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CARDIAC GLYCOSIDES AS A PROPERTY OF ANTICANCER AGENTS

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ABSTRACT

Long ago William Withering (1741-1799) recognised the usefulness of foxgloven (Digitalis) extracts, particularly for the treatment of congestive heart failure. The Cardiac glycosides are a diverse family of naturally derived compounds that bind to and inhibit Na+/K+- ATPase. Members of this family have been in clinical use for many years for the treatment of heart failure and atrial arrhythmia, and the mechanism of their positive inotropic effect is well characterized. Exciting recent findings have suggested additional signalling modes of action of Na+/K+- ATPase, implicating cardiac glycosides in the regulation of several important cellular processes and highlighting potential new therapeutic roles for these compounds in various cancer diseases. Synthesis of more active and selective derivative cardiac glycosides with antitumor activity, suggesting a new chapter in cancer therapy and side effects can be minimized.

Key words: Atrial arrhythmia, Therapeutic roles, Cardiac glycosides.

INTRODUCTION

Cardiac glycosides are organic compounds containing a glycoside (sugar) that act on the contractile force of the cardiac muscle.Cardiac glycosides contain a common molecular structure comprised of a steroid nucleus, an unsaturated lactone ring at the C-17 position, and one or more glycosidic residues at the C-3 position. Chemically cardiac glycosides are divided into two types . They are Cardenolides,Bufadienolides. Cardenolides have an unsaturated butyrolactone ring(5-membered unsaturated lactone). Common cardenolides include digoxin, digitoxin, digitoxigenin, lantoside C and ouabain .From a therapeutic point of view, the most important cardiac glycosides are digoxin and digitoxin as they are both used for the treatment of cardiac congestion and some types of cardiac arrhythmias, such as atrial fibrillation. Cardiac glycosides have been used in the treatment of cardiac disease for more than 200 years and were already known to the ancient Egyptians over 3000 years ago.Eg: Digoxin ,Digitoxin. Bufadienolides are C - 24 steroids, its characteristic structural feature is a doubly unsaturated six membered lactone ring having a 2- pyrone group attached at the C-17 β position of the perhydrophenanthrene nucleus . C-24 derivatives are collectively known as bufadienolides, including many in the form of bufadieno lide glycosides (bufadienolides that contain structural groups derived from sugars). These are a type of cardiac glycoside, bufadienolides and their glycosides are toxic specifically, they are heart-arresting [1-5].

THERAPEUTIC ACTIVITY OF CARDIAC GLYCOSIDES

Cardiac glycosides are most preferable drugs to heart problems and used even for the patients having hepatic insufficiency. Digitoxin is however, preffered in the cases having renal impairment. Ouabain is reserved for acute heart failure. Slow digitalization is always preferred over faster digitalization to avoid cardiotoxicity. Cardiac glycosides used in the treatment of congestive heart failure .Digitalis is a drug of choice for low output heart failure due to HT,IHD or arrhythmias. It provides relief from dyspnoea and cyanosis by subsiding pulmonary congestion. It increases urinary output and reduces oedematous fluid by increasing renel perfusion. These glycosides used in treatment of supraventricular tachycardia and also used for atrial flutter and atrial fibrillation .These are effectively treated with digitalis as it decreases conduction velocity and increases ERP of AV node. Digitalis is still the most preferred drug for patients

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having dilated heart and low ejection fraction as it is helpful in restoring cardiac compensation. It is usually prescribed when the patient condition is not controlled by diuretic or ACE inhibitor. Most recent studies concluded that other than the activity on cardiovascular system this cardiac glycosides is also used as anticancer agents [6,7].

