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B. Pampaloni[1], C. Mavilia[1], E. Bartolini[1], G. Palmini[1],P. Vignolini[2], A. Romani [2], A. Tanini[1], and M.L. Brandi[1]*

1 Departments of Internal Medicine, V.le Pieraccini, University of Florence, Florence, Italy; 2 Department of Pharmaceutical Science, Via U. Schiff, 6, University of Florence, Florence, Italy

*corresponding author: m.brandi@dmi.unifi.it

Introduction: Studies on the relationship between Food and Health highlighted the importance of some biologically active components contained in food matrices. To date, the most studied biologically active polyphenols are flavonoids, some of which have shown clear estrogen-like activity, why are today defined as "phytoestrogens". It has been demonstrated that this components of plants, with molecular structures similar to steroids, could be critical modulators of human hormonal system and hormonal action on target tissue (1;2). Phytoestrogens have been widely studied for their potential therapeutic use in the prevention of cardiac disease, menopausal symptoms, osteoporosis, and some carcinomas since they show some of the protective effects of estrogens in absence of the side effects associated to estrogen administration (3). In effect many Epidemiological and experimental studies suggest a protective role of estrogens against colorectal cancer. Colorectal cancer is a multifactorial disease that results from interactions of different factors such as aging, family history and dietary style. Men tend to have a slightly higher incidence of colorectal cancer than women of similar age (American Cancer Society, 2007) and oestrogen seem to be implicated for this decreased risk in women. Epidemiological studies and results of Women's Health Initiative (WHI) clinical trial, provide strong evidence that the colorectal cancer is hormone sensitive because the cancer risk is reduced in women who take HRT (Hormon Replacement Therapy) (4). Estrogen receptors alpha (ERα) and beta (ERβ) are the 2 known subtypes of ERs, through which estrogens exert their effects on various tissues. Experimental data have demonstrated that CRC express an elevated number of estrogen receptors (ERs), in particular estrogen receptor β. The observation that the level of ERβ protein is lower in malignant tumors than in normal tissue of the same organ, has fostered the hypothesis that ERβ may function as a tumor suppressor, protecting cells against malignant transformation and uncontrolled proliferation. It has been suggested that the protective effect of estrogens may be mediated through activation of ERβ, which has been shown to be the predominant subtype of ER in the gastrointestinal tract (5).

Soy and soy foods contain a wide variety of chemical compounds, biologically active, that may contribute, individually or synergistically, to the health benefits of this plant; in particular polyphenols are considered to possess chemopreventive and therapeutic properties against cancer. Among these compounds there are isoflavones, the most important and abundant of which is Genistein that have also estrogenic properties. Phytoestrogens, present in soy based food, may act through hormonal mechanisms to reduce cancer risk by binding to estrogen receptors (ER) or interacting with enzymes involved in sex steroid biosynthesis and metabolism (6). Moreover, genistein may inhibit cancer progression by inducing apoptosis or inhibiting proliferation, and the mechanisms by which genistein exerts its anti-tumor effects has been the subject of considerable interest (7). Quercetin is a flavonoid present in many plant sources at levels sufficient so that it is considered to be the major flavonol present in the human diet. Warren (8) and others (9) have demonstrated the ability of quercetin to suppress the formation of early neoplastic lesions of colon cancer (aberrant crypt foci, ACF) through an inhibition of proliferation and apoptosis.

Materials and Methods: We examined the activities of two of the flavonoids most common in food matrices, genistein and quercetin, on the colon cancer cells line HCT8-β8 engineered to overexpress ERβ, assuming that these compounds may play a protective action on colonic mucosa mediated by binding estrogen receptor β (ERβ). The aim of our study was to evaluate the *in vitro* effects of genistein (5, 25, 50, 75, 100 μM), quercetin (5, 25, 50, 75, 100 μM) and 17βE2 (10 nM) as a control, on a human colon cancer cell line, on cells proliferation. Lucyferase assay test was performed to study the interaction between genistein and quercetin and estrogen receptor β (ERβ). The presence of the ER-β and its levels of expression after stimulation with quercetin and genistein (50 μM) were assessed by the preparation of a reaction of Real-time qPCR absolute. Data were expressed as mean. Analysis of variance were performed. Differences were significant at $p<0.05$.

