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The dietary biogenic amines tyramine and histamine show synergistic toxicity towards intestinal cells in culture

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ABSTRACT

Tyramine and histamine are the biogenic amines (BA) most commonly found at high concentrations in food; they may even appear together at toxic concentrations. The present work examines, via real-time cell analysis, whether histamine and tyramine show synergistic toxicity towards intestinal cell cultures. Employing a constant equipotency ratio, their interaction was examined via the combination index (CI) method of Chou & Talalay. Co-treatment with tyramine and histamine was associated with a stronger cytotoxic effect than was treatment with either BA or on its own. Indeed, a synergistic interaction (CI < 1) was observed in the range of concentrations found in foods. The results also show that histamine, at concentrations below the legal limit, increases the cytotoxicity of tyramine at concentrations frequently reached in some foods. The synergistic cytotoxicity of tyramine and histamine should be taken into account when establishing legal limits designed to ensure consumer safety.

1. Introduction

Biogenic amines (BA) are biologically active nitrogenous organic compounds that may accumulate in food, via the enzymatic decarboxylation of their precursor amino acids by certain spoilage microorganisms (Alvarez & Moreno-Arribas, 2014; Linares, Martin, Ladero, Alvarez, & Fernandez, 2011). Fermented foods and beverages, fish and fish products, can accumulate high BA concentrations (Bover-Cid, Hugas, Izquierdo-Pulido, & Vidal-Carou, 2001; Capozzi et al., 2011; Fernandez, Linares, del Rio, Ladero, & Alvarez, 2007; Ladero, Fernandez, Cuesta, & Alvarez, 2010; Ladero, Linares, Fernandez, & Alvarez, 2008; Ladero et al., 2011, 2012; Silla Santos, 1996). The ingestion of BA-rich food can cause adverse toxicological reactions and intoxications harmful to health (Ladero, Calles-Enriquez, Fernandez, & Alvarez, 2010). Indeed, the Food and Agriculture Organization of the United Nations (2014), has declared BAs in food pose a biological hazard.

Tyramine and histamine are the most common BAs found in foods (Linares et al., 2011, 2012). The European Food Safety Authority (EFSA) deems them to be the most toxic of all BAs, and to have a negative impact on food safety (European Food Safety Authority, 2011). The consumption of foods rich in tyramine can cause toxicological reactions (headaches, migraine, neurological disorders, nausea, vomiting, respiratory disorders and hypertension) together referred to as the "cheese reaction" (Broadley, 2010; Finberg & Gillman, 2011; Shalaby, 1996). Similarly, the ingestion of histamine-rich food can cause scombroid syndrome, a condition characterized by adverse neurological, gastrointestinal, circulatory and respiratory symptoms, flushing, rashes and urticaria (Ladero, Calles-Enriquez et al., 2010; Shalaby, 1996; Stratta & Badino, 2012; Visciano, Schirone, Tofalo, & Suzzi, 2014).

Although the presence of BAs in foods poses a threat to human health, legislation establishing their maximum legal limit remains wanting. In the European Union, histamine is the only BA for which legal limits have been set (by the EFSA), and then only for scombrid fish (200 mg/kg) and fish products (400 mg/kg) (European Commission, 2005). The US Food and Drug Administration (FDA)

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suggests 500 mg/kg histamine in fish to be a health threat (Food and Drug Administration, 2001, 1996). For tyramine, only recommended limits have been proposed (European Food Safety Authority, 2011).

The EFSA Panel on Biological Hazards (BIOHAZ) indicates that further research is needed regarding BA toxicity (European Food Safety Authority, 2011). Our group recently examined the toxicity of tyramine and histamine in human intestinal cell cultures via real-time cell analysis (RTCA) (Linares et al., 2016). That work revealed i) both tyramine and histamine to be toxic towards HT29 intestinal cell cultures at concentrations found in BA-rich food, and that ii) tyramine cytotoxicity was due to the induction of necrosis, while histamine exerted its effects via the induction of apoptosis (Linares et al., 2016).

Importantly, tyramine and histamine commonly appear together (along with other BAs, such as putrescine and cadaverine) at high concentrations in fermented foods, such as cheese (Alvarez and Moreno-Arribas, 2014; Ladero et al., 2008, 2011; Ladero, Fernandez et al., 2010; Ladero, Martinez, Martin, Fernandez, & Alvarez, 2010; Linares et al., 2011). Knowledge of the toxicity of dietary BA combinations, however, is limited. The aim of the present work was therefore to examine, via RTCA, the effect of tyramine and histamine in combination on human intestinal cell cultures. The nature of their interaction (synergistic, additive or antagonistic) was determined via the combination index (CI) method of Chou and Talalay (1984). Finally, work was performed to determine whether histamine, at concentrations below the legal limit, increases the cytotoxicity of tyramine at the concentrations reached in some foods.

