

## Cytotoxicity of Bismuth Compounds to Cultured Cancer Cells

Jun Kobayashi<sup>1\*</sup>, Keichi Ikeda<sup>2</sup> and Hideo Sugiyama<sup>3</sup>

<sup>1</sup>Faculty of Veterinary Medicine, Nippon Veterinary and Life Science University, Tokyo, Japan

<sup>2</sup>Faculty of Pharmaceutical Sciences, Hokuriku University, Ishikawa, Japan

<sup>3</sup>Graduate School of Health Sciences, Matsumoto University, Nagano, Japan

### Abstract

Bismuth (Bi) is a ubiquitous metal, but its effects on human health are unknown. We examined the cytotoxicity of Bi compounds to cultured 8505C human thyroid cancer cells. Treatments of 0.05–0.1 mM of Bi subgallate and free gallic acid resulted in cell death in approximately 100% of cells.

**Keywords:** Bismuth; Cytotoxicity; Bismuth subgallate; Gallic acid

### Introduction

Bismuth (Bi) occurs in the earth's crust at a concentration of approximately 100 µg/kg, and is a Group 15 metal element with properties similar to those of lead and mercury [1]. Humans ingest 5–20 µg/day of Bi, mainly through food, and some Bi is thought to be absorbed and accumulated locally in the body. Whether Bi is an essential nutrient in humans is unknown [1,2]. Environmental pollution of Bi has not been a problem, as it is only used in small amounts in industrial settings, and is very low in environmental emissions. In Japan, Bi is not included in any regulatory criteria such as water quality standards or monitoring item guidelines. Entities such as the World Health Organization (WHO), European Union (EU), and the United States Environmental Protection Agency (USEPA) have likewise published no regulatory provisions regarding bismuth [3]. Bismuth is known to exert toxicity when administered in large quantities or for an extended period [1,2,4,5]. Bi subgallate has been used as a medicine for syphilis, peptic ulcer, and skin diseases for decades, and Bi subsalicylate and Bi citrate are used as anti-gastritis and antidiarrheal agents [1,5,6]. In recent years, Bi compounds have been used in cosmetic products, bactericides, dyes, and alloy materials. In addition, the use of Bi in semiconductor manufacturing, the chemical industry, and the nuclear fuel industry is increasing [5,7], potentially resulting in environmental release. However, there are few published reports on the toxicity of Bi compounds.

In this study, we assessed the cytotoxicity of a Bi compound standard product to cultured cells.

### Methods

#### Reagents and apparatus

We were supplied the 8505C thyroid cancer cell line from the Cell Resonance Center for Biomedical Research of Tohoku University, and used for all experiments. Reagents included RPMI-1640 culture medium (Sigma-Aldrich, St. Louis, MO, USA), antibiotics (streptomycin and penicillin, Wako Pure Chemical Industries, Osaka, Japan; biochemistry grade), 0.25% trypsin/1 mM ethylenediaminetetraacetic acid (EDTA) (Gibco, Carlsbad, CA, USA), and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Dojindo Laboratories, Kumamoto, Japan). Commercially available special grade products containing Bi (Bi acetate, Bi chloride, Bi subgallate, and Bi subsalicylate) were used. Water purified from an Elix/Milli-Q Element purifier (Merck-Millipore, Billerica, MA, USA; specific resistance value  $\geq 18$  M $\Omega$ -cm) was used for all experiments.

Twenty-four-well plates (Iwaki, Tokyo, Japan; bottom area of each well: 1.9 cm<sup>2</sup>) were used for culturing and experiments. All glassware were disposable or autoclaved to sterilize.

