Biotemas, 28 (4): 1-8, dezembro de 2015
ISSNe 2175-7925
http://dx.doi.org/10.5007/2175-7925.2015v28n4p1

Rutin increases neural crest stem cell survival against damage caused by aflatoxin B_1

Jader Nones [1]*

Janaína Nones [2]

Andrea Trentin [1]

Federal University of Santa Catarina
[1] Department of Cell Biology, Embryology and Genetics, Center of Biological Sciences
CEP 88040-900, Florianópolis – SC, Brazil
[2] Department of Chemical Engineering, Center of Technology
* Corresponding author
jnones@cidasc.sc.gov.br

Submetido em 01/05/2015
Aceito para publicação em 29/09/2015

Resumo

Rutina aumenta a sobrevivência das células-tronco da crista neural e atua contra danos causados pela aflatoxina B_1. A crista neural (CN) corresponde a um conjunto de células progenitoras multi e oligopotentes dotadas com potenciais neural e mesenquimal. Os derivados da CN incluem neurônios e células gliais do sistema nervoso periférico, melanócitos, células da musculatura lisa e algumas células endócrinas. No presente trabalho, investigamos pela primeira vez a influência da aflatoxina B_1 (AFB_1) e do flavonoide rutina na sobrevivência e proliferação da CN e de melanócitos derivados deste tipo celular. Para tal, culturas de células da CN de codornas foram tratadas com AFB_1 (30 μM) e/ou rutina (20 μM) durante seis dias. A viabilidade celular foi avaliada por análises de MTT e azul de tripan e a proliferação celular através de marcação com BrdU. Melanócitos foram identificados com uso do marcador celular específico MelEM. O tratamento com a AFB_1 diminuiu a viabilidade e proliferação das células da CN. O número total de células MelEM-positivas foi também reduzido após este tratamento, efeito parcialmente revertido através da adição de rutina. No entanto, uma melhor compreensão referente aos mecanismos envolvidos nas interações entre AFB_1 e rutina precisarão ser realizados.

Palavras-chave: Aflatoxina B_1; Crista neural; Diferenciação; Proliferação; Rutina; Viabilidade celular

Abstract

The neural crest (NC) corresponds to a collection of multipotent and oligopotent progenitors endowed with both neural and mesenchymal potential. The derivatives of the NC at the trunk level include neurons and glial cells of the peripheral nervous system, melanocytes, smooth muscle cells and some endocrine cells. The present work investigated, for the first time, the influence of aflatoxin B_1 (AFB_1) and the flavonoid rutin on the survival and proliferation of NC and NC-derived melanocytes. Quail NC cell cultures were treated with AFB_1 (30 μM) and/or rutin (20 μM) for 6 days. Cell viability was assessed by MTT and trypan blue analyses and cell proliferation by BrdU staining. Melanocytes were identified by immunocytochemistry

against the melanocyte-specific cellular marker MelEM. The AFB_1 treatment decreased both NC cell viability and proliferation. The total number of MelEM-positive cells was also reduced after this treatment, an effect partially prevented by the addition of rutin. Our results demonstrated that rutin increases the survival of the NC after damage caused by AFB_1. However, additional studies are needed to better understand the mechanisms involved in AFB_1 and rutin interactions.

Key words: Aflatoxin B_1; Cell viability; Differentiation; Neural crest; Proliferation; Rutin

Introduction

The neural crest (NC) is a population of highly multipotent cells that originate from dorsal neural folds during vertebrate neurulation that give rise to a diverse array of cell types, including neurons and glial cells of the peripheral nervous system, vascular smooth muscle and melanocytes (LE DOUARIN; KALCHEIM, 1999; TRENTIN; CALLONI, 2013). Subsequent to the epithelial-mesenchymal transition, NC cells migrate through specific routes along the vertebrate axial body. Growth factors and extracellular matrix molecules are essential for the migration and differentiation of NC cells (LE DOUARIN; KALCHEIM, 1999; COSTA-SILVA et al., 2009; BITTENCOURT et al., 2013; RAMOS-HRYB et al., 2013).

Abnormal migration, differentiation, division or survival of NC cells lead to organ and tissue dysplasia with highly diverse clinical and pathological features, referred to as neurocristopathies (ETCHEVERS et al., 2006). Therefore, exposure to drugs or environmental chemicals during early embryogenesis, such as ethanol, heavy metals and toxins, causes significant cell death within the NC that might contribute to multiple neurocristopathies (GARIC-STANKOVIC et al., 2006; WENTZEL; ERIKSSON, 2009; FLENTKE et al., 2011; GARIC et al., 2011; NONES et al., 2013).

Mycotoxins are secondary metabolic substances resulting from various strains of filamentous fungi (QING-HUA et al., 2012; BOEVRE et al., 2015). Aflatoxin B_1 (AFB_1) is among the most abundant and toxic mycotoxins (KARAMI-OSBOO et al., 2012), and causes carcinogenicity, hepatotoxicity and mutagenicity (GHADERI et al., 2010; LI et al., 2011), as well as multiple developmental diseases (CILIEVICI et al., 1980; DIETERT et al., 1985). Recently, we demonstrated that AFB_1 affects NC cell development *in vitro* by decreasing the number of neurons and glial cells (NONES et al., 2013). The co-administration of the flavonoid hesperidin (NONES et al., 2013) or the bentonite clay (NONES et al., 2015) partially prevents cell death caused by this mycotoxin.