Results/Discussion: The results showed an anti-proliferative activity of genistein (Fig 1) and quercetin (Fig 2) at the concentration of 50, 75 and 100 µM, and 17βE2 at concentration 10 nM.

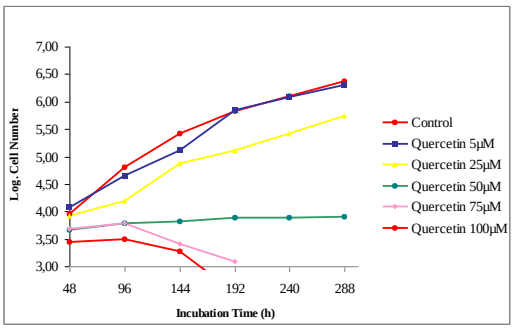

Fig 1. Effects of genistein on HCT8-β8 cells proliferation

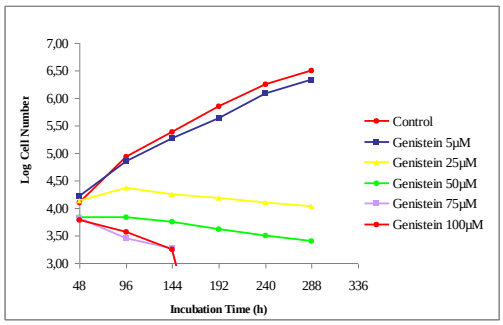

Fig 2. Effects of quercetin on HCT8-β8 cells proliferation

The polyphenols genistein and quercetin showed an inducing effect on ERβ, at concentration of 50 µM, as demonstrated by the increase in luciferase expression compared with control. A superimposable effect was present when cells were stimulated with 10 nM 17βE2, used as a positive control. (Fig. 3). Evaluation of expression levels of ERβ in cells stimulated with genistein and quercetin was performed. The results of qPCR, obtained normalized to a housekeeping gene, have demonstrated a significant increase of ERβ levels after 6 days compared to control in cells treated with 50 and 100 µM genistein and quercetin (Fig. 4).

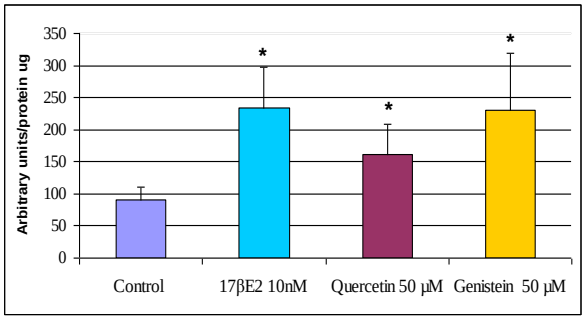

Fig 3. Effects of genistein and quercetin (50 µM) transactivation in HCT8-β8 cells. * p<0.05 vs genistein cells, p<0.05 vs 17βE2-treated cells.

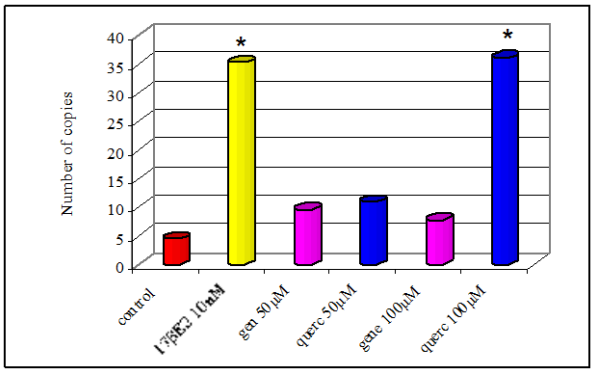

Fig 4. ERβ levels, evaluated by qPCR, in on HCT8-β8 cells treated with 17βE2, untreated and quercetin (after six days) (*p<0.05).

In conclusion our study shows that genistein and quercetin are able to induce a significant inhibition of colon cancer cell proliferation. Inhibition of proliferation may be through a series of events, encompassing transactivation, and increase in the ER β expression.

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