Anti-angiogenic effect of therapeutic concentrations of digitoxin

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Cardiac glycosides (CGs), digoxin, digitoxin and ouabain, are Na+/K+-ATPase inhibitors that have been used to treat congestive heart failure and cardiac arrhythmias. Therapeutic effects of this class of drugs have been related to Na+/K+-ATPase inhibition, that leads to an increase of intracellular Na+, which in turn induces higher levels of intracellular Ca2+. Besides this well-known mechanism, a large number of studies showed that cardiac glycosides affect several cellular processes (including cell survival or death, differentiation, proliferation and migration) with mechanisms not related to pump inhibition but associated to the activation of signalling proteins (such as Src, ERK, PI3K), indicating that Na+/K+-ATPase can act as a signal transducer (Schoner and Sheiner-Bobis, 2007). Interestingly, low concentrations of CGs, not leading to calcium overload, have a selective cytotoxic and antiproliferative effect against cancer cells, suggesting their use as anticancer drugs (Newman et al., 2008; Trenti et al., 2014). Accordingly, epidemiological studies showed that patients on digitalis therapy are more protected from cancer (Menger et al., 2013).

Angiogenesis, the process where blood vessels arise from pre-existing ones, plays an important role in tumour growth and progression, providing nutrients and oxygen to the growing mass and allowing the spread of cancer cells through the circulation. The effect of clinically used CGs on angiogenesis has not yet been explored. We previously demonstrated that in human umbilical vein endothelial cells (HUVECs) ouabain activates signalling pathways that promote cell survival such as ERK and Akt (Trevisi et al., 2004), suggesting a potential pro-angiogenic activity. On the other hand, bufadienolides (bufalin and arenobufagin), another group of CGs used in traditional Chinese medicine to treat cancer, have shown a direct anti-angiogenic action towards endothelial cells (Lee et al., 1997; Li et al., 2012). Based on these data, we studied the effect of digoxin, digitoxin and ouabain on angiogenesis using both in vitro and in vivo models. We also explored the signalling proteins involved, in particular those activated by the Na+/K+-ATPase signalosome and those linked to angiogenesis, such as focal adhesion kinase (FAK).

Digitoxin and ouabain (1-100 nM) inhibited HUVEC migration in a concentration-dependent manner without affecting cell viability, while digoxin induced apoptosis at the same concentrations. Digitoxin (1-25 nM) antagonized growth factor-induced migration (stimulated either by VEGF, bFGF, and EGF) and capillary-like tube formation. The anti-angiogenic effect of digitoxin was confirmed by in vivo studies, using the Matrigel sponge model enriched with IGROV-1 ovarian cancer cells. Digitoxin (10 nM) induced Src, Akt and ERK phosphorylation, suggesting Na+/K+-ATPase signalosome activation. Furthermore, digitoxin did not affect FAK autophosphorylation at Tyr397, but caused a significant reduction of growth factor-induced phosphorylation of FAK at Tyr576/Tyr577, thus preventing FAK maximal catalytic activity, which is congruous with the observed inhibition of cell migration and tubularization.

Our results show that digitoxin at concentrations within its plasma therapeutic range, inhibits angiogenesis and FAK activation by diverse pro-angiogenic stimuli. These novel findings suggest a potential repositioning of digitoxin as a broad-spectrum antiangiogenic drug for diseases where pathological angiogenesis is involved.

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