Rutin is a natural polyphenolic compound of the human diet that is found in a variety of fruits, vegetables, cereals, teas and wines (NONES et al., 2010; ALMEIDA et al., 2015). We previously demonstrated that rutin reduces NC cell death resulting in enhanced neuronal and Schwann cell populations (NONES et al., 2012a; 2012b). The precise cellular mechanisms of flavonoids during embryonic development are unknown. It has been suggested that rutin can modulate intracellular signaling pathways dependent of PI3K and ERK (HAVESTEEN, 1983; SCHROETER et al., 2002; VAUZOUR et al., 2007; ZAHO et al., 2010; NONES et al., 2011; 2012a).

However, there is no information concerning the effects of AFB_1 or flavonoids in NC-derived melanocyte populations. Therefore, in the present study we investigated the damage caused by AFB_1 on NC and melanocytes *in vitro* and the possible effect of rutin in preventing this damage. Our data show for the first time that the concomitant treatment of rutin with AFB_1 improves NC survival and proliferation against cell death caused by AFB_1.

Material and Methods

Rutin (C10H30016) was obtained from Sigma (number 207671-50-9) and maintained in a stock solution of 10 mM diluted in dimethyl sulfoxide (DMSO) (Sigma) that was kept at -20 °C and protected from light.

Quail NC cell cultures were performed as described by Trentin et al. (2004) and Nones et al. (2013). Briefly, neural tubes obtained from quail embryos (18-25 somite stage) were dissected at the trunk level and plated in plastic culture dishes (Corning). After 24 h, emigrated NC cells were harvested for secondary plating (400 cells per well of a 96-well plate). Cultures were maintained for an additional 6 days in a medium containing the following: α-minimum essential medium (α-MEM; Gibco) enriched with 10% fetal bovine serum (Cultlab), 2% chicken embryonic extract, penicillin (200 U/mL) and streptomycin (10 µg/mL) (all from Sigma). Cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere. The medium was changed every 2 days. Each culture was incubated with DMSO (control group) or with 20 µM of rutin. In order to determine the role of rutin in cell survival, 20 µM of rutin was added (alone or concomitant) to cells treated with AFB_1 (30 µM).

The viability of NC cells was determined by trypan blue staining (YOUN et al. 2013) and by 3-(4,5-dimethyl-2-yl)-2, 5-diphenyl-2 H-tetrazolium bromide (MTT) (NONES et al. 2012a; 2013) assays. For the trypan blue analysis, NC cells were treated with rutin (20 µM) and AFB_1 (30 µM) alone or in combination, for 6 days, trypsinized and subsequently collected by centrifugation. After washing in PBS, cells were stained with a 0.4% trypan blue solution at room temperature for 3 min, and then counted using a hemocytometer and a light microscope. At least one thousand cells were observed and the percentages of unstained (viable) and stained (nonviable) cells were determined. For the MTT assay, 0.5 mg/mL of MTT solution was added to the culture medium 2 h before completing the treatment described above. The medium was then gently removed, 100 µL of DMSO was added to each well, and the resulting product was incubated for 10 min. The formazan product generated was then solubilized with DMSO and the absorbance was measured at 570 nm.

Cultured cells were fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.2% Triton-X-100 (Vetec Química Fina Ltda) for 5 min at room temperature. Subsequently, cells were incubated with 5% bovine serum albumin (BSA, Sigma) in PBS (blocking solution) for 1 h, followed by overnight incubation at 4 ºC with the melanoblast/melanocyte early marker (MelEM) monoclonal (mAb) antibody (NATAF et al., 1993) diluted in blocking solution. Cells were then extensively washed in PBS and incubated for 2 h with goat anti-mouse IgG-Alexa Fluor® 488 (obtained from Invitrogen). Detailed procedures are described elsewhere (TRENTIN et al., 2004; COSTA-SILVA et al., 2009; NONES et al., 2012a). Cell nuclei were stained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma). Fluorescent labelling was observed with an epifluorescent microscope (Olympus IX71). The negative control was obtained by omitting the primary antibody. No reactivity was observed.

Cell proliferation was analyzed as described by Costa-Silva et al. (2009). Briefly, NC cell cultures were incubated with 5-bromo-2-deoxyuridine (BrdU; 10 µM, 12 h), fixed, and immunostained with mouse anti-BrdU (Calbiochem) and rabbit anti-mouse IgG-FITC (Southern Biotechnology) antibodies. Cell nuclei were stained with DAPI and viewed as described above.

Cell death was quantified by assessing the characteristic nuclear changes (e.g., chromatin condensation and nuclear fragmentation) using DAPI nuclear binding dye as previously described (COSTA-SILVA et al., 2009; NONES et al., 2012a). Briefly, cells were fixed with 4% paraformaldehyde and washed in PBS. The cell nuclei were then stained with DAPI and visualized/analyzed with an epifluorescent microscope (Olympus IX71).