The X-ray crystal structure of Na+/K+-ATPase (at 3.5 Å resolution) has been recently resolved. It is an oligomer composed of at least two polypeptides: the α-subunit and the β-subunit. The α-subunit is the catalytic moiety of the enzyme. Homologous to single-subunit P-type ATPases, it bears the binding sites for Na^+, K^+, mg^{2+}, ATP and the highly conserved cardiac glycoside binding site. The binding site is formed by the extracellular loops of the m1/m2, m3/m4 and m5/m6 moieties, as recently revealed by elegant functional studies. Several additional regulatory sites are also found on the α-subunit, including phosphorylation sites for numerous signal transducing kinases (such as phosphoinositide 3-kinase (PI3K), protein kinase C (PKC) and PKA), caveolins and ankyrins. These sites are important for the formation of the Na+/K+-ATPase signalosome. The regulatory β-subunit is a singlespan glycoprotein with a chaperone-like activity that is unique to the K+-counter-transporting P-type ATPases. It is mainly important for the recruitment of the α-subunit to the plasma membrane and for the occlusion of potassium ions34. Finally, the FXYD proteins are single-span, type I transmembrane proteins, which are often associated with the α β-complex and seem to act as modulators of the kinetic properties of the pump. Notably, both the β-subunit and the FXYD subunit are found to affect the binding affinity of cardiac steroids to Na+/K+-ATPase. It is postulated that the tissue-specific expression of these subunits might account for the differential physiological responses of tissues to the effects of cardiac glycosides. In addition to pumping ions, it is now established that Na+/K+-ATPase acts as a scaffold for the assembly of a multiple-protein signalling domain that transmits signals to various intracellular compartments. Several members of this complex have now been identified, including SRC kinase, epidermal growth-factor receptor (EGFR), inositol 1,4,5-triphosphate (IP3) receptor and caveolins. These are all engaged in the formation of this signaling domain, which is localized in the coated pits of the plasma membrane. Conformational changes on binding of cardiac glycosides trigger a downstream protein interplay ultimately results in the activation of intracellular signal transduction cascades. Interestingly, the signal transduction activity of this enzyme occurs through properties that are independent of its function as an ion pump. Indeed, doses of cardiac steroids — at concentrations that result in only subtle changes to the pumping activity of Na+/K+-ATPase — activate downstream signal transduction cascades and regulate many cellular processes including cell growth, cell motility and apoptosis. Two of the most established signaling avenues are described below [8,9].

Signalling through alterations in intracellular calcium oscillations

In 2001, a new signalling mechanism forcardiac glycosides was revealed by the exciting findingfrom Aizman and colleagues that ouabain at concentrationsthat confer only partial or no inhibition of Na+/K+-ATPase can trigger intracellular calcium oscillationsin renal proximal tubule cells52. more recently, similaroscillations were reported in human endothelial cells53and in CoS-7 cells54.It is now established that the binding of nano molarconcentrations of ouabain to Na+/K+-ATPase triggersan allosteric conformational change at the N-terminaltail of the catalytic α-subunit, which activates the neighbouringSRC protein. In parallel, in a way that is not yet fully defined, phospholipase C (PlC) and IP3 are also recruited, resulting in the formation of a functional microdomain that brings the cytosolic part of thesodium pump in direct contact with the IP3 receptor of the endoplasmic reticulum. At this point, single or repeated transient increases in levels of intracellular calcium are produced. Calcium oscillations are a universal mode of signaling that mediate a diverse range of cellular functions such as cell proliferation, differentiation and apoptosis. The ultimate response of the cell is dependent on the periodicity of the calcium oscillations; depending on the stimulus they can vary from seconds to hours. It is established that low concentrations of ouabain trigger low-frequency calcium oscillations (~4–6 min). In this range, the calcium-dependent transcription factor nuclear factor-κB (NF-κB) is activated and mediates transcription of several anti-apoptotic and proliferation inducing genes. Indeed, ouabain (0.1–10 nm) was reported to induce the proliferation of and protected kidney cells from serum deprivation-induced apoptosis in an NF-κB-dependent manner. Abnormal calcium homeostasis is linked to the pathogenesis of many diseases, and a plethora of therapeutic approaches aim to re-establish normal calcium homeostasis. G-protein-coupled receptors (GPCRs) are common drug targets owing to their ability to activate intracellular calcium release through the activation of IP3 receptors. The new findings on the signalling properties of Na+/K+-ATPase qualify this molecule as an alternative mediator of IP3-receptor-mediated calcium release and a potential new therapeutic target for calcium-related pathologies [10, 11].

