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Cytotoxicity of 1-dodecyl-3-methylimidazolium bromide on HepG2 cells

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ABSTRACT. We evaluated the cytotoxicity of 1-dodecyl-3-methylimidazolium bromide ([C_{12}mim][Br]) on HepG2 cells and its influence on plasma membrane permeability. The results showed that [C_{12}mim][Br] inhibited HepG2 cell growth and decreased cell viability in a concentration-dependent manner. The results also revealed that [C_{12}mim][Br] exposure induced apoptosis in [C_{12}mim][Br]-treated HepG2 cells. In addition, the results showed that [C_{12}mim][Br] increased membrane permeability in HepG2 cells. These results suggest that plasma membrane permeability may be responsible for apoptosis induced by [C_{12}mim][Br] in HepG2 cells.

Key words: Apoptosis; Cytotoxicity; HepG2 cells; Ionic liquids; Plasma membrane permeability

INTRODUCTION

Ionic liquids are low-melting point salts composed of organic cations and organic or inorganic anions. Because they exist in the liquid state at room or low temperature (<100°C), they are typically referred to as room-temperature ionic liquids (Bonhôte et al., 1996; Li et al., 2012b; Jing et al., 2013a). Common cations include quaternary ammonium salt cations, quaternary phosphine salt cations, imidazole salt cations, and pyrrole salt cations, whereas common anions include halogen anions, tetrafluoroborate anions, and hexafluorophosphate anions (Jiang and Yu, 2008). Imidazole ionic liquids are the most stable and extensively applied type among the various ionic liquids (García-Lorenzo et al., 2008; Samori et al., 2010). Therefore, we used the imidazole ionic liquid 1-dodecyl-3-methylimidazolium bromide ([C_{12}mim][Br]) in this study.

Compared with traditional organic solvents, room-temperature ionic liquids acting as reaction mediators or catalysts have a wider liquid range and almost negligible vapor pressure. Furthermore, they can be repeatedly used, which is particularly significant to reduce environmental pollution, which have attracted increasing attention. Therefore, they are referred to as green solvents and have extensive application prospects (Sheldon, 2001).

However, the environmental issues pertaining to ionic liquids have been gradually recognized. The synthesis, purification, recycling, and application of ionic liquids have some limitations, and if the liquids run off into the water reservoirs, they can affect hydrobios and the water environment (Pham et al., 2010; Samori et al., 2010; Li et al., 2012a).

Although the toxic effects of ionic liquids on organisms have been reported in numerous studies (Luo et al., 2008; Ventura et al., 2010), studies on their cytotoxicity are rare. In this study, we investigated the toxic effect of [C_{12}mim][Br] on HepG2 cells and its influence on plasma membrane permeability. Our results provide useful information for further investigating the molecular mechanisms underlying the toxic effect of ionic liquids.

MATERIAL AND METHODS

Ionic liquids and reagents

[C_{12}mim][Br] (purity > 99%) was purchased from Hubei Hengshuo Chemical Co., Ltd. (Hubei, China). The structure of [C_{12}mim][Br] is shown in Figure 1. High-glucose Dulbecco's modified Eagle's medium (H-DMEM) and fetal bovine serum were obtained from Invitrogen (Carlsbad, CA, USA). Hoechst33342 (catalog #C0222) was purchased from Beyotime Institute of Biotechnology (Haimen, China). Fluorescein diacetate (FDA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Keeasy Economic & Trade Co., Ltd (Zhengzhou, China). All other reagents were of analytical grade and were obtained from commercial sources.

Cell culture

HepG2 cells were obtained from the Henan Key Laboratory for Heredity Diseases and Molecular Targeted Medicines and cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum at 37°C in an incubator with humidified air and 5% CO_2.

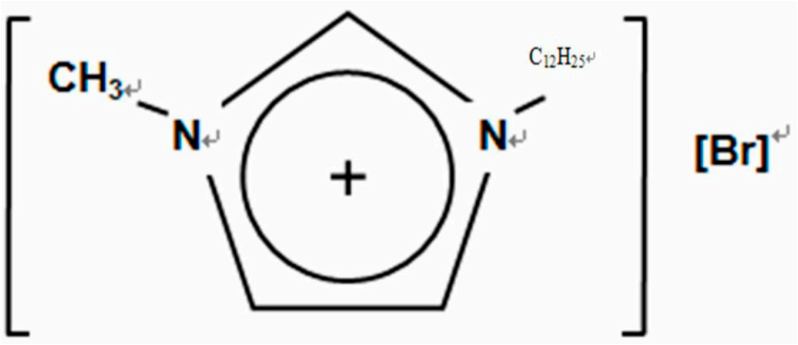

Figure 1. Structure of [C_{12}mim][Br].