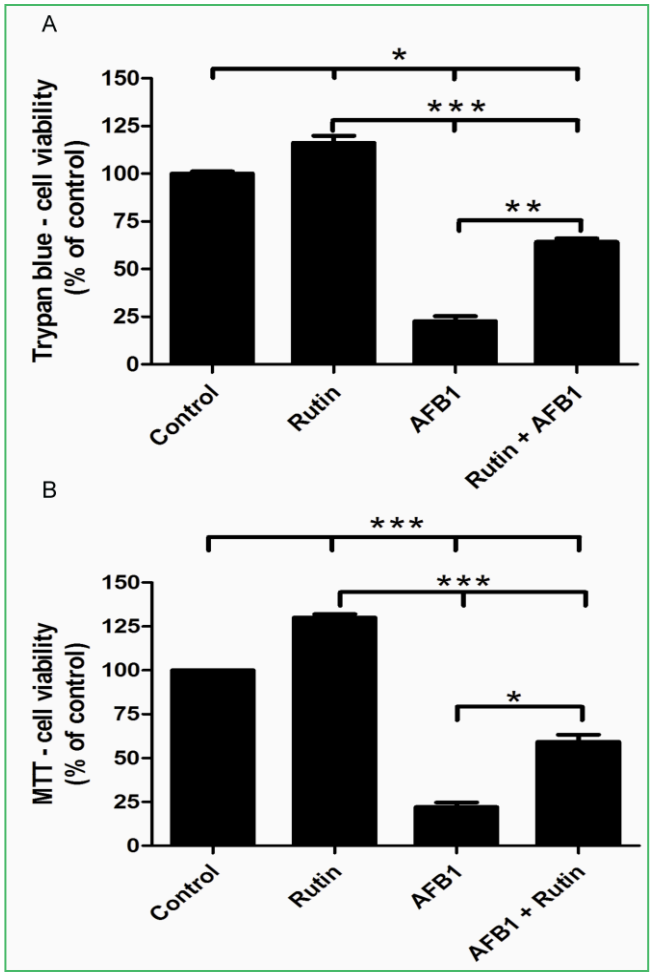
Statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, when appropriate, using GraphPad Prism 4.0. $P < 0.05$ was considered statistically significant. The experiments were performed in triplicate and each result represents the mean of at least three independent experiments.

Results

Our previous results showed that hesperidin decreases (0.64-fold) the number of apoptotic cells of NC, suggesting partial protection. This effect was also observed for the MTT assay, in which hesperidin

(20 µM) added concomitantly to aflatoxin B_1 (30 µM) promoted a 1.4-fold increase in cell viability compared to the treatment with only 30 µM of aflatoxin (0.31 and 0.22 absorbance, respectively) (NONES et al., 2013). In order to evaluate whether rutin influences NC cell viability, cultures of quail trunk NC cells were incubated with DMSO (control group) or with 20 µM of rutin, as described in the Material and Methods. Rutin increased the viability of NC in relation to the control group for the trypan blue assay (Figure 1A). On the other hand, and similar to our previous results (NONES et al., 2013), a significant decrease in NC cell viability (4.5 fold reduction) in relation to the control was observed after the treatment with AFB_1. This effect was partially prevented by the concomitant addition of rutin resulting in an NC cell viability increase (2.9-fold) compared to the AFB_1 treatment alone (Figure 1A). These findings were confirmed by the MTT analysis, in which rutin improved the NC cell viability compared to the control condition (Figure 1B). Instead, the AFB_1 treatment resulted in a 4.5-fold reduction in these values. As for the trypan blue assay, the concomitant addition of rutin resulted in values 3.1-fold greater than those where the mycotoxin was used alone (Figure 1B).

FIGURE 1: Rutin increases NC cell survival after damage caused by AFB_1. Secondary cultures of quail trunk NC cells were incubated with DMSO (control group), 20 µM of rutin, 30 µM of AFB_1 or 20µM of rutin concomitantly added with 30 µM of AFB_1. After 6 days of treatment, cell viability was analyzed using trypan blue (A) and MTT assays (B). The results represent the mean of three independent experiments performed in triplicate ± SEM. ***P<0.01; **P<0.05 or *P<0.1 compared to control or treatment groups.



