Analysis of individual and combined toxicity of the dietary biogenic amines tyramine and histamine

Beatriz del Rio, Begoña Redruello, Daniel M. Linares, Victor Ladero, Maria Fernandez, M. Cruz Martin, Patricia Ruas-Madiedo and Miguel A. Alvarez*
Dairy Research Institute (IPLA-CSIC), Paseo Rio Linares s/n, 33300, Villaviciosa, Spain
*Email: maag@ipla.csic.es

INTRODUCTION

Tyramine and histamine are the biogenic amines (BA) most commonly found at high concentrations in foods. The ingestion of histamine-rich food can cause scombroid syndrome characterized by adverse neurological, gastrointestinal, circulatory and respiratory symptoms. Similarly, the consumption of foods rich in tyramine can cause toxicological reactions (headaches, migraine, neurological disorders...) together referred as the "cheese reaction". In fact, the European Food Safety Authority (EFSA) deems them to be the most toxic of all BA and to have a negative impact on food safety. However, histamine is the only BA for which legal limits have been set (by the EFSA), and only for scombrid fish (200 mg/kg) and fish products (400 mg/kg), although it can also be present at high concentrations in fermented foods. Recently, the EFSA Panel on Biological Hazards (BIOHAZ) conducted a qualitative risk assessment for BA in fermented foods, and concluded that our present knowledge of their toxicity is limited and further research is needed. Therefore, the goal of this study was to investigate the *in vitro* cytotoxicity of tyramine and histamine on human intestinal cells using the Real-Time Cell Analyser (RTCA) technology, and to determine their mode of action (Linares *et al*., 2016). Importantly, tyramine and histamine commonly appear together at high concentrations in fermented foods, such as cheese. However, the knowledge of the toxicity of dietary BA combinations is even more limited. Therefore, we further examined if there could be some kind of interaction (synergistic, additive or antagonistic) between histamine and tyramine (del Rio *et al*., 2017).

OBJECTIVES

- Determine the *in vitro* cytotoxicity of tyramine and histamine and their mode of action
- Analyse the cytotoxicity of these dietary BA in combination and the nature of their interaction

RESULTS

Tyramine and histamine are toxic at concentrations commonly reached in BA-rich foods

To investigate the cytotoxicity of BA, an *in vitro* model of the human intestinal epithelium was developed based on the HT29 cell line and the RTCA technology. Tyramine (Fig. 1A) and histamine (Fig. 1B) were found to be cytotoxic at concentrations that can be easily found in BA-rich foods as some cheeses. Surprisingly, tyramine had a stronger and more rapid cytotoxic effect than histamine, the only BA for which legal limits have been established, both in Europe and USA.

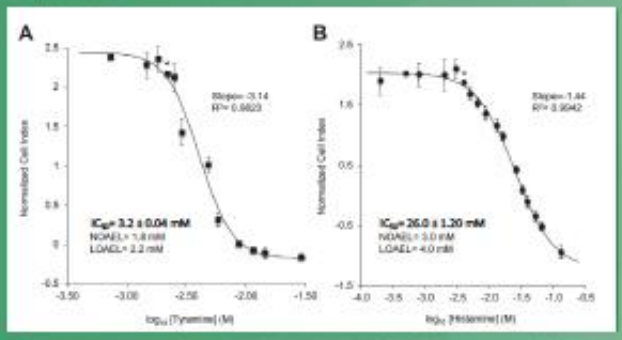

Fig. 1. Dose-response curves for tyramine and histamine in HT29 cells. Cell cultures were treated with a range of tyramine (A) or histamine (B) concentrations for 24 h. IC_{50}: concentration of BA required to achieve half of the strongest cytotoxic effect observed by RTCA. NOAEL: the highest concentration of BA that caused no detectable adverse effect. LOAEL: the lowest BA concentrations that produced a detectable adverse effect.

The mode of action of tyramine and histamine is different

While tyramine caused the necrosis of the HT29 cells (Fig. 2), histamine induced apoptosis (Fig. 3).

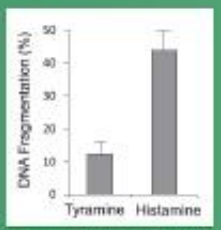

Fig. 2. Apoptotic DNA-fragmentation induced by tyramine and histamine in HT29 cells.

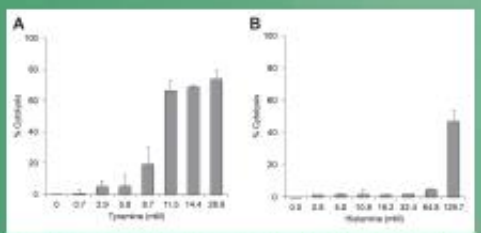

Fig. 3. Determination of the cytolytic effect of tyramine (A) and histamine (B) on HT29 cells. Necrosis was measured by quantifying lactate dehydrogenase (LDH) activity.

