**Genistein effects on Ca\textsuperscript{2+} handling in human umbilical artery: inhibition of sarcoplasmic reticulum Ca\textsuperscript{2+} release and of voltage-operated Ca\textsuperscript{2+} channels**

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Isoflavones are a group of natural phytoestrogens including the compound genistein. Health beneficial effects have been attributed to the consumption of this compound, but the fact that it has estrogen-like activity has raised doubts regarding its potential risk in infants, newborns, or in the fetus and placenta during pregnancy. This work is aimed at studying genistein effects on Ca\textsuperscript{2+} handling by smooth muscle cells of the human umbilical artery (HUA). Using fluorometric techniques, we found that in these cells genistein reduces the intracellular Ca\textsuperscript{2+} peak produced by serotonin. The same result could be demonstrated in absence of extracellular Ca\textsuperscript{2+}, suggesting that the isoflavone reduces Ca\textsuperscript{2+} release from the sarcoplasmic reticulum. Force measurement experiments strengthen these results, since genistein reduced the peak force attained by intact HUA rings stimulated by serotonin in a Ca\textsuperscript{2+}-free solution. Moreover, genistein induced the relaxation of HUA rings precontracted either with serotonin or a depolarizing high-extracellular K\textsuperscript{+} solution, hinting at a reduction of extracellular Ca\textsuperscript{2+} entry to the cell. This was confirmed by whole-cell patch-clamp experiments where it was shown that the isoflavone inhibits ionic currents through voltage-operated Ca\textsuperscript{2+} channels. In summary, we show that genistein inhibits two mechanisms that could increase intracellular Ca\textsuperscript{2+} in human umbilical
Isoflavones are a group of natural phytoestrogens including the compound genistein. This is a nonsteroidal estrogen-like compound present in significant quantities in human diet, being soy products the major dietary sources of isoflavones. This molecule exists in different forms depending on its glycosilation and processing conditions, and hydrolysis within the digestive tract can transform it and increase the aglycone fraction (10, 23).

In humans, after a single soy meal the isoflavone concentration rises slowly and reaches maximum values around the micromolar range at 7-8 hours (12). The presence of genistein has been detected in plasma, urine (1, 27), human milk (9) and in prostatic tissue (3) of subjects that consume such products, as well as in amniotic fluid during the second trimester of pregnancy (8) and at birth (2). A wide spectrum of health beneficial effects have been attributed to the consumption of this natural compound, most of them related to a decrease in the risk of cardiovascular diseases and cellular proliferation (15). However, since genistein is a phytoestrogen, and hence capable of stimulating cellular estrogen receptors (4), controversy still exists regarding the effects of these compounds on human immature estrogen sensitive target tissues as well as their potential effects on the fetus due to in utero exposition. For instance, it has been reported that the placenta is a target tissue for genistein action during gestation (22), while exposure to genistein resulted in significant feminization of the male mammary glands in rats (26).

Regarding its mechanism of action, genistein is a well-known non-selective tyrosine kinase inhibitor, and there are numerous reports showing different genistein-induced cellular effects mediated by this inhibition of tyrosine kinases. There are also descriptions of other non-genomic effects of this compound related to its capability to inhibit Ca\(^{2+}\) pathways directly or through hyperpolarization induced by the opening of K\(^+\) channels, like some estrogen compounds do (i.e. 17b-estradiol) (5). Particularly, genistein has shown relaxing effects mediated by a direct block of Ca\(^{2+}\) channels in rabbit basilar artery (25).

Our work is focused on the effects of genistein on a fetal vessel, such as the human umbilical artery (HUA), with special attention on the mechanisms involved in the regulation of intracellular Ca\(^{2+}\) concentration and force development. Using microfluorimetry, isometrical force measurements and patch-clamp techniques, we present data showing that genistein, acting on different cell structures involved in the handling of intracellular Ca\(^{2+}\), is able to induce a decrease in intracellular Ca\(^{2+}\) concentration and consequently produce a relaxation of this vessel. Since the isoflavones are able to cross the placental barrier (8, 22), these findings may contribute to the understanding of the...