2. Material and methods

2.1. Cell line and growth conditions

The intestinal cell line HT29 (ECACC 91072201), derived from a human colorectal adenocarcinoma, was purchased from the European Collection of Cell Cultures and used to create an *in vitro* model of the intestinal epithelium. These cells were routinely cultured in McCoy's 5a medium supplemented with 10% heat-inactivated foetal bovine serum plus a mixture of antibiotics (50 μg/ml penicillin, 50 μg/ml streptomycin, 50 μg/ml gentamicin and 1.25 μg/ml amphotericin B). All media and reagents were purchased from Sigma-Aldrich (Madrid, Spain). All manipulations required for culturing, passaging (144–149 passages were performed) and maintenance of the cell line were undertaken in a 5% CO_2 atmosphere at 37 °C, within an SL Waterjacked CO_2 Incubator (Sheldon Mfg. Inc., Cornelius, OR, USA), following standard procedures (Ruas-Madiedo et al., 2010).

2.2. Real-time cell analysis

The RTCA system is used to gather information on cell proliferation, migration and cytotoxicity via changes in cell morphology and adhesion (Atienzar et al., 2011). An xCelligence Real-Time Cell Analyzer (ACEA Bioscience Inc., Roche Applied Science, Germany) was used as previously described (Linares et al., 2016) to detect any changes in the proliferation, adhesion or morphology of the HT29 intestinal cells following their treatment with different doses of tyramine [4-(2-aminoethyl)phenol hydrochloride] (Acros Organics, Belgium), histamine [2-(4-Imidazolyl) ethylamine dihydrochloride] (Sigma-Aldrich) or their combination. Cells were seeded in 16-well E-Plates (Roche Applied Science) equipped with gold microelectrodes that generate an electric field (<20 mV). Real-time measurements of the electrical impedance (referred to as the cell index) across the interdigitated microelectrodes allows for the monitoring of the variables mentioned above.

Briefly, HT29 cells were seeded at a density of 2×10^4 cells/well in the above E-Plates containing 100 μl of medium per well. They were then incubated and monitored in a Heracell-240 Incubator (Thermo Electron LDD GmbH, Langenselbold, Germany) at 37 °C in a 5% CO_2 atmosphere (Hidalgo-Cantabrana et al., 2014). Stock solutions of tyramine and histamine were dissolved in water and adjusted to pH 6.8. Approximately 20 h after seeding, the proliferating cells were treated for 24 h with 14 different concentrations of tyramine, histamine or their combination (Table 1). Combination studies were performed employing a constant equipotency ratio (Chou & Talalay, 1984), thus ensuring that the contribution of each BA to the combined toxic effect was equal. To define the equipotency ratio and keep it constant, the assayed concentrations of histamine and tyramine were based on their previously assessed IC_{50} values (the concentration of BA required to achieve half the most potent cytotoxic effect observed by RTCA): tyramine 3.2 mM, and histamine 26.0 mM (Linares et al., 2016). The tyramine:histamine equipotency ratio ($IC_{50(T)}$ /$IC_{50(H)}$) was therefore 1:8.125. The concentrations assayed in the co-treatments were 0.03, 0.05, 0.07, 0.11, 0.17, 0.25, 0.33, 0.4, 0.5, 0.67, 1, 1.5, 2 and 2.5 times the IC_{50} values (Table 1). Tyramine and histamine were also analyzed independently at these concentrations.

After the addition of the BA, the cell index was monitored for 24 h; this was normalized to the time point just before the addition of the BA, and set to 1. All tests were performed in triplicate.

Dose-response curves for tyramine, histamine and their combination were constructed using RTCA software, plotting the normalized cell index at 24 h of treatment against the value for the corresponding BA concentration. The IC_{50} values for tyramine, histamine and their combination were calculated using RTCA software.

2.3. Live cell microscopy

Cells were seeded at a density of 2×10^4 cells/well and incubated in flat-bottomed 96-well microplates under identical conditions to those used in the RTCA studies. After 20 h of incubation the cells were treated with concentrations of tyramine and histamine at 0.33, 0.4, 0.5, 0.67 and 1 times their IC_{50} values (Table 1). Tyramine and histamine were also tested independently at these concentrations. At 48 h post-treatment, live cells were visualised using an inverted LumaScope-600 Series optical microscope (Etaluma, Carlsbad, CA) with a 40× objective.

2.4. Determination of combination index values

The nature of the tyramine-histamine interaction was determined via the CI method, using CompuSyn software (ComboSyn, Inc, Paramus, NJ) as previously described (Chou, 2006). The nor-

Table 1
Doses of tyramine and histamine used in co-treatment experiments. BAs were used either alone or combined at a fixed ratio of 1:8.125 (tyramine:histamine).

	Fold reduction or increase in the IC_{50}[a]													
	0.03×	0.05×	0.07×	0.11×	0.17×	0.25×	0.33×	0.4×	0.5×	0.67×	1×	1.5×	2×	2.5×
Tyramine (mM)	0.11	0.16	0.24	0.36	0.53	0.80	1.10	1.30	1.60	2.10	3.20	4.80	6.40	8.00
Histamine (mM)	0.86	1.28	1.93	2.89	4.33	6.50	8.70	10.40	13.00	17.30	26.00	39.00	51.90	64.00

[a] IC_{50} values for tyramine and histamine were previously obtained via RTCA analysis [$IC_{50(T)}$ = 3.2 mM and $IC_{50(H)}$ = 26.0 mM respectively] (Linares et al., 2016).