

Quercetin and Rutin Affect the Survival and Proliferation of Human Skin-Derived Multipotent Mesenchymal Stromal Cells

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Abstract: Flavonoids are phenolic compounds with biological and pharmacological properties, such as antioxidant and antiviral effects. In the present work, we evaluated the effect of the flavonoids quercetin and rutin in human SD-MSCs (skin-derived-multipotent mesenchymal stromal cells). Cultured SD-MSCs were exposed to different concentrations of flavonoids (80 to 320 μ M) for 2 days *in vitro*. Cell viability was assessed by MTT assay and cell proliferation by BrdU staining. Cell death was quantified by the analysis of picnotic nuclei. In this paper, we demonstrated for the first time that both quercetin and rutin affect the viability of SD-MSCs, although high concentrations of quercetin (320 μ M) promoted increased values of picnotic nuclei. Quercetin treatment increased cell proliferation and, in contrast, rutin in the same concentration decreased these values. Our results may aid the comprehension of flavonoids effect in SD-MSCs. However, a better understanding of the mechanisms involving flavonoids and SD-MSCs interactions are necessary.

Key words: Bioactive substances, cell survival, cytotoxicity, flavonoids, human mesenchymal stromal cells.

1. Introduction

MSCs (multipotent mesenchymal stromal cells) constitute a heterogeneous subset of stromal precursor cells present in connective tissue compartments [1]. These cells are plastic-adherent and can differentiate to osteoblasts, adipocytes and chondroblasts [2]. Therefore, MSCs are an attractive cell source for the treatment of a variety of injuries and diseases because of their immunomodulatory, regenerative potential, multipotentiality, anti-inflammatory capabilities and tissue preservation properties [3-6].

The nature of mesenchymal cells, together with their multipotentiality, may render these cells an excellent choice for many strategies and cell-based therapies [7]. Similarly, stromal cells exhibit MSCs characteristics, such as immunosuppressive properties,

transdifferentiation potential and cell phenotype, thus, they are functionally comparable to MSCs [5, 8]. Therefore, the effect of different substances, such as flavonoids, in human SD-MSCs (skin-derived multipotent mesenchymal stromal cells) are an interesting subject, because these substances may provide viable strategies for MSC culture and perhaps, be useful against injuries.

Flavonoids are a class of phenolic compounds widely distributed in human diet with multiple health benefits [9-12]. The biological activities of flavonoids cover a very broad spectrum, due to their neuroprotective, cardioprotective, and chemopreventive effects [10, 13-16].

In vitro and animal studies have shown that flavonoids, including quercetin and rutin, are antioxidants and anti-inflammatory agents [10, 11, 14, 17, 18] and can relieve nuclear condensation, decrease oxidative stress, and strengthen the barrier integrity and

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functions, as well are involved in important pathways that regulate cell division and proliferation [10, 19-21]. On the other hand flavonoids can be harmful, exhibiting *in vitro* cytotoxicity to many human cell lines [14, 22, 23].

This discrepancy in the effects of flavonoids may be attributed to the fact that the positive and negative actions of these phytochemicals are also dependent on cell types and metabolic disorders involved [7]. Due to conflicting results with regard to the adverse properties of flavonoids, it is important to evaluate its safety [17, 24]. Considering these aspects, the aim of this study was to evaluate the effect of quercetin and rutin on SD-MSCs, with emphasis on cell viability, survival and proliferation.

Our data demonstrates that quercetin and rutin affect SD-MSC viability in a dose-dependent manner. On the other hand, in the higher concentrations, fragmented nuclei were observed indicating cell death. Quercetin increased the SD-MSC proliferation and rutin decreased it. Taken together, our results may aid the comprehension of the flavonoids effects on SD-MSCs, and offer new perspectives for the use of these substances to improve culture conditions and may provide the first steps for its application in future therapies.

2. Material and Methods

Two flavonoids (all from Sigma[®]) from different chemistry groups were used: rutin (C₁₀H₃₀O₁₆), number 207671-50-9; and quercetin (C₁₅H₁₀O₇), number 117-39-5. The flavonoids were maintained in a stock solution of 100 mM diluted in DMSO (dimethyl sulfoxide) (Sigma[®]) and kept at -20 °C protected from light.

