Invited commentary

Olive oil phenolics: effects on DNA-oxidation and redox enzyme mRNA in prostate cells

The health benefits of the 'Mediterranean diet' have been recognized since the mid-1970s with many reviews on the topic being produced up to the present (Trichopoulou *et al.* 2001). Benefits relate principally to prevention of cardiovascular disease and colo-rectal, breast and prostate cancers. Disease prevention has been linked to characteristic features of a typical Mediterranean diet, including tomatoes, fruits and vegetables, seafood, wine and olive oil. These foods contain a wide range of potentially protective factors such as *n*-3 fatty acids, low saturated fat content, soluble fibre, glucosinolates, carotenoids, tocopherol, vitamin C and a variety of phenolic compounds. Virgin olive oil is a particularly rich source of the phenolics caffeic acid, oleuropein, tyrosol and hydroxytyrosol.

For many years now, the dogma has been that the protective effects of phenolics and many vitamins are derived from their antioxidant capacity (Visioli & Galli, 2001). There is no doubt that these compounds can act as antioxidants in the test-tube, or even in cell culture systems, where pre-treatment of isolated DNA, lipids or whole cells with potential antioxidants blocks the effect of adding a pro-oxidant such as H_2O_2. But the question must be posed: is this purely a chemical phenomenon that is totally predictable or does it have real biological meaning? Often the doses used of both pro- and anti-oxidant have been supra-physiological, and the effects difficult to relate to responses found *in vivo*. The authors of the paper by Quiles *et al.* (2002) in the present issue of the *British Journal of Nutrition* have made a commendable effort to relate this type of study to physiological levels of phenolics in plasma. However, their proposed values of approximately $100 \cdot 0 \, \mu M$ are perhaps optimistic. Maximal intake of olive oil is likely to be in the region of 50 ml containing about $4 \, \mu mol$ hydroxytyrosol. Assuming 60 % of this crosses the intestinal mucosa and then is diluted into 5 litres blood, one might expect a concentration in the region of $0 \cdot 5 \, \mu M$ in plasma, not allowing for dilution effects in the meal or the time over which absorption takes place. This value is very much in keeping with values reported by Scalbert & Williamson (2000) for polyphenolics, although there is some evidence in rats that quercetin can accumulate over time to reach concentrations of $20 \cdot 0 \, \mu M$ (Manach *et al.* 1997). There is clearly a great need for actual measurements of phenolic levels in plasma before we can be comfortable with extrapolating from cell line work to effects in man.

The role of oxidation in the aetiology of atherosclerosis is well established, and the important function provided by antioxidants in the protection of LDL from free-radical attack is widely recognized (Trichopoulou *et al.* 1999). However, the role that oxidative damage plays in the aetiology of cancer is far from clear. Cancer is a multi-step process starting with initiation of DNA damage, but then progressing through a number of stages involving an accumulation of genetic damage and loss of control of cell proliferation and apoptosis (Johnson *et al.* 1994). There is little evidence to support or refute the importance of DNA oxidation in the development of cancers. A major source of cellular free-radical attack is likely to be the endogenous production of superoxide radicals during mitochondrial respiration and the production of hydroxyl radicals. Hydroxyl radical generation will be exacerbated by the presence of unchelated transition metals, particularly Fe and Cu. Early studies showed that DNA can be oxidized in cell free systems or in cell culture, and formation of 7,8-dihydro-8-oxo-guanine can lead to point mutations, typically G to T substitution. Such changes have a significant impact. For example, k-*ras*, which is mutated in about 40 % of colo-rectal tumours involves predominantly G to A transitions (Esteller *et al.* 2000), but carries G to T substitutions in the most aggressive forms (Kusaka *et al.* 2000). The frequency of such mutations in the sporadic induction of cancer overall is unclear. Moreover, G to T substitutions may be caused by other mechanisms, including exposure to (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). The presence of 8-hydroxy-2.-deoxy-guanosine in urine does, however, suggest that DNA oxidation occurs widely (Halliwell, 1998), but that there is effective removal of oxidized guanine adducts by cells.

Research into the mechanisms underlying the influence of phenolic compounds on carcinogenesis has, until recently, been constrained by the perception that oxidative 'stress' is always detrimental to the cell or whole organism. There is, however, a growing realization that phenolics may not act purely by preventing oxidative damage and that they may have a more subtle role in controlling cell phenotype, whilst also acting as metal chelators. The putative chemo-protective effects of phenolics could therefore act at many stages in the progression of a cancer. The response of cell lines to oxidative stress is very dose-dependent (Dypbukt, 1994), so by modifying the redox state of the cell, phenolics are likely to impact on other signalling pathways that are dependent on redox potential, including expression of phase 2 enzymes and control of mitosis and apoptosis. The role of dietary antioxidants in relation to redox-dependent signalling pathways has been

reviewed as part of a European Concerted Action on anti-oxidants (Jackson *et al.* 2002). The effect of each phenolic on a particular end-point is difficult to predict (Kuntz *et al.* 1999), although recent work by Depeint *et al.* (2002) reports some consistent structure–function relationships. Structure will affect bioavailability, antioxidant capacity and ability to induce antioxidant enzymes. Some phenolics, such as the phyto-oestrogens, may interact with cell signalling pathways in a manner unrelated to their antioxidant capacity. In the case of the anti-inflammatory effects of phenolics the antioxidant capacity does appear to be important (Ma & Kinneer, 2002) and this would influence cancer risk. The functioning of caspases is integral to the induction of apoptosis and dependent on cell redox state (Carmody & Cotter, 2001). The failure to induce apoptosis can lead to necrosis and potentially inflammation (Hirsch *et al.* 1997) or simply to survival of damaged cells; thus, work on cancer cell lines must be interpreted with caution.

In the study by Quiles *et al.* (2002), the simple phenolics had no effect on cell viability but did affect cell number, indicating an effect on mitosis rather than apoptosis, which is consistent with results for other phenolic compounds (Depeint *et al.* 2002). The antiproliferative effect of phenolic compounds is probably a very important protective function. It should be noted however that pro-oxidants might also be protective against carcino-genesis. Addition of eicosapentaenoic acid (20:5) to rat diets, following initiation of carcinogenesis, decreases aberrant crypt foci. This is associated with an increase in apoptosis, which is enhanced by reducing intracellular glutathione levels (Latham *et al.* 2001). Whether dietary anti-oxidants at physiological doses would have the same effect *in vivo* is not known but this hypothesis should be investigated in relation to excessive intake of dietary antioxidant supplements.

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