Ouabain induces autophagic cell death in A549 lung cancer cells through a mechanism dependent on JNK activation

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Autophagy is a cellular self-digestive process that ensures degradation of long-lived or damaged proteins and organelles; it is also induced under certain stress conditions, such as nutrient deprivation, to ensure energy balance [1]. Although autophagy was initially described as a protective mechanism, studies have demonstrated that persistent stress can promote autophagic (programmed type II) cell death. The regulation of autophagy in cancer cells is complex since it can enhance cell survival in response to stress, but it can also suppress the initiation of tumor growth [2]. Cardiac glycosides such as ouabain, digoxin and digitoxin, are a class of naturally derived compounds that bind and inhibit Na/K-ATPase and are clinically used for congestive heart failure and atrial arrhythmia. Recent findings have demonstrated that, besides its pumping function, Na/K-ATPase acts as a signal transducer, converting cardiac glycosides binding into signalling cascades involved in regulation of cell proliferation, differentiation and death [3]. Interestingly, in vitro studies have demonstrated that cardiac glycosides show a selective cytotoxicity against cancer cells. These studies are consistent with epidemiological data reporting protection from some types of cancer (i.e. breast. lymphoma/leukemia, prostate/urinary) in patients who are on cardiac glycoside treatment [4]. We studied the anticancer effect of ouabain in A549 lung cancer cells and the signalling pathways involved. Ouabain inhibited cell proliferation at concentrations below 50 nM as shown by MTT test, trypan blue exclusion assay and clonogenic assay. Treatment with 100 nM ouabain for 24 h induced cell death, confirmed by flow cytometric analysis of annexin V and propidium iodide binding. Cell death was caspase-independent and showed classical patterns of autophagy: conversion of LC3-I to LC3-II, increase of LC3 puncta and increase of autophagic flux. Moreover, cell death was completely blocked by class III phosphatidylinositol-3 kinase inhibitor 3-methyladenine. Western blotting analysis showed that ouabain caused the stimulation of AMP activated protein kinase (AMPK). Accordingly, ouabain induced the activation of the autophagy-initianting kinase Ulk1 (evidenced by increase of Ser 555 phosphorylation and the decrease of Ser 757 phosphorylation of Ulk1 protein). Furthermore, ouabain reduced Bcl-2 protein levels and did not change the expression of the autophagic protein Beclin 1. Early signalling events were ERK and JNK activation, however, their role on ouabain-induced cell death were opposite. In fact, MEK inhibitor U0126 did not antagonize cell death and, on the contrary, enhanced the cytotoxic effect of the glycoside indicating a pro-survival role of ERK. On the other hand, inhibition of JNK with SP600125 was able to prevent conversion of LC3-I to LC3-II, Bcl-2 decrease and cell death. We hypothesize that JNK activation by ouabain leads to decrease of Bcl-2 levels and disruption of the inhibitory interaction of Bcl-2 with Beclin 1 thus promoting autophagy.

[1] Klionsky DJ, Emr SD (2000). Science. 290, 1717-1721.
[2] Turcotte S, Giaccia AJ (2010) Curr Opin Cell Biol. 22, 246-251.
[3] Schoner W, Scheiner-Bobis G. (2007) Am J Physiol Cell Physiol. 293, C509-536.
[4] Newman RA et al (2008) Mol Interv. 8, 36-49.