Antioxidant Activity of Pomegranate (*Punica granatum* L.) Extracts in Cell-free and Cell Culture Systems

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Punica granatum L. (Punicaceae) fruits have been widely used in folk medicine; a number of therapeutic properties of pomegranate preparations has been reported (Duke and Ayensu, 1985), including vermifugal, taenicidal, astringent, antispasmodic, antihysteric, diuretic, carminative, emmenagogue and antiinflammatory. More recently, De Nigris *et al*. (2005) reported that pomegranate juice may exert a preventive role in atherosclerotic disease. These actions have been ascribed to the presence of anthocyanins, cyanidin, ellagitannins, punicalagin and vitamin C. It has been reported (Gil *et al*., 2000) that industrial pomegranate juice (obtained from both arils and rinds) displays antioxidant activity against reactive oxygen species; this activity has been found to be higher than that of red wine and green tea; arils-only juice seems to be less active than whole fruit juice. Further studies, including one from our group (Ricci *et al*., in press), dealt with the antioxidant activity of whole, arils or rinds pomegranate juices/extracts: however, these research works have been carried out exclusively in cell-free systems.

The lack of data on the actual antioxidant activity of pomegranate preparations in biological systems prompted us to determine the antioxidant potential of experimental, microfiltrated whole fruit (FJ), arils-only (AJ) juices, or rinds-only (RE) extract in cultured mammalian cells (human promonocytic U937 and endothelial HUVEC cells) exposed to hydrogen peroxide or *tert*-butylhydroperoxide (tB-OOH), and compare these results with those from cell-free systems. The results obtained indicate that AJ (up to 1% v/v final concentration), which in cell-free systems is known to display antioxidant activity, failed to protect cultured cells from the insult elicited by the two oxidants. By contrast, in analogy with cell-free data, FJ and RE (up to 1% v/v) increased the survival of oxidatively injured cells; the activity in cultured cells was higher against hydrophilic-(H_2O_2) compared with lipophilic-oxidants (tB-OOH). A finding of particular interest is that RE-afforded cytoprotection was far higher than that of FJ, which contains only a small fraction of rind components. A necessary condition for maximal cytoprotection to occur was that FJ and RE had to be given to cells during the oxidant challenge stage: indeed preincubation (up to 3 h) with FJ or RE before treatment with the oxidants in an extract-free medium resulted in a significant loss of cytoprotection. This finding would suggest that the active components rapidly egress from the cell. As to the mechanism of RE antioxidant activity, it can be attributed either to the scavenging action of the OH-groups of polyphenols, or to their iron chelating activity, which has been spectrophotometrically and biologically confirmed with an experimental approach described elsewhere (Sestili *et al*., 2002).

Taken together, these results indicate that the antioxidant activity of pomegranate in cultured cells is mainly due to the presence of rind components: as a consequence, future research on the possible therapeutic applications of pomegranate preparations should focus on rinds extracts and/or derivatives.

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