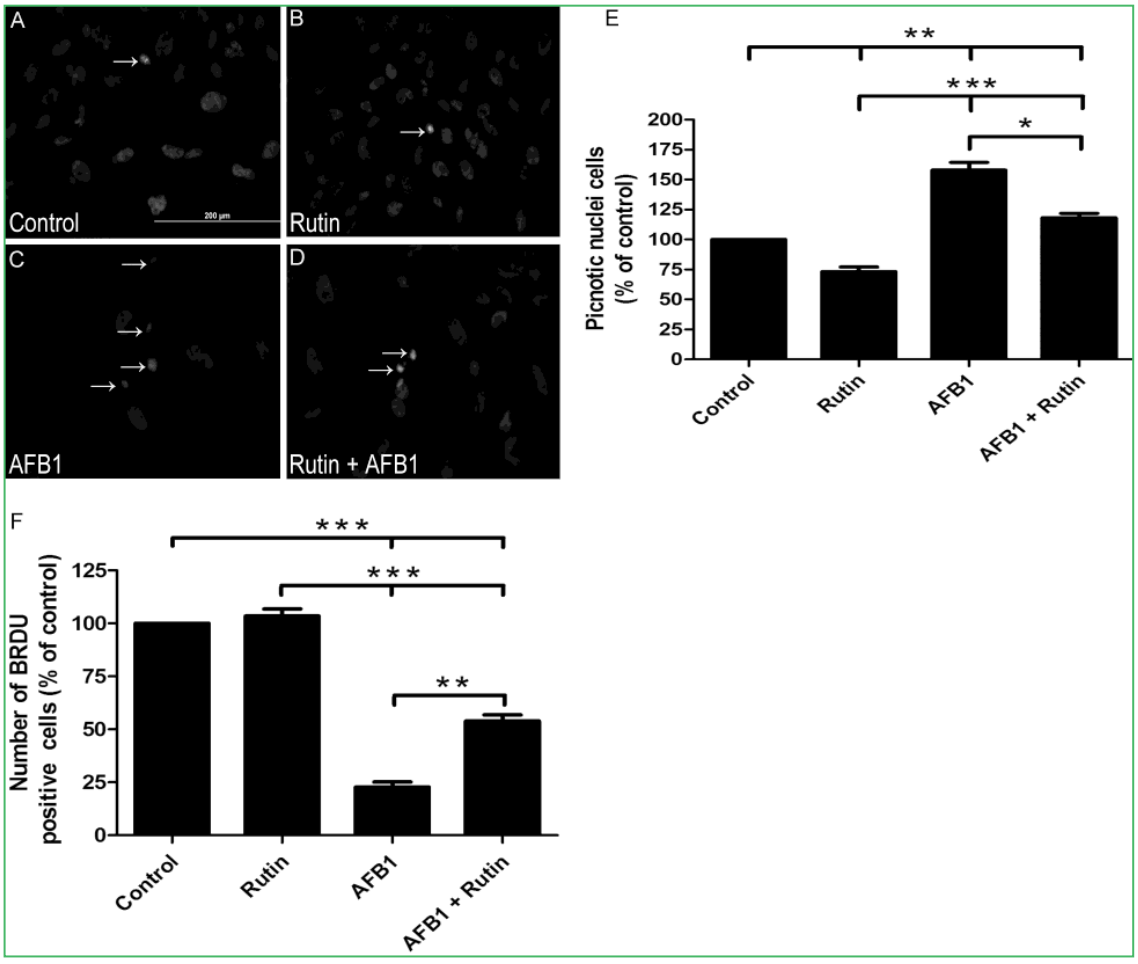
We therefore postulated that the effects of rutin in NC viability could be due to (1) decreased cell death and/or (2) enhanced cell proliferation.

NC cell death was then assessed by the quantification of picnotic nuclei after DAPI staining in a 6-day culture (Figure 2 A-E). The proportion of dead cells was reduced 0.73-fold in NC cell cultures treated with rutin compared with the control condition (Figure 2, A-B, E). As expected, AFB_1 increased the percentage of picnotic nuclei by 1.58-fold when compared with the control (Figure 2A-C, E). The co-administration of rutin with AFB_1, however, reduced this value by 0.75-fold, corroborating the previous results and further demonstrating its effect in promoting NC cell survival from the damage caused by AFB_1 (Figure 2D-E).

Next, we evaluated the possible effect of the flavonoid on NC cell proliferation using a BrdU incorporation assay (Figure 2F). Similar proportions of BrdU-positive cells were observed in control cultures and after treatment with rutin. AFB_1 reduced the percentage of BrdU-positive cells 4.5 and 4.4-fold when compared to the control and rutin-treated cultures, respectively. This effect was partially prevented when rutin was concomitantly added to AFB_1, resulting in values 1.88-fold higher.

Previously, we demonstrated that AFB_1 reduces the viability of neurons and glial cells derived from the NC, an effect partially prevented by the flavonoid hesperidin (NONES et al., 2013). Hence, we investigated here the