Signalling through Ras activation.

Na+/K+-ATPase can also relay signals through activation of other multiple protein–protein interactions. The initial event, following binding of cardiac glycosides, is the release of the cytoplasmic tyrosine kinase SRC from the complex signalosome45. SRC kinase is activated upon phosphorylation at Tyr418 and, in turn, activates the proximal EGFR. Activated EGFR sequentially recruits the adaptor proteins SHC, growth factor receptor-bound protein 2 (GRB2) and SoS until eventually the signal activates the Ras–RAF–MAPK (mitogen-activated protein kinase) cascade3,58. Activation of Ras stimulates several downstream signalling cascades. In cardiac myocytes, ouabain induced activation of Ras triggers the opening of the ATP-sensitive mitochondrial potassium channels, resulting in a concomitant production of mitochondrial

reactive oxygen species (RoS). RoS in turn activate NF-κB, which stimulates the transcription of several cell-growth-related and differentiation genes, in parallel with the calcium-induced NF-κB activation. RoS production is also the result of a third described pathway overall, it is now clear that the ultimate response to cardiac glycoside treatment is dependent on the tissue, exposure time and dose [12,13].

EVIDENCE THAT SUPPORT CARDIAC GLYCOSIDES AS ANTICANCER ACTIVITY

Marije slingerland *et al* proposed that the decrease in intracellular K^+ and increase in intracellular Na^+ and Ca^{2+} following inhibition of the Na^+/K^+-ATPase may also induce apoptosis. Inhibition of the Na^+/K^+-ATPase by digitoxin and subsequent increase in intracellular Ca^{2+} led to the induction of apoptosis of prostate cancer cells. Besides inducing apoptosis by intracellular decrease of K^+ and of Na^+ and intracellular Ca^{2+}, cytotoxic mechanisms of action include intracellular acidification; inhibition of IL-8 production and the TNF-α/NF-κB pathway; inhibition of DNA topoisomerase II and activationof the Src kinase pathway.[1,2,3]

Shah VO *et al* carried out anticancer activities on some cardiac glycosides such as digitoxin and ouabain.They investigated the anticancer activity of cardiac glycosides. It has been proposed that the anti-cancer properties of cardiac glycosides involve selective inhibition of glycolysis. There is considerable interest in targeting cancer cell metabolism, including glycolysis, as an approach to development of new cancer drugs. Cardiac glycosides were also identified in a recent study that was designed to identify new inhibitors of the activation of NF-kB. In this study, 2800 clinically approved and bioactive compounds were screened in an NF-κB reporter assay. This screen identified digitoxin and ouabain.This study has identified a broad anti-inflammatory activity of cardiac glycosides in protecting whole blood against LPS-induced production of pro-inflammatory cytokines TNF(IL-1 and IL-6). This appears primarily to involve the NF-kB signaling pathway [4-6].

Afaq *et al.*have suggested that oleandrin might serve as an effective agentfor the prevention or treatment of skin cancer . Their researchinvestigated the topical application of oleandrin to CD-1 mice tocounteract the effects of TPA (12-*0*-tetradecanoylphorbol-13-acetate),a widely used skin tumor promoter. The topical applicationof TPA to mouse skin or its treatment in certain epidermal cells isknown to result in several biochemical alterations, changes in cellularfunctions, and histological changes leading to dermal tumor promotion.Theyclearly show that application of oleandrin to skin prior to TPA administration affords significant inhibition of TPA-induced skin edema, hyperplasia, epidermal ornithine decarboxylase (ODC) activity, and protein expression of ODC and cyclooxygenase-2 (COX-2), classical markers of inflammation and tumor promotion. They show that topical application of oleandrin prior to TPA inhibits activation of PI3K and phosphorylation of Akt, activation of NF-κB, and degradation and phosphorylation of the inhibitor of NF-κB α protein . They are recommend the use of chemopreventive agents (i.e., oleandrin) in formulations such as emollients or patches for the prevention or treatment of skin cancer [7-10].

