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Letter

Citrulline enhances methylmercury toxicity in HEK293 and C17.2 cells

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ABSTRACT — We identified 3-phenylpropionic acid, citrulline, lactic acid, ornithine, proline, and beta-alanine as low-molecular weight substances that are released from cells treated with methylmercury. In this study, we studied their effect on cellular sensitivity to methylmercury. Treating HEK293 and C17.2 cells with each of the six substances minimally affected the proliferation of both cell lines. Among these six substances, however, only citrulline slightly but significantly increased the sensitivity of C17.2 and HEK293 cells to low levels of methylmercury. Citrulline is thought to be a methylmercury toxicity-enhancing factor whose extracellular release is enhanced by methylmercury.

Key words: Methylmercury, Toxicity, Citrulline, HEK293 cells, C17.2 cells

INTRODUCTION

Methylmercury is a well-known environmental pollutant that produces characteristic and severe central nervous system (CNS) damage (Bakir *et al.*, 1973; Harada, 1995). However, its toxicity-causing mechanism is not well understood at the cellular level. By using metabolome analysis, we searched for low-molecular weight substances that are subject to extracellular release enhanced by treating HEK293 cells (human embryonic renal epithelial cells) with methylmercury, and found an increase in the levels of 3-phenylpropionic acid, citrulline, lactic acid, ornithine, proline, and beta-alanine in the culture media (Toyama *et al*., 2015b). In this study, we studied the effect of these six substances on the sensitivity of HEK293 cells and C17.2 cells (mouse cerebral nerve precursor cells) to methylmercury.

MATERIALS AND METHODS

Materials

Methylmercuric chloride (MeHgCl) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Alamar blue was purchased from Invitrogen (Carlsbad, CA, USA). All other reagents used were of the highest grade available.

Cells and cell culture

Human embryonic kidney cells (HEK293) and mouse neural stem cells (C17.2) were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 0.3% L-glutamine and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). Cells were maintained at 37°C in a humidified incubator under an atmosphere of CO_2 (5%) and ambient air (95%).

Measurement of cell viability

Cell viability was determined by Alamar blue assay. HEK293 and C17.2 cells were seeded at a density of 5×10^3 cells/well/100 μL of DMEM on a 96 well plate and incubated for 24 hr. Next, cell media were changed to Alamar blue solution (Invitrogen, CA, USA), and the fluorescence was measured (Ex: 545 nm, Em: 590 nm) after a 2 hr incubation.

Statistical analysis

Statistical significance was assessed using a *t*-test. All *p* values are two-tailed.

RESULTS AND DISCUSSION

We identified 3-phenylpropionic acid, citrulline, lactic acid, ornithine, proline, and beta-alanine as low-molecular weight substances that are released from cells treated with methylmercury (Toyama *et al*., 2015a; Toyama *et al*., 2015b). In this study, we studied their effect on cellular sensitivity to methylmercury. In our results, when

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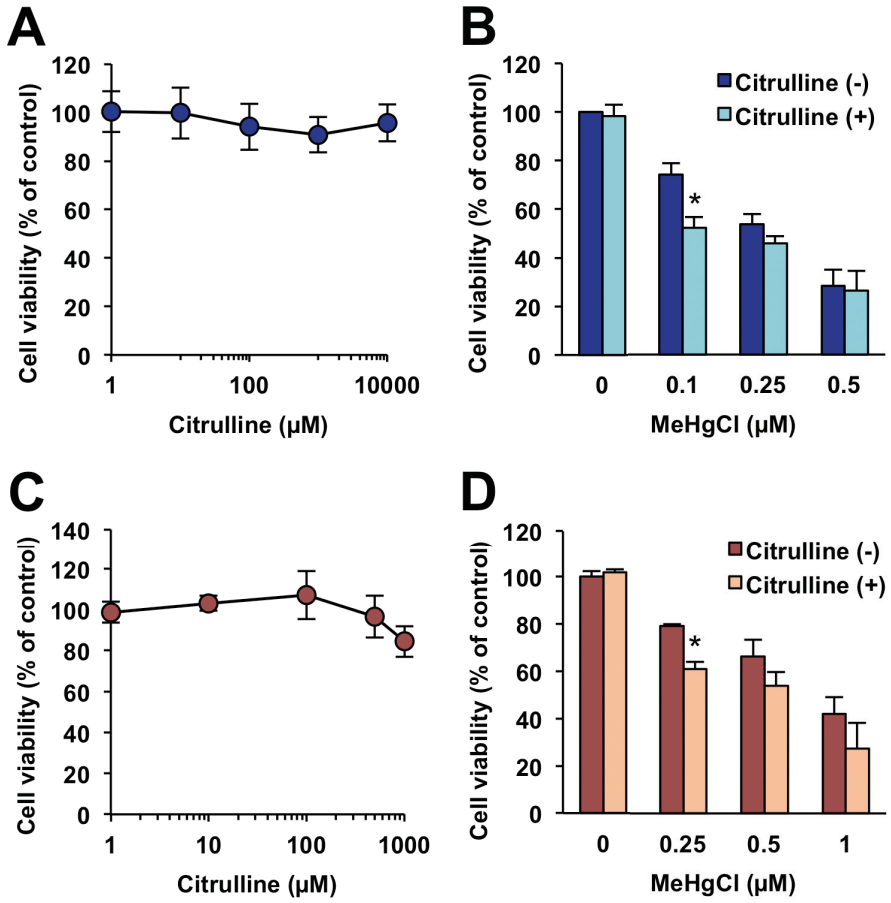

Fig. 1. Effect of citrulline on methylmercury cytotoxicity. C17.2 cells (A) and HEK293 cells (C) were incubated with citrulline for 48 hr, and cytotoxicity was examined using an Alamar blue assay. C17.2 cells were exposed to methylmercuric chloride (MeHgCl) in the presence or absence of citrulline (10 μM) for 48 hr, and cytotoxicity was determined (B). HEK293 cells were exposed to MeHgCl with 50 μM of citrulline for 48 hr, and cytotoxicity was determined (D). Mean ± S.D., n = 3; *$P < 0.05$.

3-phenylpropionic acid, lactic acid, ornithine, proline, and beta-alanine were individually added into the culture media, each compound minimally affected the cellular sensitivity of cultured HEK293 and C17.2 cells to methylmercury (data not shown). However, citrulline slightly but significantly increased cellular sensitivity of C17.2 and HEK293 cells to low-level methylmercury (Fig. 1). In considering the toxicity of methylmercury, it is interesting to note that citrulline, which is subject to extracellular release enhanced by methylmercury, increases cellular sensitivity to methylmercury in non-toxic concentrations.

In vivo, citrulline is produced together with nitric oxide (NO) from arginine by nitric oxide synthase (NOS) (Bush *et al.*, 1992). It is also known that citrulline increases intracellular NO production (Husson *et al.*, 2003). Since enhanced NO production induces cell death (Boje and Arora, 1992), NO may be involved in citrulline's action to enhance methylmercury toxicity. We expect that a more detailed investigation on the role of citrulline in developing methylmercury toxicity will clarify part of the molecular mechanism for developing methylmercury toxicity.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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