#### Bi cytotoxicity assay

The 8505C cells were cultured to a subconfluent state in an incubator (37°C, 5% carbon dioxide, humidified) and stripped from the culture plates with trypsin/EDTA. Cells were centrifuged at 800 rpm for 5 min and the supernatant removed. The medium was replaced with RPMI-1640 containing 10% fetal bovine serum (FBS). One milliliter of the cell suspension ( $5 \times 10^4$  cells/ml) was added to each well of a 24-well plate. After overnight incubation, the supernatant of culture medium was replaced with the experimental medium (RPMI-1640 containing no FBS) and Bi (0–0.1 mM Bi subgallate, Bi subsalicylate, Bi acetate, and Bi chloride). After 24 h, live cells were measured using the MTT assay [8].

### Results and Discussion

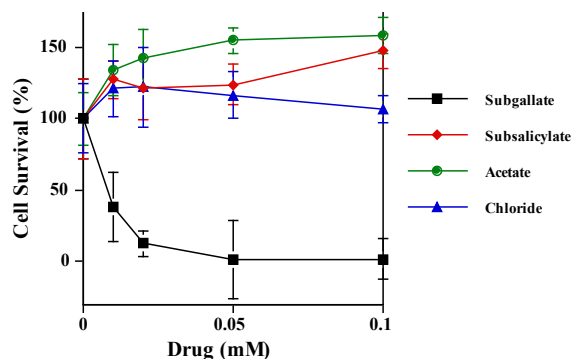
Cell death was observed only in cells treated with Bi subgallate (Figure 1). To ascertain whether the cytotoxicity was attributable to Bi or gallic acid, or to a synergistic effect of both, we investigated the cytotoxicity of gallic acid alone and gallic acid in combination with Bi chloride or Bi subgallate. Gallic acid displayed substantial toxicity, followed by the toxicity of Bi subgallate combined with gallic acid (Figure 2). This finding indicates that Bi can mitigate the cytotoxicity of gallic acid; perhaps ionic Bi forms a complex with free gallic acid, regulating its permeation of the cell membrane [8,9]. It seems that not contrary to the fact that the ratio of free Bi ions in the culture solution is low in the case of organic acid salts such as subgallate. Bi can be detoxified by chelation [10,11], although some amino acids and soil fungi have been shown to enhance its toxicity [8,9]. In this study, we conducted only a very basic examination of cytotoxicity in a human thyroid cancer cell line. Experiments using free gallic acid and other cell lines are necessary to identify the molecular effects of Bi compounds.

**\*Corresponding author:** Jun Kobayashi, School of Veterinary Nursing and Technology, Faculty of Veterinary Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan, Tel: +81422314151; Fax: +81422332094; E-mail: [junkoba@nvlu.ac.jp](mailto:junkoba@nvlu.ac.jp)

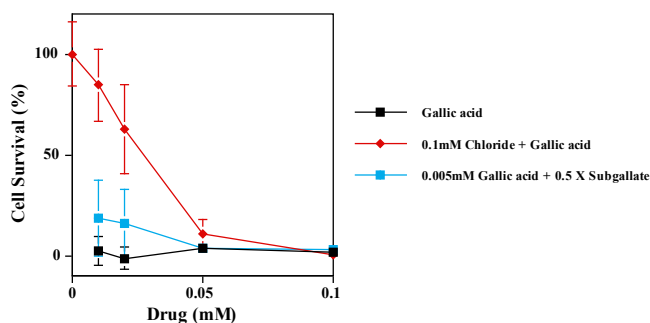
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**Figure 1:** Effect of bismuth salts on 8505C human thyroid cancer cells. Data were calculated and scaled linearly using negative (only medium, 0%) and positive (untreated cells in medium, 100%) controls to yield the survival rate (%). Data are presented as means  $\pm$  standard deviation of three experimental replicates (n=3).



**Figure 2:** Effect of gallic acid on 8505C human thyroid cancer cells. Data were calculated and scaled linearly using negative (only medium, 0%) and positive (untreated cells in medium, 100%) controls to yield the survival rate (%). Data are presented as means  $\pm$  standard deviation of three experimental replicates (n=3).

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