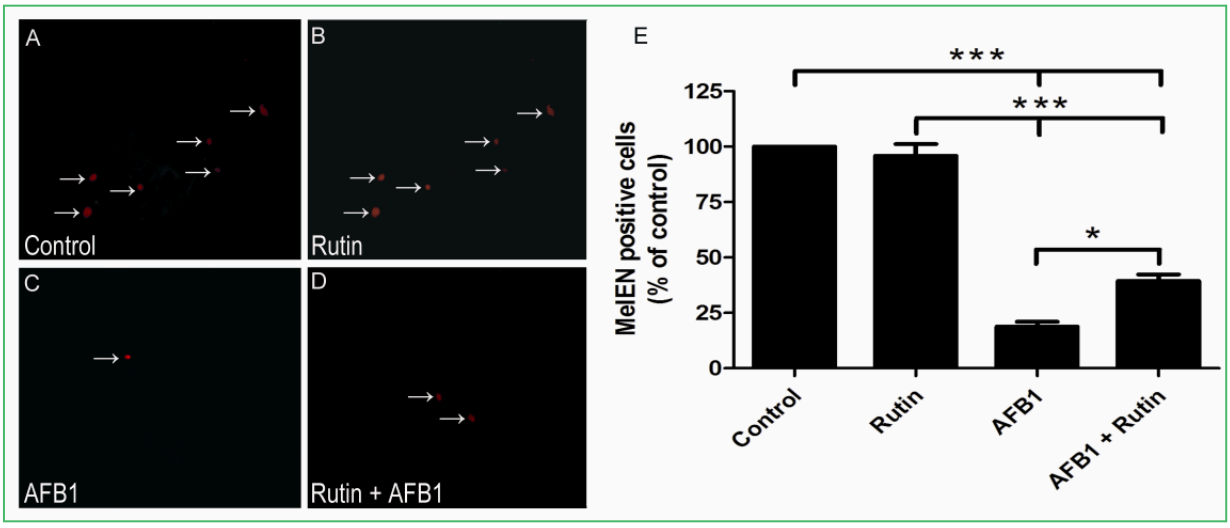
FIGURE 2: AFB_1 reduces NC cell proliferation and rutin partially prevents this effect. Secondary cultures of quail trunk NC cells were incubated with DMSO (control group), 20 µM of rutin, 30 µM of AFB_1 or 20 µM of rutin concomitantly added with 30 µM of AFB_1 (A-D). After 6 days of treatment, picnotic nuclei cell (E) and cell proliferation (BrdU) was analyzed (F). The results represent the mean of three independent experiments performed in triplicate ± SEM. ***P<0.01; **P<0.05 or *P<0.1 compared to control or treatment groups. Scale bar = 200 µm.



influence of rutin and AFB_1 in melanocyte populations, another cell phenotype that originates from the NC (TRENTIN; CALLONI, 2013). Secondary cultures of quail trunk NC cells were incubated with the vehicle (control group) or with 20 µM of rutin. After 6 days of treatment, MelEN-positive cells were analyzed by immunocytochemistry.

Figure 3 shows representative pictures of melanocytes identified by the expression of their phenotypic marker MelEM (Figure 3 A-D) and the corresponding quantitative analysis (Figure 3E). In rutin treated cultures, the MelEM-positive cells per field was similar to the control group (Figure 3 A-B, E). The total melanocyte population was reduced 5.5-fold by the AFB_1 treatment compared to control cultures (Figure 3 C, E). This effect was partially prevented by the concomitant addition of rutin, resulting in a 2.16-fold increase in value (Figure 3 D, E).

FIGURE 3: Rutin increases the survival of NC-derived-melanocytes after damage caused by AFB_1. Cultures of quail trunk NC cells were incubated for 6 days with DMSO (control group) or 20 µM of rutin, 30 µM of AFB_1 or 20 µM of rutin concomitantly added with 30 µM of AFB_1. Representative pictures (A-D) and quantitative analysis of MelEN-positive melanocytes (E). The results represent the mean of three independent experiments performed in triplicate ± SEM. ***P<0.01 or *P<0.1 compared to control or treatment groups. Scale bar = 200 µm.



Discussion

In a previous study we demonstrated that hesperidin increases the survival of central nervous system neurons, although rutin had no influence (NONES et al., 2011). In our recent work, hesperidin enhanced NC cell survival when concomitantly added with AFB_1 (NONES et al. 2013). The present study demonstrates for the first time that rutin promotes survival of NC cells and protects against the toxic effects of AFB_1. Rutin also protects the NC-derived melanocytes from AFB_1 toxicity. Hesperidins (flavanone group) have a saturated heterocyclic C ring and consequently a lack of conjugation between the A and B rings, in contrast to rutins (flavone group), which are defined by their UV spectral characteristics as well as by their lower antioxidant activity (RICE-EVANS et al., 1996). Similarity in the expression, affinity and/or chemical structures of putative receptors of flavonoids might explain the similar effects between these flavonoids (NONES et al., 2010; 2011).

Mycotoxins influence cell division, membrane integrity and viability; moreover, they induce apoptosis in several human and animal cells (KÖNIGS et al., 2007; CHEN et al., 2014; NONES et al., 2015; SUN et al., 2015). Aflatoxins are highly electrophilic molecules able to react with the nucleophilic sites of macromolecules, thus creating the basic mechanisms for cell death, mutagenesis and carcinogenesis (LI et al., 2011; AGAR et al., 2013). These data attest to the high toxicity of aflatoxin B_1 demonstrated in our results.

Rutin, on the other hand, can protect neuronal cells through its antioxidant effects (NONES et al., 2011; 2012a; 2012b). Indeed, the involvement of rutin in the modulation of cell death, through activation of the ERK and PI3 kinase pathways has recently been demonstrated (NONES et al., 2011; 2012a). Further, we have verified that rutin can partially protect NC cells from the toxicity of aflatoxin B_1. However, additional studies are necessary to identify the pathways involved with these effects.