In vitro cytotoxicity of tyramine and histamine in combination

Since tyramine and histamine may appear together in food at toxic concentrations, we further examined the *in vitro* cytotoxicity of both BA in combination. The co-treatment with tyramine and histamine was associated with a stronger cytotoxic effect than was the treatment with either BA by its own (Fig. 4).

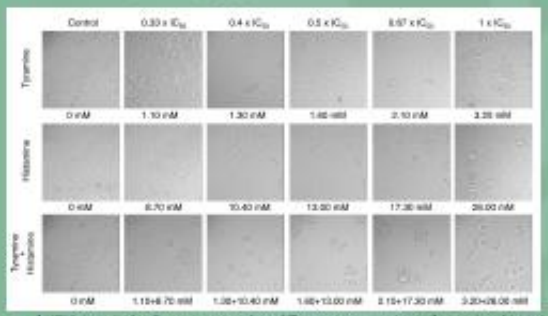

Fig.4. Cultures of HT29 intestinal cells were exposed to different concentrations of tyramine, histamine and their combination. To ensure that the contribution of each BA to the combined toxic effect was equal, a constant equipotency ratio based on their previously assessed IC50 values (Fig. 1) was used. Live cells were visualised using an inverted optical microscope (40 magnification) and analysed by RTCA (data not shown).

Histamine and tyramine have synergistic cytotoxic effect

The nature of the tyramine-histamine interaction was determined using the combination index (CI) method of Chou &Talalay (2006). A synergistic interaction (CI<1) between tyramine and histamine was observed in the range of concentrations that can be found in foods (Fig. 5). In fact, histamine at concentration below the European legal limit established by the EFSA (1.71 mM = 200 mg/Kg), increased the cytotoxicity of tyramine at a concentration frequently found in foods (Fig. 6).

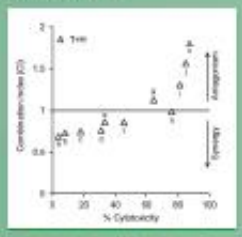

Fig. 5. Combination index (CI) analysis of tyramine and histamine co-treatment. CI values were calculated for the tyramine and histamine combinations (T + H) used in the RTCA analysis, i.e., corresponding to the following proportions of the IC_{50}: a, $0.11xIC_{50}$; b, $0.17xIC_{50}$; c, $0.25xIC_{50}$; d, $0.33xIC_{50}$; e, $0.4xIC_{50}$; f, $0.5xIC_{50}$; g, $0.67xIC_{50}$; h, $1xIC_{50}$; i, $1.5xIC_{50}$; j, $2xIC_{50}$; and k, $2.5xIC_{50}$. CI values of <1, =1 and >1 indicate synergy, additivity and antagonism respectively

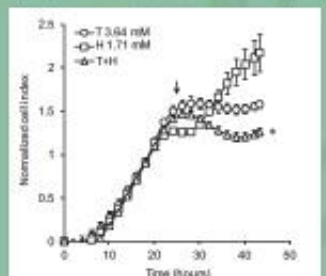

Fig. 6. Real-time cell analysis of the cytotoxic effect of histamine at a concentration below the legal limit combined with a tyramine concentration commonly reached in food. Cells were co-treated with 1.71 mM histamine and 3.64 mM tyramine (T+H). Single tyramine (T) and histamine (H) were also tested. The BA administration point is shown by a vertical arrow.

CONCLUSIONS

The RTCA technology was shown to be a useful way to assess the toxicity of BA on an *in vitro* model of the human intestinal epithelium. Using this technology we found that unexpectedly, tyramine was more cytotoxic than histamine. In both cases, the concentrations found to be toxic are commonly reached in BA-rich foods. Their mode of action was also different, while tyramine caused cell necrosis, histamine induced apoptosis. Moreover, we found that tyramine and histamine have synergistic cytotoxicity in the range of concentrations that can be found in foods. The results of this work should be taken into account to establish the legal limits of both BA in food.

REFERENCES

- Linares D.M., del Rio B., Redruello B., Ladero V., Martin M.C., Fernandez M., Ruas-Madiedo P., Alvarez M.A. 2016. Comparative analysis of the *in vitro* cytotoxicity of the dietary biogenic amines tyramine and histamine. *Food Chemistry*, 197: 658-663
- del Rio B., Redruello B., Linares D.M., Ladero V., Fernandez M., Martin M.C., Ruas-Madiedo P., Alvarez M.A. 2017. The dietary biogenic amines tyramine and histamine show synergistic toxicity towards intestinal cells in culture. *Food Chemistry*, 218:249-255
- Chou T.C. 2006. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacological Reviews*, 58(3), 621-681

ACKNOWLEDGEMENTS

This work was funded by the Spanish Ministry of Economy and Competitiveness (AGL2013-45433-R & AGL2015-64901-R) and by the GRUPIN14-137 project (which is co-financed by the Plan for Science, Technology and Innovation of the Principality of Asturias 2013-2017 and European Regional Development Funds).