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Lycopene supplementation (passata sauce) reduces apoptosis but does not affect oxidant-responsive heme oxygenase-1 in human lymphocytes

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Abstract

Objective: We tested the hypothesis that lycopene supplementation reduces the expression of oxidant-responsive heme oxygenase-1 (HO-1) in basal conditions and in response to an oxidant challenge and determined whether this is temporally associated with increased cell viability.

Methods: We determined basal and stimulated ex vivo expression of HO-1 and cell viability in lymphocytes from volunteers after lycopene supplementation. Twenty-four healthy young men on a low lycopene diet consumed 1) 170 g of passata sauce with butter or 2) butter alone for 3 wk in a randomized crossover design.

Results: Plasma lycopene concentrations at the end of the tomato and control trials were 0.54 ± 0.20 versus 0.20 ± 0.15 μmol/L, respectively ($P < 0.05$). There was a significant increase in the proportion of live cells (91 ± 5% versus 87 ± 9%) and a corresponding reduction in apoptosis (6 ± 4% versus 11 ± 9%) in untreated lymphocytes after supplementation ($P < 0.05$), with no effect on cell viability in response to hydrogen peroxide treatment. HO-1 protein expression in basal conditions and induction of HO-1 after hydrogen peroxide treatment was not different between trials.

Conclusion: Lycopene supplementation did not affect basal oxidative stress or susceptibility to oxidant-induced stress as indicated by the expression of the oxidant-responsive protein HO-1 and cell viability in response to hydrogen peroxide treatment. However, lycopene supplementation significantly reduced apoptosis in freshly harvested untreated lymphocytes. We conclude that this was not through an oxidant-mediated mechanism because of the lack of an effect on oxidant-responsive HO-1.

Keywords: Carotenoids; Mononuclear cells; Apoptosis; Necrosis; Insulin-like growth factors

Introduction

The carotenoid lycopene is a potent antioxidant found particularly in tomatoes and there are many observational studies that suggest that tomato and lycopene consumption has the capacity to modulate the risk of developing certain types of cancers [1], cardiovascular disease [2], and type 2 diabetes [3]. However, investigations that have directly manipulated tissue concentrations of this antioxidant have shown conflicting findings concerning the protective role of tomatoes and lycopene; hence, the evidence for this association has been described as limited [4–7]. Clearly, there is a need for studies to examine whether the association between consumption of lycopene-rich tomato products and reduced risk of chronic disease reported in observational studies can be corroborated through direct laboratory-based intervention studies employing appropriate tools and endpoints.

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One possible mechanism for the protection afforded through lycopene supplementation is through attenuation of oxidative stress [8]. There is strong evidence that lycopene has powerful antioxidant properties, but the evidence to show that this is the primary mechanism that underpins the biological activity of this compound in vivo is far less convincing [8]. The assessment of oxidative stress and damage in biological systems is a difficult task. In contrast to the determination of specific endproducts of oxidative modification (e.g., lipid hydroperoxides), it may be possible to use the altered expression of oxidant-responsive genes as tools to determine changes in oxidative stress [9]. The induction of heme oxygenase-1 (HO-1) represents a general response to oxidant stress in mammalian cells [10] and its altered expression is used as a marker of oxidative stress in vitro [11] and in vivo [12]. Indeed, in cell-based experimental models, HO-1 is by far the most responsive gene to oxidative stress [9,13,14].

If the antioxidant properties of lycopene were sufficient to affect oxidative stress, then we would expect manipulation of tissue lycopene concentrations to alter the expression of HO-1. In general, the literature indicates a suppressive effect of supplementation with antioxidants such as β-carotene and α-tocopherol on the expression of HO-1, and this has been observed in animal models, cell culture studies, and human trials [15,16]. Although counterintuitively lycopene has been reported to increase HO-1 expression in mouse fibroblasts [17], comprehensive studies in human fibroblasts clearly demonstrate that when adequate precautions are taken to prevent oxidation, then carotenoids protect against oxidant-mediated HO-1 induction [18]. Therefore, lycopene has the capacity to lower the expression of HO-1 in cell systems and this is probably through some form of antioxidant-mediated effect, although there is no evidence that this occurs in humans in vivo.

Oxidative stress causes cellular damage and under the right conditions has the capacity to affect cell viability. For example, it is well known that ex vivo treatment of human lymphocytes with hydrogen peroxide (H_2O_2) causes cell death by apoptosis and necrosis [19] and that antioxidant supplementation before H_2O_2 exposure in vitro can reduce apoptotic cell death [20,21]. If lycopene is protective through lowering oxidative stress, we might anticipate that supplementation with this antioxidant would consequently increase cell viability when cells are exposed to an oxidant challenge.

The main objective of the present study was to examine whether the manipulation of tissue lycopene concentrations through daily consumption of tomato-based passata sauce (rich in lycopene) reduces oxidative stress and modulates lymphocyte viability in humans. We hypothesized that lycopene supplementation would attenuate oxidative stress and that this would reduce the expression of the oxidant-responsive gene HO-1 in lymphocytes in response to an oxidant challenge (H_2O_2). We also hypothesized that increased cellular protection as a result of lycopene supplementation would increase lymphocyte viability in response to an oxidant challenge (H_2O_2).

Materials and methods

Subjects

Twenty-four healthy male volunteers provided written informed consent to take part in this investigation, which was approved by the local ethics committee. All participants were non-smokers and were not taking regular prescribed medication or dietary supplements. Participants' mean ± SD values for age, height, and mass were 26 ± 4 y, 180 ± 10 cm, 76.9 ± 10.8 kg.

Experimental design and procedures

The study comprised two 3-wk intervention periods separated by a 2-wk washout in a randomized, open-label crossover design. Low-dose lycopene supplementation has been reported to reduce lipid peroxidation, protein thiols, and DNA damage, and based on these findings recommendations for a daily lycopene intake of 5 to 10 mg have been made [22]. Supplementation (+TOM) consisted of 170 g of passata tomato sauce (containing approximately 7 mg of lycopene and 0.3 mg of β-carotene; H.J. Heinz, United Kingdom) taken with 10 g of butter (Anchor, Arla Foods, Leeds, United Kingdom), whereas in the no-supplementation trial (control; −TOM) 10 g of butter alone was received per day.

The passata tomato product was made from the same batch of tomatoes and was consumed with butter to ensure there was minimal loss of bioavailability of lycopene from ingestion to absorption [23]. Subjects were given a variety of recipe ideas for the consumption of the passata tomato sauce. During the entire investigation, volunteers were provided with a list of all lycopene-rich foods to avoid for the full duration of the trial to ensure that this was a comparison of dietary lycopene supplementation through passata sauce (+TOM) versus control (−TOM).

Venous blood samples were collected at baseline before commencing the first intervention period, with further samples taken at the end of the first intervention period, after a 2-wk washout before the second experimental period, and then after a further 3-wk experimental period. Comparisons were made between the two post-intervention time points (post-supplementation versus post-control). Subjects were asked to complete a weighed food and fluid record for at least 3 d of each week during the experimental periods (total ~9 d per trial period), which was used to monitor compliance. During the 72-h period before the first trial (supplementation/control), subjects maintained a record of food and fluid intake that was reproduced as closely as possible in the 72 h before the subsequent trial. During the washout between trials, subjects did not weigh their food