In addition to the effects on cell death, our results show that rutin can prevent the decrease of NC proliferation caused by AFB_1. Similar studies have shown that AFB_1 also inhibits the proliferation of other cell types, such as kidney cells of rats (CHOU et al., 1993), bovine mammary epithelial cells (FOROUHARMEHR et al., 2013) and human lymphoblastoid cells (LUONGO et al., 2014). On the other hand, studies have also shown that this effect can be avoided by co-treatment with

flavonoids, such as ternatin (SOUZA et al., 1999) and hesperidin (NONES et al., 2013).

Microenvironmental factors that affect cell survival and proliferation during embryonic development can be extremely significant (CHEN; SULIK, 1996; MALLO, 1997; HAZELTINE et al., 2014). Substantial evidence supports the critical role of environmental chemicals in promoting birth defects by affecting the viability of NC cells (WEST et al., 1994; KOTCH et al., 1995; CHEN; SULIK, 1996; DUPIN et al., 2010). In this study we demonstrate a new role of rutin in promoting the survival of NC cells and NC-derived melanocytes *in vitro*. The flavonoid rutin may be useful in the protection against toxic elements, like AFB_1, and, thus, in the prevention of malformations and/or neurocristopathies.

Acknowledgments

This study was supported by grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil), Ministério da Ciência, Tecnologia e Inovação/Conselho Nacional de Desenvolvimento Científico e Tecnológico (MCTI/CNPq/Brazil), MCTI/INFRA (Brazil), PRONEX/CNPq and Instituto Nacional de Neurociência Translacional (INNT, Brazil).

References

AGAR, G.; ALPSOY, L.; BOZARI, S.; ERTURK, F. A.; YILDIRIM, N. Determination of protective role of selenium against aflatoxin B1-induced DNA damage. **Toxicology and Industrial Health**, Princeton, v. 29, n. 5, p. 396-403, 2013.

ALMEIDA, P. A.; NONES, J.; TRENTIN, A.; NONES, J. Quercetin and rutin affect the survival and proliferation of human skin-derived multipotent mesenchymal stromal cells. **Journal of Pharmacy and Pharmacology**, London, v. 3, p. 237-242, 2015.

BITTENCOURT, D. A.; COSTA, M. C.; CALLONI, G. W.; ALVAREZ-SILVA, M.; TRENTIN, A. G. Fibroblast growth factor 2 promotes the self-renewal of bipotent glial smooth muscle neural crest progenitors. **Stem Cells and Development**, Larchmont, v. 22, n. 8, p. 1241-1251, 2013.

BOEVRE, M. D.; GRANICZKOWSKA, K.; SAEGER, S. D. Metabolism of modified mycotoxins studied through in vitro and in vivo models: An overview. **Toxicology Letters**, Amsterdam, v. 233, n. 1, p. 24-28, 2015.

CHEN, K.; FANG, J.; PENG, X.; CUI, H.; CHEN, J.; WANG, F.; CHEN, Z.; ZUO, Z.; DENG, J.; LAI, W.; ZHOU, Y. Effect of selenium supplementation on aflatoxin B_1-induced histopathological lesions and apoptosis in bursa of Fabricius in broilers. **Food and Chemical Toxicology**, Oxford, v. 74, p. 91-97, 2014.

CHEN, S. Y.; SULIK, K. K. Free radicals and ethanol-induced cytotoxicity in neural crest cells. **Alcoholism Clinical and Experimental Research**, New York, v. 20, n. 6, p. 1071-1076, 1996.

CHOU, M. W.; LU, M. H.; PEGRAM, R. A.; GAO, P.; CAO, S.; KONG, J.; HART, R. W. Effect of caloric restriction on aflatoxin bi-induced DNA synthesis, AFB_1-DNA binding and cell proliferation in fischer 344 rats. **Mechanisms Ageing and Development**, Oxford, v. 70, n. 1-2, p. 23-33, 1993.

CILIEVICI, O.; GORDOS, I.; GHIDUS, E.; MOLDOVAN, A. The toxic and teratogenic effect of aflatoxin B1 on the chick embryo development. **Morphologie et Embryologie**, Bucuresti, v. 26, n. 4, p. 309-314, 1980.

COSTA-SILVA, B.; COSTA, M. C.; MELO, F. R.; NEVES, C. M.; ALVAREZ-SILVA, M.; CALLONI, G. W.; TRENTIN, A. G. Fibronectin promotes differentiation of neural crest progenitors endowed with smooth muscle cell potential. **Experimental Cell Research**, New York, v. 315, n. 6, p. 955-967, 2009.

DIETERT, R. R.; QURESHI, M. A.; NANNA, U. C.; BLOOM, S. E. Embryonic exposure to aflatoxin B1: mutagenicity and influence on development and immunity. **Environmental Mutagenesis**, New York, v. 7, n. 5, p. 715-725, 1985.

DUPIN, E.; CALLONI, G. W.; LE DOUARIN, N. M. The cephalic neural crest of amniote vertebrates is composed of a large majority of precursors endowed with neural, melanocytic, chondrogenic and osteogenic potentialities. **Cell Cycle**, Georgetown, v. 9, n. 2, p. 238-249, 2010.

ETCHEVERS, H. C.; AMIEL, J.; LYONNET, S. Molecular bases of human neurocristopathies. **Advances in Experimental Medicine and Biology**, New York, v. 589, p. 213-34, 2006.

