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Reversal of hypermethylation of *p16^{INK4A}* and *ESR1* genes by selenium and iberin in Caco-2 cells

Lawrence N. Barrera[1,2], Nigel J. Belshaw[2], Yongping Bao[1], Aedin Cassidy[1] and Ian T. Johnson[2]
[1]*University of East Anglia, Norwich, UK and* [2]*Institute of Food Research, Norwich, UK*

Dietary isothiocyanates, have been demonstrated to possess cancer-preventive activities, including inhibition of carcinogen-activating enzymes, induction of carcinogen-detoxifying enzymes, induction of apoptosis and arrest of cell-cycle progression[(1)]. Similarly, research has shown that Se possesses a chemoprotective effect at various stages of carcinogenesis[(2)]. Recently, studies have suggested that these bioactive food components can modify epigenetic patterns in complex ways[(3)]. Currently, transcriptional silencing of tumour suppressor genes by CpG island promoter hypermethylation and the concomitant overall hypomethylation of the genomic DNA are the most-studied epigenetic alterations that play an important role in the development of carcinogenesis[(4)] and represent a key mechanism that underlies most sporadic colo-rectal cancers[(5)]. Since epigenetic events are susceptible to modification they represent excellent targets to explain how environmental factors, such as diet, may modify cancer risk. In this context Se has been shown as an effective inhibitor of human DNA methyltransferase 1[(6)] whereas isothiocyanates such as sulforaphane act as an inhibitor of histone deacetylase in cultured cancer cells[(7)]. Despite the documented presence of these changes in cancer cells as a result of these compounds, the mechanisms of these events remain unclear. To gain insights into the molecular mechanisms mediated by these factors and to establish whether a combination of food components offer additive or synergistic effect to regulate epigenetic marking, two biological replicates of cultured Caco-2 cells were exposed to a nutritionally-relevant concentration of Se (200 nM) and/or the isothiocyanate iberin (6 μM) for 15 d and changes to gene-specific (*p16^{INK4A}* and *ESR1*) and global methylation were quantified using real-time PCR-based assays.

Data revealed that in control samples the CpG islands of *p16^{INK4A}* and *ESR1* in Caco-2 cells were heavily methylated while treatment with Se, iberin and iberin + Se reduced *p16^{INK4A}* methylation by 15%, 12% and 10% respectively (Fig. 1). The demethylation effect of the treatments at the ESR1 CpG island, although not significant, showed a similar trend, with a reduction of 20% for Se, 30% for iberin and 8% for iberin + Se (Fig. 2).

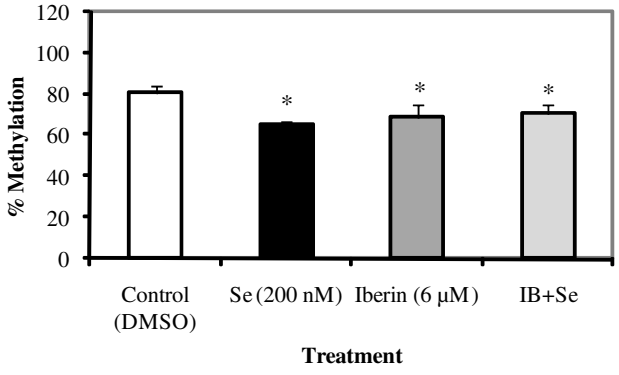

Figure 1. *p16* Methylation reduction after treatment in Caco-2 cells.

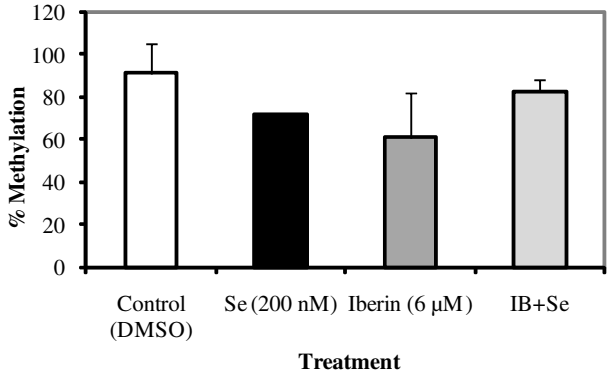

Figure 2. *ESRI* methylation reduction after treatment in Caco-2 cells.

A significant reduction in global methylation was observed for all treatments ($P<0.05$) with Se leading to 11% decrease, iberin 8% decrease and iberin + Se 9% decrease. These results suggest that the doses of Se or isothiocyanates supplied do not offer a synergistic effect when combined in Caco-2 cells. The reduction of both global and gene-specific DNA methylation is modulated perhaps through a modification of the expression and/or activity of the DNA methyltransferase enzymes. On-going research is investigating this hypothesis.

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