The procedure of isolation and culture of SD-MSCs was approved by the Ethics Committee of the Federal University of Santa Catarina and performed as previously described [5], with the following modifications: cells were plated in standard medium consisting of DMEM (Dulbecco's Modified Eagle's Medium; Invitrogen) supplemented with 10% FBS

(fetal bovine serum; Vitrocell), penicillin (200 U/mL; Invitrogen) and streptomycin (10 mg/mL; Invitrogen) and maintained until confluence at 37 °C in a humidified 5% CO₂ atmosphere. Cells were expanded up to 10 multiple passages.

SD-MSCs (7,000-10,000 per well in 96-well plates) were cultured in DMEM supplemented with 10% FBS for 24 hours. Then, the medium was removed and SD-MSCs were cultured for 48 hours in DMEM supplemented with 10% FBS and different concentrations of quercetin and rutin (80, 160, 240 and 320 µM). After treatment with flavonoids, cell viability was determined by MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay. Briefly, cells were incubated with MTT (Sigma; 10 µL for each 100 µL of medium) for 4 hours. After that, the medium was removed and 100 µL of DMSO was added to each well to dissolve the blue formazan crystals. After about 20 minutes at room temperature, the absorbance was determined at 570 nm with an ELISA reader.

Cell death was quantified by analyzing the characteristic nuclear changes (e.g., chromatin condensation and nuclear fragmentation) using the nuclear binding dye DAPI [10, 20, 25] and fluorescence microscopy.

Cell proliferation was analyzed by BrdU (5-bromo-2'-deoxyuridine) incorporation as previously described [10] with a few modifications. Briefly, cells (4,000 per well in 96-well plates) were incubated with BrdU (10 µL/mL) for 4 hours. Then, cells were fixed with 4% paraformaldehyde (15 minutes), permeabilized with 0.25% Triton X-100 (Sigma), and incubated with a blocking solution (PBS with 5% FBS; 60 min, room temperature). Then, cells were incubated with 2 N HCl (15 min at 37 °C). After washing in PBS, cells were immunostained with anti-BrdU mAb (Calbiochem; 60 min) followed by anti-mouse IgG1-Alexa Fluor 594 (60 min).

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 software [20]. *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.

3. Results

In order to evaluate whether quercetin and rutin could influence SD-MSCs viability, cultures were incubated with DMSO (vehicle, control group) or with 80-320 μM of flavonoids (quercetin or rutin) as described in material and methods.

In SD-MSCs exposed to 80 μM of quercetin was observed an increase of 1.3-fold in MTT values that at the concentration of 320 μM were 1.7-fold higher than the control group (Fig. 1a). In SD-MSCs treated with rutin however, significant increase in cell viability was observed at the flavonoid concentration of 320 μM (1.4-fold of the control value) (Fig. 1b).

Next we evaluated if nuclear condensation and fragmentation, indicative of cell death, could be observed in the presence of the flavonoids. To this end, SD-MSCs were stained using DAPI nuclear binding dye and cell death was then assessed by the quantification of picnotic nuclei (Figs. 2a-2e). Quercetin and rutin decreased the total number of DAPI-labeled nuclei when compared to the control group (Figs. 2a-2d). Accordingly, the number of picnotic nuclei was significantly increased (3.9-fold) in quercetin-treated cells compared to the control group (Figs. 2a, 2b and 2e). On the other hand, the number of picnotic nuclei was similar in rutin and control cultures (Figs. 2a-2c and 2e).

Next, we evaluated the effect of the flavonoids on SD-MSC proliferation by BrdU incorporation assay (Figs. 3a-3d). We observed that quercetin increased the proliferation of SD-MSCs by 2.6-fold compared to control cultures (Figs. 3a, 3b and 3d). On the other hand, rutin treatment significantly reduced these values (2.0-fold compared to control) (Figs. 3a-3d).

4. Discussion

The present study demonstrates for the first time that the flavonoids quercetin and rutin affect SD-MSC

viability in a dose-dependent manner, although increased values of picnotic nuclei could be observed at the higher concentration (320 μM) of quercetin, suggesting that it may cause some toxicity. At 320 μM , quercetin increased SD-MSC proliferation whereas rutin decreased it.

