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Apple juice prevents oxidative stress induced by amyloid-beta in culture

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Abstract. Increased oxidative stress contributes to the decline in cognitive performance during normal aging and in neurodegerative conditions such as Alzheimer's disease. Dietary supplementation with fruits and vegetables that are high in antioxidant potential have in some cases compensated for oxidative stress. Herein, we examined whether apple juice could alleviate the neurotoxic consequences of exposure of cultured neuronal cells to amyloid-β (Aβ), since at least a portion of the neurotoxicity of Aβ is due to oxidative stress. Apple juice concentrate (AJC; 70 degree brix) was diluted into culture medium of SH-SY-5Y human neuroblastoma cells that had been differentiated for 7 days with 5 μM retinoic acid concurrent with the addition of 20 μM Aβ. AJC prevented the increased generation of reactive oxygen species (ROS) normally induced by Aβ treatment under these conditions. AJC also prevented Aβ-induced calcium influx and apoptosis, each of which results in part due to increased ROS. These findings suggest that the antioxidant potential of apple products can prevent Aβ-induced oxidative damage.

Keywords: Amyloid-β, oxidative stress, antioxidant, apples, neuroblastoma, Alzheimer's disease

1. Introduction

Oxidative stress is a major factor contributing to the age-related decline in cognitive performance [2–4,12, 29,30] and is a pivotal factor in Alzheimer's disease (AD) [27,28,35]. Pharmacological approaches or supplementation with antioxidants and vitamins represents one potential approach to alleviate oxidative stress [13, 18,2–26,33–35,41]. In addition, consumption of fruits and vegetables that are high in antioxidant potential can be effective against oxidative stress [5,6,8,14–16, 27,37–40,44]. This latter approach has been demonstrated effective against memory impairment and concentration difficulties [17,18,31] and Alzheimer's disease (AD) progression [16,17,30,31,38], and, in some cases has provided superior antioxidant activity than dietary supplements of purified antioxidants [8].

One hallmark of AD is the accumulation of amyloid-β (Aβ), which invokes a cascade of oxidative damage to neurons that can eventually result in neuronal death (for reviews, see 1,29). Aβ neurotoxicity can be alleviated in culture by co-treatment with antioxidants such as vitamin E and N-acetyl cysteine [7,12,13,20]. Herein, we tested whether or not supplementation with apple juice, which contains antioxidant activity [42, 43], could compensate for Aβ neurotoxicity in cultured human neuroblastoma cells.

2. Materials and methods

SH-SY-5Y cells were differentiated for 7 days with 5 μM RA in DMEM containing 10% fetal bovine serum. Alternate cultures then received 20 μM Aβ 25–35 [10] and/or apple juice concentrate (AJC) from a stock of 70 degree brix (generous gift of Veryfine Inc. Littleton MA), diluted into culture medium at final concentrations ranging from 1:50 to 1:1000. Since a portion of the antioxidant activity of apples is recovered in apple juice [8,24,40,42,43], AJC was uti-

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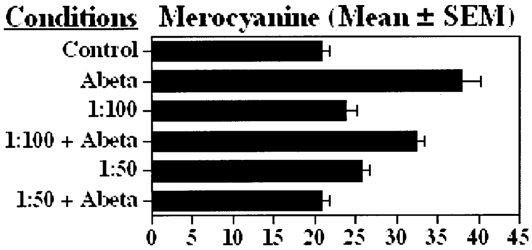

Fig. 1. AJC diminishes Aβ-induced apoptosis Differentiated SH-SY-5Y cells were treated with 20 μM Aβ and/or 1:100 and 1:50 dilutions of AJC for 2hr. The graph presents densitometric values [presented as the mean $\pm$ standard error of the mean (SEM) in arbitrary densitometric units] compiled from a total of 4 values from 2 independent experiments in which merocyanine fluorescence (proportional to the level of externalized phosphatidyl serine, an early index of apoptosis) was quantified in $\geqslant$50 individual cells in 2 cultures per experiment. As shown previously, Aβ induced a significant increase in externalized phosphatidyl serine ($p < 0.05$ vs. untreated cells), this increase was progressively reduced by co-treatment with 1:100 AJC and eliminated by 1:50 AJC.

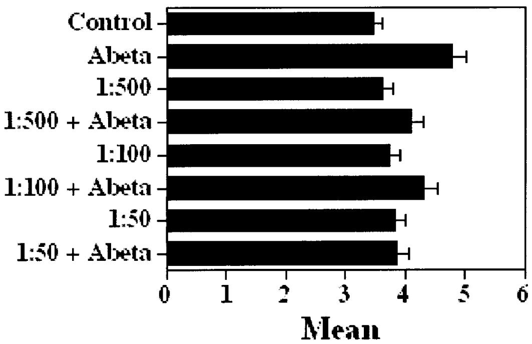

Fig. 2. AJC diminishes the increase in cytosolic calcium induced by Aβ Differentiated SH-SY-5Y cells were treated with 20 μM Aβ and/or 1:100 and 1:50 dilutions of AJC for 2hr. The graph presents densitometric values [presented as the mean $\pm$ standard error of the mean (SEM) in arbitrary densitometric units] compiled from 2–4 values from 2 independent experiments in which cytosolic calcium was quantified by Fluo-3 fluorescence in $\geqslant$50 individual cells in at least 2 cultures per experiment. Note that, while Aβ induced a significant increase in cytosolic calcium ($p < 0.05$ vs. untreated cells), this increase was progressively reduced by co-treatment with 1:500 and 1:100 AJC, and eliminated by 1:50 AJC.