Sreenivasan *et al*, shown that oleandrin produced an increase in expression of Fas and Tumor Necrosis Factor Receptor 1 (TNFR1), resulting in potentiation of apoptosis in tumor cells but not in normal primary cells, such as peripheral blood mononuclear cells or neutrophils . Fas–Fas ligand and TNF–TNFR1 death pathways are important mediators of apoptosis . Also shown that oleandrin, bufalin, digoxin, and digitoxin initiate apoptosis induced by Apo2L/TNF- related apoptosis-inducing ligand (TRAIL) in non-smallcell lung cancer cells by increasing the expression of death receptors . Because Apo2L/TRAIL induces apoptosis in tumor cells with little if any toxicity to normal cells. The selective cardenolide activation of death receptors may very well contribute to the observation that compounds such as oleandrin are relatively selective in their cytotoxic activity. Oleandrin elicits caspase associated apoptosis in human prostate carcinoma cells . Interestingly, however, treatment of human PANC-1 pancreatic cancer cells produces clear hallmarks of autophagy, including formation of autophagosome bodies with damaged mitochondria and expression of light chain-1 protein, an early indicator of autophagosome formation [11-13].

Li wang *et al* investigated into the anticancer activity of cardiac glycosides. They demonstrated that 24-h treatment with HCS led to dose-dependent cytostatic or cytotoxic responses, with an IC50 of approximately 20 mg/ml for H460 , A549, and H1299 human lung cancer cell lines, suggesting that this effect of HCS appeared to be independent of the p53 status. However, a 24-hn pretreatment with HCS strongly enhanced the radiosensitivity of H460 and A549 cells, with enhancement factors of 1.50 and 1.44, respectively, at 0.1 survival fraction, with a dose range of 2-6 Gy γ-radiation. This radiosensitizing effect was stronger than the reported enhancement values of other cardiac glycosides such as ouabain and oleandrin . HCS did not affect the radiosensitivity of H1299 cells, suggesting a role for p53 in mediating cellular response to HCS-induced radiation sensitivity.Oleandrin and ouabain induces cytotoxicity under radiation [14, 15].

Steffen frese *et al* investigated that non–small cell lung cancer(NSCLC) cells can be sensitized to Apo2L/TRAIL-induced apoptosis by combined treatment with different cardiac glycosides. Induction of the transcription factors c-jun and c-fos and an activation of activator protein-1 (AP-1) after treatment with the glycoside ouabain.They reported an inhibition of the transcription factors AP-1, nuclear factor-nB (NF-nB), and the MAPK c-Jun by the glycoside oleandrin . They show that in NSCLC cell lines cardiac glycosides up-regulate DR4 and DR5, which is responsible for sensitization to Apo2L/TRAIL-mediated apoptosis. Up-regulation of DR4 and DR5 by cardiac glycosides was initiated on transcriptional level; however, the participating transcription factors have not been identified. They

suggest that MAPKs and their respective transduction pathways might be not crucially involved in glycoside-mediated sensitization to Apo2L/TRAIL-induced apoptosis [16, 17].

Chen *et al* investigated the anticancer activity of cardiac glycoside such as bufadienolide bufalin as a potential agent for the treatment of pancreatic cancer in combination with the standard anticancer drug gemcitabine. They found that bufalin inhibited the growth on three pancreatic cancer cell lines (Bxpc-3, Mia PaCa-2, and Panc-1) and it synergistically increased gemcitabine-induced cancer cell growth inhibition and apoptosis.The combination of bufalin with gemcitabine was also found to significantly reduce tumor growth in mice bearing human Mia Paca-2 pancreatic cancer cells [18].