Metabolic specificities of each cell type interfere directly with the effect of flavonoids [7]. It was shown that quercetin at low concentrations stimulate the proliferation of human cells and was suggested for the treatment of certain diseases, in contrast to the higher concentrations that induce apoptosis and was suggested as potential anticancer drug [18]. Similar results were described by Srivastava et al. [7], who observed that this flavonoid increase the proliferation of mouse bone

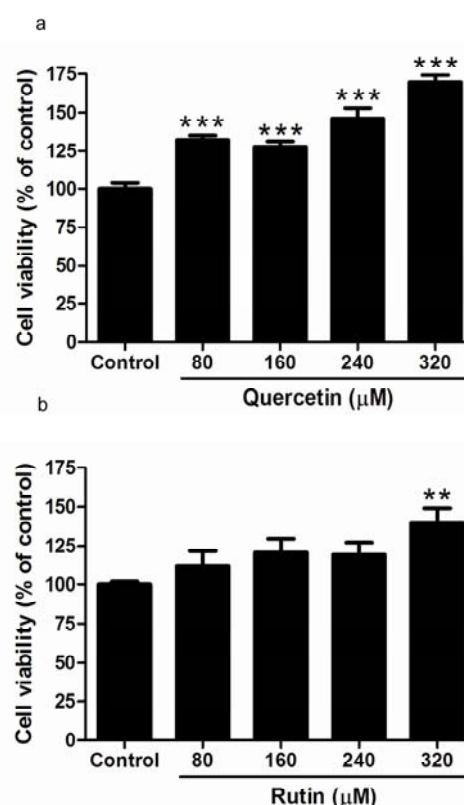


Fig. 1 Quercetin and rutin affect SD-MSCs viability. SD-MSCs cultures were incubated with 80-320 μM of flavonoids and vehicle (DMSO, control group). After 2 days of treatment, cell viability was observed by MTT assay. Two different treatments were analyzed: quercetin (a) and rutin (b). The results represent the mean of three independent experiments performed in triplicate \pm SEM. ** $P < 0.05$ or *** $P < 0.01$ when compared with control group.

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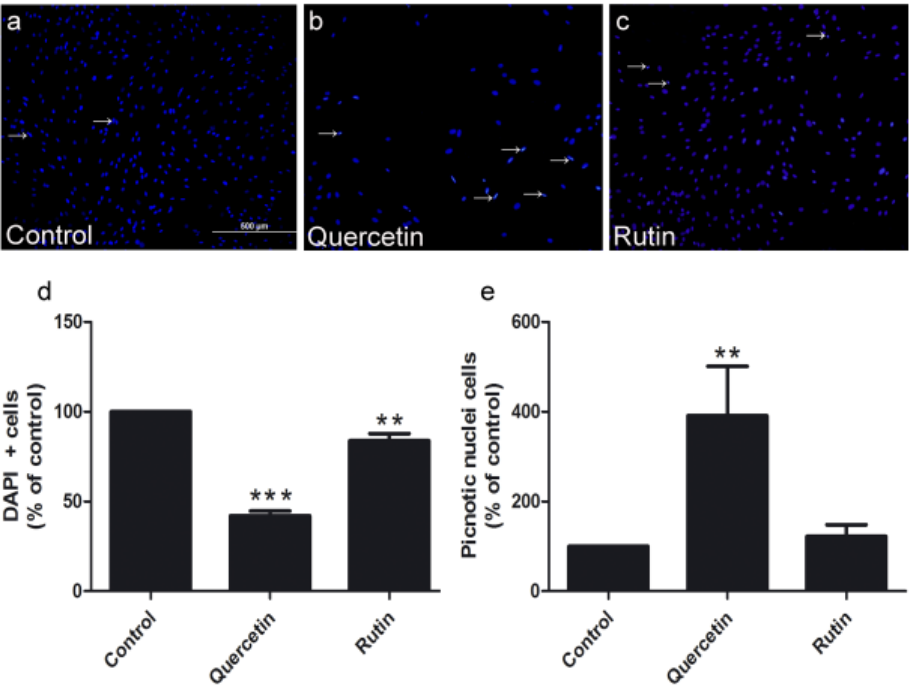


Fig. 2 Quercetin and rutin decrease the number of SD-MSCs. SD-MSCs cultures were incubated with DMSO (control group) or 320 μM of quercetin or rutin. After 2 days of flavonoids treatment, cells were stained by DAPI (a-d) and the proportion of picnotic nuclei (dead cells) was quantified (a-c, e). The results represent the mean of three independent experiments performed in triplicate \pm SEM. ** $P < 0.05$ or *** $P < 0.01$ when compared with control group. Scale bar = 500 μm .

