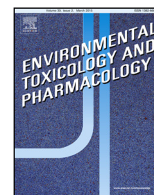
Environmental Toxicology and Pharmacology 48 (2016) 17–21

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap



Chlorhexidine possesses unique cytotoxic actions in rat thymic lymphocytes: Its relation with electrochemical property of membranes

Kayo Nonami [a], Shohei Saitoh [b], Yumiko Nishimura-Danjobara [c], Shiro Ishida [d], Yasuo Oyama [a,e,*]

[a] Faculty of Integrated Arts and Sciences, Tokushima University, Tokushima 770-8502, Japan
[b] Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan
[c] Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima 734-8553, Japan
[d] Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8512, Japan
[e] Faculty of Bioscience and Bioindustry, Tokushima University, Tokushima 770-8513, Japan

ARTICLE INFO

Article history:
Received 17 April 2016
Received in revised form 9 August 2016
Accepted 30 September 2016
Available online 1 October 2016

Keywords:
Chlorhexidine
Hydrogen peroxide
Cytotoxicity
Lymphocytes
Phosphatidylserine

ABSTRACT

Chlorhexidine (CHX) is an antibacterial agent used in various types of pharmaceutical products. Therefore, CHX is easily found around us. Owing to its positive charge, the electrochemical property of cell membranes was assumed to be a key point of cytotoxic action of CHX. Depolarization of membranes attenuated the cytotoxic action of CHX in rat thymic lymphocytes. CHX interfered with annexin V binding to membranes. Manipulations to induce exposure of phosphatidylserine on the outer membrane surface augmented the cytotoxic action of CHX, indicating that changes in the electrochemical property of membranes affected the cytotoxic action of CHX. Hence, CHX might kill cells physiologically undergoing apoptosis, resulting instead in necrotic cell death. However, the threshold CHX concentration in this in vitro study was slightly higher than blood CHX concentrations observed clinically. Therefore, these results may support the safety of CHX use although CHX possesses unique cytotoxic actions described in this study.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Chlorhexidine (CHX) is a widely used antibacterial agent commonly found in disinfectants, cosmetics, and pharmaceutical products (Lowbury and Lilly, 1973; Fardai and Turnbull, 1986; Opstrup et al., 2015). Therefore, CHX is easily found in our environment. Mouthwash typically contains 0.02–0.2% CHX digluconate as an antimicrobial agent (for a review, Jones, 1997), while vaginal wash during labor is performed usually with 0.2% CHX digluconate (Burman et al., 1992). In gel formulation for single topical application, the concentration of CHX ranges from 1 to 2% (Jones, 1997). CHX digluconate or CHX diacetate of 2–4% is used for full body bath in hospitalized patients (Cowen et al., 1979; Climo et al., 2013) and medical device sterilization (Chapman et al., 2013). No significant adverse events induced by CHX have been reported except for contact dermatitis, and there are no clinical data to discourage the use of CHX. Therefore, the effect of CHX on intact cells may be ignored. The number of cells in multicellular organisms is regulated by controlling the rates of cell division and cell death. Such cell death that is programmed is called as apoptosis. Apoptosis occurs in developing and adult tissues (Elmore, 2007). Therefore, the chemical compounds affecting the process of apoptosis may disturb normal development.

At physiological pH range, CHX is positively charged and binds non-specifically to negatively charged membrane phospholipids (Hjeljord et al., 1973; Jones, 1997). Distribution of phospholipids in the membrane lipid bilayer of eukaryotic living cells is asymmetric under normal physiological conditions, where phosphatidylcholine and phosphatidylserine are found in the outer and inner monolayers, respectively. However, phosphatidylserine is exposed on the outer monolayer in cells at the early stage of apoptosis. Apoptosis occurs normally during development and aging, and is a homeostatic mechanism to maintain cell populations in tissues (Elmore, 2007; Tiwari et al., 2015). Phosphatidylcholine is zwitterionic while phosphatidylserine is anionic. Therefore, electrochemical property

* Corresponding author at: Faculty of Bioscience and Bioindustry, Tokushima University, Tokushima 770-8513, Japan.
E-mail address: oyamay@tokushima-u.ac.jp (Y. Oyama).

http://dx.doi.org/10.1016/j.etap.2016.09.024
1382-6689/© 2016 Elsevier B.V. All rights reserved.

of membranes is changed in cells undergoing apoptosis. If positively charged CHX binds to anionic phosphatidylserine in high preference to zwitterionic phosphatidylcholine, CHX would induce a more profound effect on apoptotic living cells, leading to necrosis. Necrosis is an uncontrolled and passive process that usually affects large fields of cells, whereas apoptosis is controlled, energy-dependent, and can affect individual or clusters of cells (Kono and Rock, 2008). Cells undergoing necrosis lose membrane integrity and leak their intracellular components, some of which serve as danger signals that stimulate inflammation (Kono and Rock, 2008; Moriwaki and Chan, 2013). To test the hypothesis, we examined the effects of CHX on rat thymocytes simultaneously incubated with agents that induce exposure of phosphatidylserine on the outer surface of membranes. This study may provide new insights into the toxicological profile of CHX for its safe use.