FLENTKE, G. R.; GARIC, A.; AMBERGER, E.; HERNANDEZ, M.; SMITH, S. M. Calcium-mediated repression of β-catenin and its transcriptional signaling mediates neural crest cell death in an avian model of fetal alcohol syndrome. **Birth Defect Research. Part A, Clinical and Molecular Teratology**, Hoboken, v. 91, n. 7, p. 591-602, 2011.

FOROUHARMEHR, A.; HARKINEZHAD, T.; QASEMI-PANAHI, B. Evaluation of STAT5A gene expression in aflatoxin B1 treated bovine mammary epithelial cells. **Advanced Pharmaceutical Bullettin**, Tabriz, v. 3, n. 2, p. 461-464, 2013.

GARIC, A.; FLENTKE, G. R.; AMBERGER, E.; HERNANDEZ, M.; SMITH, S. M. CaMKII activation is a novel effector of alcohol's neurotoxicity in neural crest stem/progenitor cells. **Journal of Neurochemistry**, Oxford, v. 118, n. 4, p. 646-657, 2011.

GARIC-STANKOVIC, A.; HERNANDEZ, M.; FLENTKE, G. R.; SMITH, S. M. Structural constraints for alcohol-stimulated Ca2+ release in neural crest, and dual agonist/antagonist properties of n-octanol. **Alcoholism: Clinical Experimental Research**, New York, v. 30, n. 3, p. 552-559, 2006.

GHADERI, M.; ALLAMEH, A.; SOLEIMANI, M.; RASTEGAR, H.; AHMADI-ASHTIANI, H. R. A comparison of DNA damage induced by aflatoxin B1 in hepatocyte-like cells, their progenitor mesenchymal stem cells and CD34(+) cells isolated from umbilical cord blood. **Mutatation Research**, Amsterdam, v. 719, n. 1-2, p. 14-20, 2010.

HAVESTEEN, B. Flavonoids a class of natural products of high pharmacological potency. **Biochemical Pharmacology**, Oxford, v. 32, n. 7, p. 1141-1148, 1983.

HAZELTINE, L. B.; BADUR, M. G.; LIAN, X.; DAS, A.; HAN, W.; PALECEK, S. P. Temporal impact of substrate mechanics on differentiation of human embryonic stem cells to cardiomyocytes. **Acta Biomaterialia**, Oxford, v. 10, n. 2, p. 604-612, 2014.

KARAMI-OSBOO, R.; MIRABOLFATHY, M.; KAMRAN, R.; SHETAB-BOUSHEHRI, M.; SARKARI, S. Aflatoxin B_1 in maize harvested over 3 years in Iran. **Food Control**, Vurrey, v. 23, n. 1, p. 271-274, 2012.

KÖNIGS, M.; LENCZYK, M.; SCHWERDT, G.; HOLZINGER, H.; GEKLE, M.; HUMPF, H. U. Cytotoxicity, metabolism and cellular uptake of the mycotoxin deoxynivalenol in human proximal tubule cells and lung fibroblasts in primary culture. **Toxicology**, Amsterdam, v. 240, n. 1-2, p. 48-59, 2007.

KOTCH, L. E.; CHEN, A. Y.; SULIK, K. K. Ethanol-induced teratogenesis: free radical damage as a possible mechanism. **Teratology**, Philadelphia, v. 52, n. 3, p. 128-136, 1995.

LE DOUARIN, N. M.; KALCHEIM, C. **The neural crest.** Cambridge: University Press, 1999. 445 p.

LI, C. H.; WANG, Y. J.; DONG, W.; XIANG, S.; LIANG, H. F.; WANG, H. Y.; DONG, H. H.; CHEN, L.; CHEN, X. P. Hepatic oval cell lines generate hepatocellular carcinoma following transfection with HBx gene and treatment with aflatoxin B1 in vivo. **Cancer Letters**, Virginia, v. 311, n. 1, p. 1-10, 2011.

LUONGO, D.; RUSSO, R.; BALESTRIERI, A.; MARZOCCO, S.; BERGAMO, P.; SEVERINO, L. In vitro study of AFB_1 and AFM_1 effects on human lymphoblastoid JurkatT-cell model. **Journal of Immunotoxicology**, London, v. 11, n. 4, p. 353-358, 2014.

MALLO, M. Retinoic acid disturbs mouse middle ear development in a stage-dependent fashion. **Development Biology**, New York, v. 184, n. 1, p. 175-186, 1997.

NATAF, V.; MERCIER, P.; ZILLER, C.; LE DOUARIN, N. M. Novel markers of melanocyte differentiation in the avian embryo. **Experimental Cell Research**, New York, v. 207, n. 1, p. 171-182, 1993.

NONES, J.; COSTA, A. P.; LEAL, R. B.; GOMES, F. C. A.; TRENTIN, A. G. The flavonoids hesperidin and rutin promote neural crest cell survival. **Cell and Tissue Research**, Berlin, v. 350, n. 2, p. 305-15, 2012a.