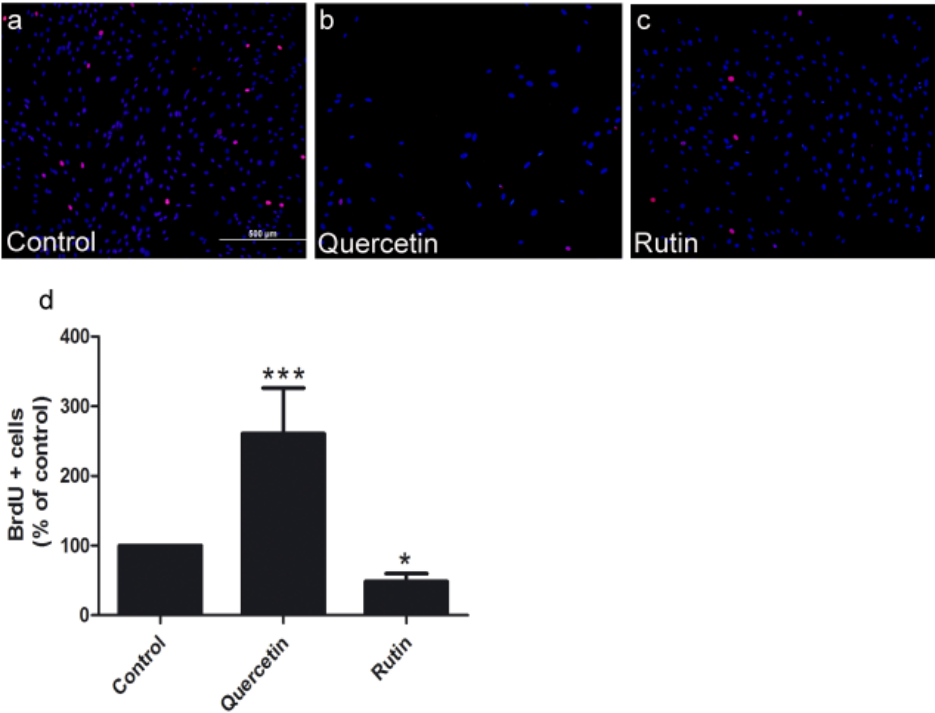


Fig. 3 Flavonoids affect SD-MSC proliferation. MSCs cultures were incubated with a vehicle (DMSO) or 320 μM of quercetin or rutin. After 2 days, cell proliferation was determined by BrdU incorporation (a-d). The results represent the mean of three independent experiments performed in triplicate \pm SEM. * $P < 0.05$ or *** $P < 0.01$ when compared with control group. Scale bar = 500 μm .

marrow MSCs in a dose dependent manner. In agreement with these studies we found that 320 μ M of quercetin increases SD-MSCs proliferation, after 2 days of treatment.

An opposite effect on SD-MSC proliferation was observed in cells treated with rutin, which promotes significant reduction on cell proliferation at 320 μ M. Similar results were reported by Marcarini et al. [17] and Santos et al. [26] in rat hepatoma and glioblastoma cells, respectively, which found reduced cell proliferation after treatment with 810 μ M and 10-100 μ M of rutin, respectively.

The cytotoxic properties of flavonoids are likely attributed to their effects on inhibiting cell cycle progression and/or triggering apoptosis [27]. Here we showed that both quercetin and rutin at 320 μ M, promoted a decrease in the total number of SD-MSCs accompanied by increased values of picnotic nuclei after 2 days of treatment. Reduction in the total number of cells treated with these flavonoids was also reported in other cell types, such as mouse neuroblastoma cells [28] and human glioblastoma [26]. The differential cytotoxic effects of flavonoids could be attributed to different rate of drug metabolism displayed by these cells [7].

5. Conclusions

Quercetin and rutin have been highlighted as potent bioactive substances because of their different biological, pharmacological, and medicinal activities [7]. Our results can improve the new comprehension of quercetin and rutin effects in SD-MSCs, which can facilitate the creation of new drugs for cell therapies. However, further investigations are required to ensure the safety of the application of these substances and understand their cellular mechanisms of action.

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