Cell viability assay

The viability of cells treated with [C_{12}mim][Br] was measured using MTT reagent, according to a previously described method (García-Lorenzo et al., 2008). This assay is based on the conversion of yellow tetrazolium salt to purple formazan crystals by metabolically active cells. Briefly, HepG2 cells were cultured in a 96-well plate (1.5 x 10^4 cells per well) for 12 h and were subsequently exposed to [C_{12}mim][Br] for 24 h. The concentrations used in the test were 0.01, 0.1, 1, 10, 20, 100, 1000 μM. Each test was conducted in octuplicate. The cells cultured in complete medium without [C_{12}mim][Br] were used as controls. After 24 h of exposure, 10 μL MTT was added to each well and incubated for 4 h, and then the medium was carefully removed and formazan crystals were dissolved in 150 μL dimethyl sulfoxide and shacked for 10 min. The absorbance at 490 nm was monitored. Cell viability was expressed as the absorption percentage of the exposed cells to the controls. EC_{50} values were calculated using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA).

Observation of cellular and nuclear morphology

HepG2 cells were cultured on 24-well plates (2.5 x 10^5 cells per well) with 0, 1.25, 2.5, 5 μM [C_{12}mim][Br] for 24 h and then collected and rinsed twice with phosphate-buffered saline (PBS). Hoechst 33342 was subsequently added to the suspensions at a final concentration of 10 μg/mL for 10 min at 37°C in the dark. Cells were then washed twice with PBS and immediately observed by using a fluorescence microscope (Nikon, Tokyo, Japan) at an emission wavelength of 521 nm. In total, 200 cells from 10 random high-power fields were counted. Apoptotic nuclei of cells were assessed by counting the number of cells displaying changes in nuclear morphology, such as chromatin condensation and fragmentation. The percentage of apoptosis was expressed as the ratio of apoptotic cells to total cells.

Determination of alterations in plasma membrane permeability

FDA staining can be used to detect alterations in plasma membrane permeability. HepG2 cells were cultured in 6-well plates (2.5 x 10^5 cells per well) with 0, 1.25, 2.5, 5 μM [C_{12}mim][Br] for 24 h and then collected and rinsed twice with PBS. FDA was subsequently added to the suspensions at a final concentration of 50 mM. After incubation for 10 min at 37°C, the cells were centrifuged at 600 *g*, the supernatant was removed, and the cells were finally resuspended in 0.5

mL PBS. The cell suspensions were transferred into a 96-well black plate and each test was conducted in quadruplicate. Fluorescence intensity (Ex: 485 nm, Em: 538 nm) was detected using a fluorescent chemical analyzer. The results were presented as normalized relative fluorescence units (U/cell).

Statistical analysis

Data were analyzed using one-way analysis of variance, followed by least significant difference determination using SPSS 17.0. P values less than 0.05 were considered statistically significant.

RESULTS

Viability of HepG2 cells exposed to [C_{12}mim][Br]

After 24 h of [C_{12}mim][Br] exposure, the viability of HepG2 cells in all treatment groups was decreased compared with that of the controls, as shown in Figure 2. The 24-h EC_{50} of [C_{12}mim][Br] for HepG2 cells was 9.8 μM. Additionally, the results also showed that the cytotoxicity of [C_{12}mim][Br] was concentration-dependent.

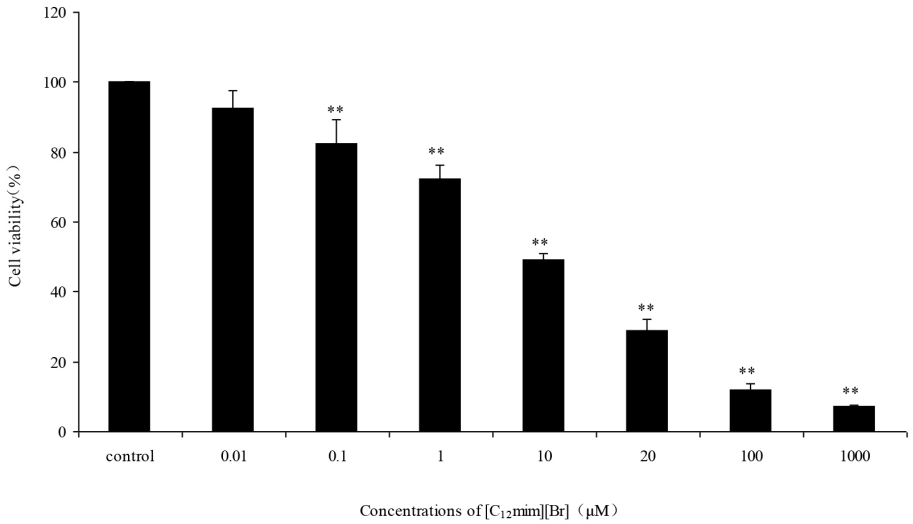

Figure 2. Viabilityof HepG2 cells exposed to variouscon centrations of [C_{12}mim][Br]. Data are shown as means ± SD. Asterisk denotes a response that is significantly different from the control (**P < 0.01).