NONES, J.; NONES, J.; RIELLA, H. G.; KUHNEN, N. C.; TRENTIN, A. Bentonite protects neural crest stem cells from death caused by aflatoxin B_1. **Applied Clay Science**, Oxford, v. 104, p. 119-127, 2015.

NONES, J.; NONES, J.; TRENTIN, A. The flavonoid hesperidin protects neural crest cells from death caused by aflatoxin B_1. **Cell Biology International**, London, v. 37, n. 2, p. 181-186, 2013.

NONES, J.; SPOHR, T. C. L.; GOMES, F. C. A. Hesperidin, a flavones glycoside, as mediator of neuronal survival. **Neurochemical Research**, New York, v. 36, n. 10, p. 1776-1784, 2011.

NONES, J.; SPOHR, T. C. L.; GOMES, F. C. A. Effects of the flavonoid hesperidin in cerebral cortical progenitors in vitro: indirect action through astrocytes. **International Journal of Developmental Neuroscience**, Oxford, v. 30, n. 4, p. 303-313, 2012b.

NONES, J.; STIPURSKY, J.; COSTA, L. C.; GOMES, F. C. A. Flavonoids and astrocytes crosstalking: implications for brain development and pathology. **Neurochemical Research**, New York, v. 35, n. 7, p. 955-966, 2010.

QING-HUA, H.; YANG, X.; DAN, W.; MIN, K.; ZHI-BING, H.; YAN-PING, L. Simultaneous multiresidue determination of mycotoxins in cereal samples by polyvinylidene fluoride membrane based dot immunoassay. **Food Chemistry**, Barking, v. 134, n. 1, p. 507-512, 2012.

RAMOS-HRYB, A. B.; DA-COSTA, M. C.; TRENTIN, A. G.; CALLONI, G. W. Matrigel supports neural, melanocytic and chondrogenic differentiation of trunk neural crest cells. **The International Journal of Developmental Biology**, Vizcaya, v. 57, n. 11-12, p. 885-90, 2013.

RICE-EVANS, C. A.; MILLER, N. J.; PAGANGA, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. **Free Radical Biology and Medicine**, Indianapolis, v. 20, p. 933-956, 1996.

SCHROETER, H.; BOYD, C.; SPENCER, J. P.; WILLIAMS, R. J.; CADENAS, E.; RICE-EVANS, C. Mapk signaling in neurodegeneration: influences of flavonoids and of nitric oxide. **Neurobiology of Aging**, New York, v. 23, n. 5, p. 861-880, 2002.

SOUZA, M. F.; TOMÉ, A. R.; RAO, V. S. Inhibition by the bioflavonoid ternatin of aflatoxin B1-induced lipid peroxidation in rat liver. **Journal of Pharmacy and Pharmacology,** London, v. 51, n. 2, p. 125-129, 1999.

SUN, L. H.; LEI, M.; ZHANG, N. Y.; GAO, X.; LI, C.; KRUMM, C. S.; SHENG, D. Individual and combined cytotoxic effects of aflatoxin B_1, zearalenone, deoxynivalenol and fumonisin B_1 on BRL 3A rat liver cells. **Toxicon**, Oxford, v. 95, p. 6-12, 2015.

TRENTIN, A.; GLAVIEUX-PARDANAUD, C.; LE DOUARIN, N. M.; DUPIN, E. Self-renewal capacity is a widespread property of various types of NC precursor cells. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 101, n. 13, p. 4495-4500, 2004.

TRENTIN, A. G.; CALLONI, G. W. The Neural crest and the stem cells of neural crest. In: GOLDENBERG, R. C. S.; CARVALHO, A. C. C. (Ed.). **Resident stem cells.** Waltham: Academic Press, 2013. p. 157-176.

VAUZOUR, D.; VAFEIADOU, K.; RICE-EVANS, C.; WILLIAMS, R. J.; SPENCER, J. P. Activation of pro-survival Akt and ERK1/2 signalling pathways underlie the anti-apoptotic effects of flavanones in cortical neurons. **Journal of Neurochemistry**, Oxford, v. 103, n. 4, p. 1355-1367, 2007.

WENTZEL, P.; ERIKSSON, U. J. Altered gene expression in neural crest cells exposed to ethanol in vitro. **Brain Research**, Amsterdam, v. 1305, p. 50-60, 2009.

WEST, J. R.; CHEN, W. J. A.; PANTAZIS, N. J. Fetal alcohol syndrome – the vulnerability of the developing brain and possible mechanisms of damage. **Metabolic Brain Disease,** New York, v. 9, n. 4, p. 291-322, 1994.

YOUN, H.; JEONG, J. C.; JEONG, Y. S.; KIM, E. J.; UM, S. J. Quercetin potentiates apoptosis by inhibiting nuclear factor-kappab signaling in h460 lung cancer cells. **Biological & Pharmaceutical Bulletin**, Tokyo, v. 36, n. 6, p. 944-951, 2013.

ZAHO, H.; LIANG, J.; LI, X.; YU, H.; LI, X.; XIAO, R. Folic acid and soybean isoflavone combined supplementation protects the post-neural tube closure defects of rodents induced by cyclophosphamide in vivo and in vitro. **Neurotoxicology**, Amsterdam, v. 31, n. 2, p. 180-187, 2010.