Calderon montano et al investigated into the anticancer activity of cardiac glycosides such as digitoxin, digoxin and ouabain were more cytotoxic on A549 lung cancer cells than on MRC5 non-malignant lung fibroblasts . These are particularly relevant forbdigitoxin, as this drug was cytotoxic on the cancer cell line at concentrations below those commonly observed in the plasma of cardiac patients treated with this drug (20-33 nM). cell lines was affected by several concentrations of each cardiac glycoside and of the anticancer drug cisplatin. Cardiac glycosides are known inhibitors of the Na+/K+ -ATPase pump glycolysis is coupled to sodium and potassium transport processes, and that some cardiac glycosides (e.g., ouabain) can inhibit glycolysis in a variety of non-malignant cells [19].

Joseph A Langerhan *et al* investigated cardiac glycosides and their anticancer activity . Cardiac glycosides shows apoptosis in cancer cells and also potentially block tumorigenesis and inflammation . Yet digitoxin mediated inhibition of the NF-kB signaling pathway in cystic fibrosis lung epithelial cells has been demonstrated to be mechanistically distinct from Na^+/K^+-ATPase inhibition. With respect to other implicated cellular players, the nonlethal cardenolide concentrations that inhibit breast cancer cell proliferation also activate Src kinase, stimulate the interaction between Na^+/K^+-ATPase, the activated Src kinase, and epidermal growth factor (EGFR), and lead to the activation of extracellular signal-regulated kinases 1 and 2 (ERK1_2) and subsequent cell cycle arrest caused by increased levels of p21. Cardiac glycosides have also been demonstrated to initiate apoptosis via the classical caspase-dependent pathways in malignant T lymphoblasts and prostate cancer cells [19].

Cao J *et al* investigated into the anticancer activity of cardiac glycosides. The reduction of HIF-1α by Ouabain occurs at thetranscriptional level, they analyzed HIF-1α mRNA levels in Ouabain treatedU2OS cells. Semi-quantitative PCR indicated thatHIF-1α mRNA levels were not significantly changed after Ouabain treatment.Based on this observation, we next investigated the effect of Ouabain onHIF-1α post transcriptional regulation. To assess the effect of Ouabain onHIF-1α degradation, U2OS cells were treated with the ribosomal inhibitor,cycloheximide (CHX), under hypoxic conditions in the presence of Ouabain. The degradation rates of HIF-1α were similar in both theCHX- treated and untreated cells, which clearly indicates that Ouabain inhibits the hypothesis that Ouabain-inhibited HIF-1α accumulation is due to reducedHIF-1α protein synthesis. U2OS cells were pretreated with the proteasomeinhibitor, MG132, followed by Ouabain treatment and hypoxia induction.Although the expression of HIF-1α was somewhat increased in Ouabain plusMG132 treated cells, MG132 did not effectively reverse the decreased HIF-1αprotein level caused by Ouabain treatment [20].

