

Effects of digitoxin on cell migration in the ovarian cancer microenvironment

1)Trenti A. 2)Boscaro C. 3)Tedesco S. 4)Cignarella A. 5)Trevisi L. 6)Bolego C.

University of Padova

Clinical and experimental evidence supports a role for cardiac glycosides (CGs) such as digoxin, digitoxin and ouabain as potential novel anticancer drugs. In particular, it is widely accepted that CGs display selective cytotoxic and anti-proliferative activity against cancer cells through mechanisms unrelated to sodium pump inhibition (1). Accordingly, we demonstrated that ouabain induces autophagic cell death in lung cancer cells (2). It has also been shown that CGs prevent cancer cell migration, and we recently observed that digitoxin at clinically relevant concentrations switches off angiogenesis hampering growth factor-induced FAK activation and endothelial cell (EC) migration and tubularization (unpublished data). However, there are no studies reporting the effect of CGs on inflammatory tumor microenvironment (TME), which plays a central role in tumor progression and invasiveness (3,4,5).

Ovarian cancer is a highly invasive tumor characterized by an unique TME enabling specific metastatic routes. Indeed, ovarian TME comprises both the intra-tumor and the surrounding tumor microenvironment, namely peritoneal fluid, which is responsible for the generation of ascites (4).

Tumor-associated macrophages (TAMs), mostly deriving from peripheral blood monocytes, are a key component of TME and represent the most abundant immune population in both human ovarian cancer and ascites (6). TAM activation is skewed by factors in the TME to adopt a spectrum of phenotypes that represent mixed forms of alternatively-activated (M2) and pro-inflammatory (M1) macrophages (5). Ascites from patients with ovarian carcinoma contain M2 macrophages, and their accumulation correlates with ovarian cancer progression (6). Indeed, TAMs supply the microenvironment with chemoattractant cytokines and growth factors, which in turn support various aspects of cancer growth and progression, including tumor cell invasion, angiogenesis and metastasis (5,7). Accordingly, the presence of TAM in several tumors including ovarian cancer correlates with poor prognosis (6,8).

We hypothesized that digitoxin treatment would hinder cancer progression by affecting a) specific pathways involved in motility and/or activation of different cell types shaping TME, and b) cancer cell invasiveness in response to TME. To test our hypothesis, we used conditioned media (CM) from polarized macrophages, and apoptotic or non-apoptotic ovarian cancer cells (SKOV3) as chemoattractants for endothelial cells, monocytes and cancer cells.

Human macrophages were obtained and polarized to M1 (LPS/IFN γ) or M2 (IL4) phenotypes as previously described (9). In order to obtain apoptotic cells, SKOV3 were challenged with 0.5 μ M staurosporin for 3 hours. Apoptosis was evaluated by a caspase 3/7 activation assay.

We demonstrated that CM from M1 and M2 polarized macrophages, which mimic inflammatory TME, increased both HUVEC migration (M1 more than M2) and tubularization (M1 similar to M2)

with respect to 1% FCS as control. Treatment of HUVECs with digitoxin (10-25 nM) counteracted these effects. Digitoxin did not significantly affect the expression of M1 (CD80/CCR2) and M2 (CD206/CD163/CX3CR1) activation markers as assessed by flow cytometry; accordingly, HUVEC migration in response to CM from digitoxin-treated macrophages was unchanged. These data point to a direct effect of digitoxin on HUVEC signaling rather than the modulation of the cytokine profile released from activated macrophages. At variance with what observed for HUVECs, digitoxin did not prevent monocyte migration induced by either specific stimuli (10 ng/ml MCP1 and VEGF; 1 μ M LPS) or SKOV3 CM. Finally, digitoxin significantly impaired SKOV3 migration towards M1 or M2 macrophage CM.

Overall, digitoxin treatment at concentrations within its plasma therapeutic range 1) reduced cancer and endothelial cell migration in response to activated macrophage CM; and 2) did not affected neither monocyte migration nor macrophage polarization. These data further support the potential use of CGs as anticancer drugs.

References

1. Newman et al., (2008), *Mol Interv*, 8;36
2. Trenti et al., (2014), *Biochem Pharmacol*, 89;197
3. Murdoch et al., (2004), *Blood*, 104;2224
4. Worzfeld et al., (2017), *Front Oncol*, 7;1
5. Noy and Pollard (2014), *Immunity*, 41;49
6. Takaishi et al., (2010), *Cancer Sci*, 101;2128
7. Orre and Rogers (1999), *Gynec Oncol*, 73;47
8. De Palma and Lewis (2013), *Cancer Cell*, 23;277
9. Toniolo et al., (2015), *JCEM*, 100;E50