lized in the present study due to ease of dispensing onto cultures. Following 2hr of incubation in the presence or absence of Aβ and AJC, cultures were analyzed for ROS, cytosolic calcium and apoptosis, all of which have been shown to result from Aβ exposure under these conditions. To monitor intracelluar peroxide concentrations (which provides an "end-point" of the cascade of ROS generated by Aβ), cultures were then treated with 2′–7′ dichlorofluorescein diacetate (DCFD; Kodak) for 20 minutes. Cultures were rinsed with serum-free medium and intracellular peroxide levels were measured under fluorescein optics [10]. Cytosolic calcium was monitored as described [10] using Flou-3 (Molecular Probes). Externalized phosphatidyl serine was monitored using merocyanine as described as an index of apoptosis [9–11]. Images were then captured with our DAGE CCL-72 cooled CCD camera via a Scion LG-3 frame grabber operated by NIH Image analysis software and stored in a Macintosh Power PC 7100AV. Analysis was carried out with NIH Image software [28]. For each of the above analyses, $\geqslant$50–100 cells individual cells in duplicate cultures were encircled with the software's freehand tool and fluorescence quantified following automated background subtraction. Two to four independent experiments were carried out for each probe, with 2–4 cultures under each condition within each experiment. Statistical analyses were carried out with Student's t test and ANOVA.

3. Results and discussion

Consistent with prior studies, Aβ induced a marked increase in ROS, and this increase was prevented by co-treatment with AJC (Fig. 1). The initial increase in cytosolic calcium following Aβ exposure is due to activation of plasma membrane-associated calcium channels (ref. 10 and refs. therein); however, the full extent of the Aβ-induced increase in cytosolic calcium is a consequence of oxidative stress, since it is quenched by co-treatment with antioxidants [10,11]. AJC decreased the increased cytosolic calcium otherwise induced by Aβ (Fig. 2). AJC also prevented apoptosis that normally results from exposure to Aβ (Fig. 3). Moreover, AJC apparently reduced all three of these neurotoxic parameters in a dose-response fashion; values of cultures treated with AJC under all concentrations presented herein differed statistically from those receiving Aβ alone ($p < 0.05$) and at an AJC dilution of $\geqslant$1:50 were restored to levels statistically identical to those of untreated control cultures ($p > 0.05$).

We have not conclusively determined which component(s) present in AJC were responsible for the neuroprotective effects demonstrated herein on cytosolic calcium and apoptosis. Quenching of ROS by AJC, and the prior demonstration that both the Aβ-induced increase in cytosolic calcium and apoptosis can be prevented by co-treatment with vitamin E [10,11], is consistent with the possibility that the antioxidant potential of apple juice was responsible for reducing the Aβ-induced increase in cytosolic calcium and apoptosis as well as quenching ROS. Since varying amounts of an-

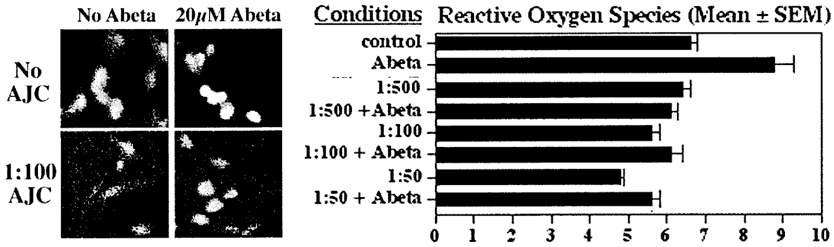

Fig. 3. AJC diminishes Aβ-induced ROS Representative micrographs are presented from untreated cultures and cultures treated for 2hr with 20 μM Aβ in the presence or absence of AJC diluted 1:50 to 1:500 as indicated in culture medium; values presented indicate the dilution of AJC). As in prior studies, ROS are largely confined to the perikaryon. The accompanying graph presents densitometric values [presented as the mean $\pm$ standard error of the mean (SEM) in arbitrary densitometric units] compiled from 2–4 values from 4 independent experiments in which ROS was quantified in $\geqslant$50 individual cells in at least 2 cultures per experiment. Note that, while Aβ induced a marked increase in ROS ($p < 0.05$ versus untreated control cultures), this increase was prevented by AJC over a range of dilutions as great as 1:1000.

tioxidant activity are detected in apple peels, peeled apples and juice [8,23,40,42,43], testing the comparative efficacy of different apple products in a model such as utilized herein, and/or by supplementation of transgenic mice expressing Aβ, would be of interest. While it would be important to confirm in situ efficacy of AJC and/or other apple products, demonstration of quenching Aβ-induced oxidative damage in culture confirms a direct protective effect on neuronal cells, rather than indirect (e.g., by protecting cerebral vasculature).

The findings derived in culture support the notion that consumption of foods rich in antioxidant potential can provide neuroprotection [3,4,14–16,30,31,38].

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