Fig 1. Cardiac glycosides as anticancer activity mechanism of action

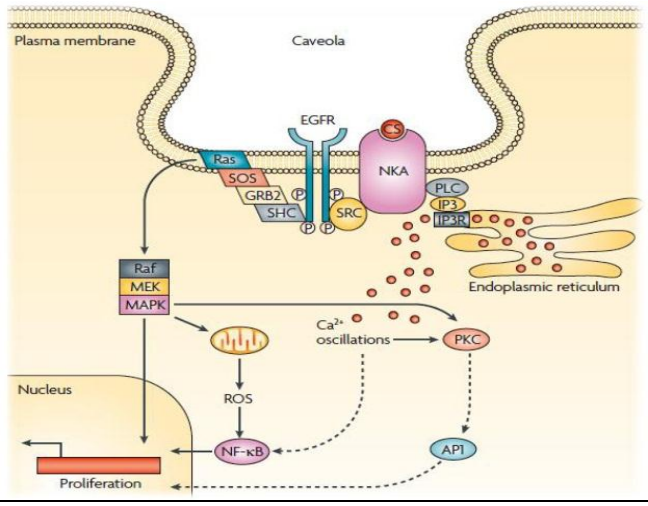

Fig 2. Signalling through Ras activation

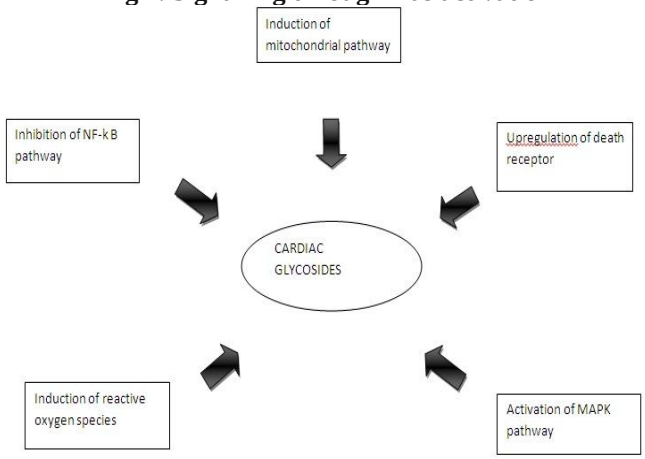

Table 1. Anti proliferative mechanism of different cardiac glycosides

Cardiac glycoside	Mechanism of action
Digitoxin	Induction of cell cycle arrest in G_2/M phase through the regulation of cyclin B_1,cdc2 and survivin. Increase in the intracellular Ca^{2+} concentration.
	Increase in the intracellular Ca^{2+} concentration

Digoxin	Inhibition of DNA topoisomerases I and II and an increase in the intracellular Ca^{2+} concentration. Induction of cell cycle arrest through the up regulation of HIF-1α.
Ouabain	Depletion of Na^{+}/K^{+}-ATPase and up regulation of p^{21} . Increase in the intracellular Ca^{2+} concentration . Inhibition of DNA topoisomerases I and II and an increase in the intracellular Ca^{2+} concentration
Oleandin	Attenuation of NF-Kb ,JNK and AP-1 (nuclear transcription factors) activation
Bufalin	Induction of cell cycle arrest in G_2/M phase through the up regulation of p^{21},WAF 1 and p^{53} and the down regulation of cyclin D. Inhibition of DNA topoisomerases I and II
Proscillaridin	Inhibition of DNA topoisomerases I and II and Increase in the intracellular Ca^{2+} concentration

CONCLUSION

Cardiac glycosides are involved in complex cell signal transduction mechanisms that may have important consequences in their application to the prevention and/or treatment of malignant diseases. The development of synthetic, semisynthetic, or naturally occurring cardiac glycosides, with assessment of their toxicity and structure activity relationships, might expand the possibilities of finding a cardiac glycoside with a wider therapeutic index. Additionally, because chemotherapy has had limited benefits in most advanced malignancies, cardiac glycosides could also be investigated for possible adjuvant therapy. These compounds have been reported to be therapeutically beneficial for the treatment of various tumor types because of their antiproliferative effects, ability to induce apoptosis, and ability to sensitize cells to chemo/radiotherapy-induced cell death. cardiac glycosides can induce apoptosis and inhibit the growth of cancer cell lines at concentrations close to those found in the plasma of patients with cardiac conditions.Furthermore, on the basis of the increased susceptibility of cancer cells to cardiac glycosides, the potential use of cardiac glycosides as anticancer agents might be associated with fewer side effects than traditional cytotoxic therapies.

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Nil

CONFLICT OF INTEREST

No interest

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