Alterations in cellular and nuclear morphology

Microscopic observation showed that different concentrations of [C_{12}mim][Br] caused changes in cell morphology (Figure 3). Regular morphology and consistent uniform size was observed in the control groups, while [C_{12}mim][Br]-treated cells became small and spherical, showing lower adhesion ability with increasing [C_{12}mim][Br] concentration. Quantitative analysis showed that the percentage of apoptotic cells in all treatment groups was higher compared to that in the controls, suggesting that [C_{12}mim][Br] exposure induces the apoptosis of HepG2 cells.

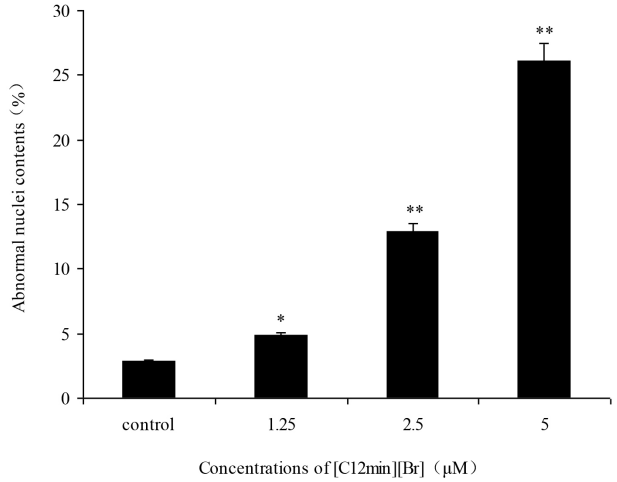

Figure 3. Alteration in the nuclear morphologyof HepG2 cells,induced by [C_{12}mim][Br]. Data are shown as the means ± SD. Asterisks denote a response that is significantly different from the control (*P < 0.05, **P < 0.01).

Plasma membrane permeability

The results of FDA staining revealed obvious fluorescein leakage in all treated groups when compared to the control groups (Figure 4), which indicated that [C_{12}mim][Br] may increase membrane permeability in HepG2 cells.

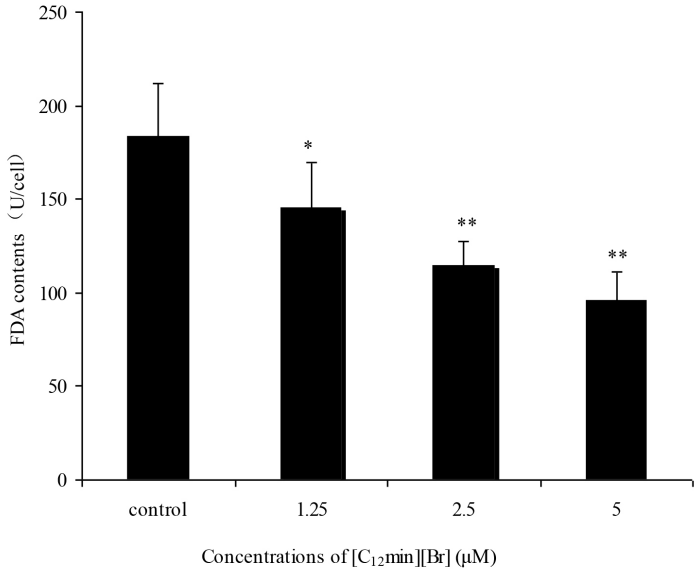

Figure 4. FDA contents of HepG2 cells exposed to various concentrations of [C_{12}mim][Br]. Data are shown as means ± SD. Asterisk denotes a response that is significantly different from the control (*P < 0.05, **P < 0.01).

DISCUSSION

Toxicity tests at the cellular level are convenient and easy to perform; they also avoid many ethical issues. Thus, these studies are useful for examining toxicity mechanisms (Guan et al., 2008). In this study, we evaluated the toxic effect of [C_{12}mim][Br] and explored its toxicity mechanisms. This study not only furthered the findings of our previous study that was conducted at an individual level (Jing et al., 2013b) but also evaluated ionic liquids for a more comprehensive analysis. In this study, MTT colorimetry showed that [C_{12}mim][Br] significantly decreased HepG2 viability when its concentration exceeded 0.1 μM.

Apoptosis is programmed cell death determined by genes. This process is very complex and is influenced by numerous internal and external factors in organisms, particularly in mammals where coordination between a higher number of genes is involved. Apoptosis is characterized by various features such as an abnormal nucleus (Fraser and Evan, 1996). In this study, observation of the cellular morphology revealed that [C_{12}mim][Br] exposure induced typical apoptosis in HepG2 cells.

Structurally, imidazolium ionic liquids are similar to cationic surfactants and can act on biomembranes, thereby increasing cellular membrane permeability (Sheldon, 2005; Jungnikcel et al., 2008; Jing et al., 2014). In this study, [C_{12}mim][Br] increased membrane permeability in HepG2 cells, resulted in abnormal physiological and biochemical reactions.

Overall, ionic liquids at a certain concentration induce cell apoptosis. Increased plasma membrane permeability may be responsible for the apoptosis induced by [C_{12}mim][Br].

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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