervix from each intervention group. The level of MDA was significantly higher in all Rhodamine B groups compared to the control group ($P < 0.05$). The MDA levels were significantly increased in a dose-dependent manner.

Table 2 presents the proliferation of cervical epithelial cells in the uterus from each intervention group. The proliferation of epithelial cells was significantly greater in the Rhodamine B groups compared with the untreated group ($P < 0.05$) in a dose-dependent manner.

Table 1
The levels of uterine cervical malondialdehyde in each experimental group.

	Rhodamine B (mg/ 200 g body weight)			
Level	Control	4.5	9	18
MDA (ng/100 mg)	0.065 ± 0.018	0.135 ± 0.057[a]	0.228 ± 0.024[a,b]	0.445 ± 0.075[a,b,c]

Note: values are presented as mean ± SD.
[a] $p < 0.05$; in comparison with the control group.
[b] $p < 0.05$; in comparison with the RB group using the first dose.
[c] $p < 0.05$; in comparison with the RB group using the second dose; MDA: malondialdehyde; RB: Rhodamine B; ng: nanogram; mg: milligram.

Table 2
Cells proliferation level in each experimental group.

	Rhodamine B (mg/ 200 g body weight)			
Level	Control	4.5	9	18
Proliferation (%)	39.58 ± 3.86	62.16 ± 5.09[a]	69.49 ± 1.94[a,b]	74.81 ± 1.79[a,b,c]

Note: values are presented as mean ± SD.
[a] $p < 0.05$; in comparison with the control group.
[b] $p < 0.05$; in comparison with the RB group using the first dose.
[c] $p < 0.05$; in comparison with the RB group using the second dose; RB: rhodamine B; %: percentage of the cells.

4. Discussion

The oxygen-derived species as well as oxidative stress have been implicated in the etiology of a wide array of human diseases. The dynamic state of cellular ROS depends on the equilibrium between the internal generation of ROS and the cell's antioxidant system. In the present study, we observed a significant increase in MDA levels in the uterine cervical epithelial cells of mice exposed to Rhodamine B. MDA is a decomposition product of peroxidized polyunsaturated fatty acid that acts as a marker for the detection of ROS' reactivity toward lipid peroxidation [9,10]. The observation of MDA levels clearly demonstrates that Rhodamine B increases lipid peroxidation as an oxidative stress marker. Our data contributes to previous data suggesting that Rhodamine B can increase ovarian MDA levels significantly compare to the control female rats ($P < 0.05$) [5]. Quinones, as Rhodamine B structure, are a compound where electron and proton carriers play a major role in aerobic metabolism of every cell and performing redox reactions in cell organelles. Quinones also enter the redox cycle with semiquinone radicals to form reactive oxygen compounds [11–13]. In addition, Rhodamine can also penetrate into the cells and accumulate in the mitochondria to disrupt the respiratory chain [14]. We found that the MDA levels in cervix were significantly increased by Rhodamine B in a dose-dependent manner. This finding indicates that there was a positive association between the dose of Rhodamine B with redox reaction and disruption of respiratory chain that produces more reactive oxygen compounds.

Both the inner (endocervix) and outer regions of the cervix are layered by squamous mucosal epithelia [15,16]. In this study, the proliferations of epithelial cells were significantly greater in the Rhodamine B group compared to the untreated group ($P < 0.05$). The NF-κB pathway is generally thought to be a primary oxidative stress-response pathway. We therefore hypothesize from these observations that the oxidative stress marked by the elevation of MDA levels will activate NF-κB pathway for cell proliferation. We also found that the proliferation of epithelial cells was increased by Rhodamine B in a dose-dependent manner.

In conclusion, subchronic oral Rhodamine B administration induces lipid peroxidation and the proliferation of cervical epithelial cells in a dose dependent manner.

Conflict of interest

The author(s) declare(s) no conflict of interests regarding the publication of this article.

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