2. Materials and methods

2.1. Animal and cell preparation

This study was approved by the Committee for Animal Experiments of Tokushima University, Tokushima, Japan (No. 14124). Experimental methods were similar to those described in previous papers (Chikahisa et al., 1996; Oyama et al., 1999; Matsui et al., 2008).

Male rats (Wistar strain, Charles River Laboratories, Yokohama, Japan) were provided with water automatically and a commercial diet (MF, Oriental Yeast, Tokyo, Japan) ad libitum. The animal room was maintained at a temperature of 23 ± 2 °C and a relative humidity of 55 ± 5%, and it was artificially illuminated with fluorescent light on a 12-h light/dark cycle (08:00–20:00 h). The total number of 8–12 week old rats sacrificed under ether inhalation anesthesia was 27.

Cell suspension was prepared as previously reported (Chikahisa et al., 1996). In brief, thymus glands dissected from ether-anesthetized rats were sliced under cold conditions. The slices were triturated in Tyrode's solution to dissociate the thymocytes. The cell suspension was incubated at 36–37 °C for 1 h before the experiment. The cell suspension (60–80 mL) was prepared from one experimental animal.

We used ether inhalation anesthesia for sacrifice. One may argue the possibility that the ether anesthesia changes the effect of drug on rat thymocytes under the in vitro condition. We previously confirmed no difference between the results obtained from the cells isolated from rats sacrificed with decapitation and ether-anesthetized ones (Matsui et al., 2008).

2.2. Chemicals

CHX digluconate was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Annexin V-FITC, propidium iodide, and 5-chloromethylfluorescein diacetate (5CMF-DA) were obtained from Molecular Probes Inc., Invitrogen (Eugene, OR, USA). Other chemicals were obtained from Wako Pure Chemicals (Osaka, Japan) unless mentioned.

2.3. Fluorescence measurements of cellular parameters

The methods for measurements of cellular and membrane parameters using a flow cytometer equipped with an argon laser (CytoACE-150, JASCO, Tokyo, Japan) and fluorescent probes were similar to those previously described (Chikahisa and Oyama, 1992; Chikahisa et al., 1996). The fluorescence was analyzed by JASCO software (JASCO). As to chemicals used in this study, there was no fluorescence detected under our experimental condition.

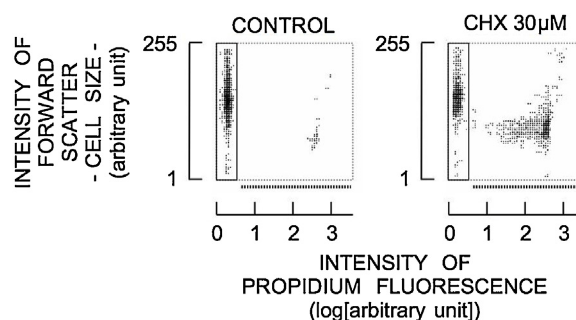


Fig. 1. CHX-induced increase in population of cells exhibiting propidium fluorescence (dead cells). The dotted bar under each cytogram indicates the population of cells stained with propidium iodide.

Table 1
CHX-induced change in cell lethality.

	MEAN	SD	VS CONT
Control	5.3	0.3	
CHX (μM)			
1	5.3	0.4	NS
3	6.0	1.1	NS
10	10.0	0.4	P < 0.01
30	53.4	2.8	P < 0.01
100	99.1	0.1	P < 0.01

NS: No siginificant difference.

To assess cell lethality (population of dead cells) using propidium iodide, the dye was added to the cell suspension at a final concentration of 5 μM at 5 min before the measurement. Exposure of phosphatidylserine on the outer surface of cell membranes, a marker of early stage apoptosis, was detected using 10 μL/mL annexin V-FITC (Koopman et al., 1994). AnnexinV-FITC was added to the cell suspension at 30 min before the measurement. Excitation wavelength for these fluorescent probes was 488 nm elicited by argon laser. Fluorescence of FITC and propidium was detected at 530 ± 20 nm and 600 ± 20 nm, respectively.

2.4. Experimental protocols

The agents were added to the cell suspension (2 ml cell suspension in each 10 ml test tube). The cells were incubated with the agent(s) at 36 °C for 1–3 h under room air condition. The incubation time was dependent on each experimental purpose. The data acquisition of fluorescence from 2500 cells by a flow cytometer required 30 s at maximum.

2.5. Statistical analysis

Statistical analyses were performed by ANOVA with post-hoc Tukey's multivariate analysis. P-values of less than 0.05 were considered significant. Values (including columns and bars in figures) were expressed as mean and standard deviation of 4–8 samples.

3. Results

3.1. CHX-induced change in cell lethality of rat thymocytes

The incubation of rat thymocytes with 30 μM CHX for 1 h increased the population of cells exhibiting propidium fluorescence (Fig. 1), indicating CHX-induced increase in cell lethality. The dose-response relationship of CHX-induced increase in cell lethality shown in Table 1. revealed statistically significant increase in cell lethality when the cells were incubated with CHX concentrations of 10 μM and higher.

Download English Version:

https://daneshyari.com/en/article/5559894

Download Persian Version:

https://daneshyari.com/article/5559894

Daneshyari.com