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Research update

Cardiac glycosides: From molecular targets to immunogenic cell death

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ABSTRACT

Cardiac glycosides (CGs) are approved for the treatment of cardiovascular alterations and their known cellular target is the alpha subunit of the sodium (Na^+)/potassium (K^+)-ATPase (NKA). Pharmacologically, they represent a well-known generation of drugs for treating cardiovascular problems, thus allowing the investigation of potential dose-dependent side effects.

Interestingly, since the end of the 1960s, epidemiological studies have indicated that anti-cancer effects were associated with the regular use of these compounds. Since then, a large body of evidence has been accumulated on the in vitro and in vivo effects of CGs in various experimental models, thus confirming their selective action on cancer cell proliferation and viability.

CGs have the potential for targeted therapeutic applications. Many of the anti-cancer activities of these compounds have been linked to the inhibition of their primary target, the NKA. A number of studies have shown a correlation between the overexpression of specific alpha subunits in cancerous versus non-cancerous cells and cancer cell responsiveness. Other findings have provided evidence of the on-target nature of the ascribed anti-cancer effects. More recently, studies have indicated additional intracellular targets for these agents, whose modulation might be, at least in some instances, unrelated to NKA targeting. These include endosomal trafficking of both NKA and Src kinase, downregulation of pro-survival Mcl-1 and Bcl-xL pro-survival proteins, and immunogenic cell death induction, among others.

This research update summarizes the current knowledge about CGs as new, targeted anti-cancer agents, alone or in combination with other chemotherapeutic compounds.

Contents

Abbreviations: 53BP1, p53 binding protein 1; ACTH, adrenocorticotropic hormone; AIF, apoptosis-inducing factor; ATG5, autophagy-related 5; Bad, Bcl-2-associated death promoter; Bcl-2, B-cell lymphoma 2; Bcl-xL, B cell lymphoma extra-large; DSB, DNA double-strand break; CamK, Ca^{2+}-calmodulin kinase; CG, cardiac glycoside; CML, chronic myeloid leukemia; EDLC, endogenous digitalis-like compound; EGFR, epithelial growth factor receptor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; HCC, hepatocellular carcinoma; HER, human estrogen receptor; HSP, heat shock protein; HIF-1α, hypoxia-inducible factor-1α; IAP, inhibitor of apoptosis; ICD, immunogenic cell death; IRE, inositol-requiring enzyme; JNK, c-Jun N-terminal kinase; LAMP, lysosomal-associated membrane protein; LE, late endosome; LMP, lysosomal membrane permeabilization; MAP1LC3 or LC3, microtubule-associated protein 1 light chain 3; MDC1, mediator of damage checkpoint protein 1; MeCP2, methyl CpG binding protein; MEK1/2, mitogen-activated protein kinase kinase 1/2; MOMP, mitochondrial outer membrane permeabilization; NKA, sodium/potassium-ATPase; NSCLC, non-small cell lung cancer; NF-κB, nuclear factor κB; p62/SQSTM1, p62/sequestosome; PAINS, pan-assay interference compounds; PI3K, phosphoinositide 3-kinase; Plk1, polo-like kinase 1; PK, protein kinase; RNF8, ring finger protein 8; PP2A, protein phosphatase 2A; ROS, reactive oxygen species; SAR, structure–activity relationship; TKI, tyrosine kinase inhibitor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; UPR, unfolded protein response; XIAP, X-linked inhibitor of apoptosis; YAP, Yes-associated protein.

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1. Fundamental mode of action of cardiac glycosides

Considering the ability of many natural compounds with chemopreventive or therapeutic potential to target multiple cellular components, it is essential to assess the anti-cancer potential of compounds with a more limited range of molecular targets allowing targeted interaction with cellular components. Among the many natural chemical entities, cardiac glycosides (CGs) have been previously described as scaffolds with a more limited target range at low nanomolar concentrations to limit off-target effects, thus representing a potential, targeted anti-cancer treatment approach.

CGs are clinically used drugs for the treatment of cardiac diseases. Their mechanism of action is activated by the binding and inhibition of or interference with the sodium (Na^+)/potassium (K^+)-ATPase (NKA) transmembrane pump that controls Na^+, K^+, and Ca^{2+} ion flows in muscle contraction as well as in excitable cells [1]. NKA targeting as the initial trigger of the anti-cancer activity of CGs is described in Section 2. Chemically, CGs are characterized by a steroidal nucleus with 4 rings (A, B, C, and D). The sugar moiety on ring A in position 3 controls the pharmacodynamics of the compound. The nature of the lactone on the D ring in position 17 divides CGs in two subgroups: cardenolides present a butyrolactone, whereas bufadienolides are characterized by an α-pyrone. The sugar and lactone moieties are in the *cis* conformation relative to the steroid core [1]. Fig. 1A depicts the core CG scaffolds.

Cardenolides are not only endogenous or exogenous compounds that inhibit the NKA pump to increase Na^+ and intracellular Ca^{2+} ions while depleting cells of K^+; recent studies have shown that this pump can activate an elaborate signalosome, leading to the inhibition of proliferation and induction of cell death [2]. A body of evidence indicates a dynamic balance between two distinct pools of functional NKA, pumping versus non-pumping, that have differential sub-cellular localizations as well as preferential α- and β-subunit isoform compositions, with important modulatory outcomes [3–5].

The inhibition of the ion pump activity versus the activation of the cell death signalosome is dependent on the nature and concentration of the cardenolide used. At elevated doses and for extended periods of time, CGs profoundly affect the vital ionic gradients leading to cell death by apoptosis or necrosis. This effect is not directly dependent on other intracellular cancer-specific pathways but merely kills cells by disrupting ionic homeostasis. At lower concentrations, selected CGs activate a precise signaling pathway or signalosome involving the α-subunit of the NKA and acting via Src-EGFR-Ras-Raf-extracellular signal-regulated kinase (ERK), AKT/Protein kinase (PK)B, and phosphoinositide 3-kinase (PI3K) to inhibit proliferation and cell survival [6]. Moreover, other reports suggest that the relative expression of specific NKA subunits might actively influence the susceptibility to specific cardenolides. The modulation of diversified, even opposite, cellular responses has been shown by several CGs including ouabain (1) [7–9] and oleandrin (2) [10] (Fig. 1B and C illustrates all CGs mentioned in this research update, following their order in the text).

A new targeted anti-cancer therapeutic approach would therefore be to (i) select CGs with a particularly high affinity for the NKA α-subunit and (ii) match those CGs to cancer types overexpressing these subunits to ultimately activate cell death [11]. Moreover, better knowledge of the specific cell death regulators expressed by target cancer types would allow the design of additional "smart" co-treatments, thereby further strengthening the anti-cancer effects of CGs.

2. Anti-cancer activity of CGs

2.1. Overview

The aim of this research update is not to give an extensive overview of existing CGs but to provide insight into the specific anti-cancer activities of this family of compounds and related identified targets. Nature is a rich source of CGs such as digitoxin (3) and digoxin (4), two cardenolides extracted from the foxglove or *Digitalis purpurea*. Digitoxin was first used in the eighteenth century by the botanist William Withering, although the active

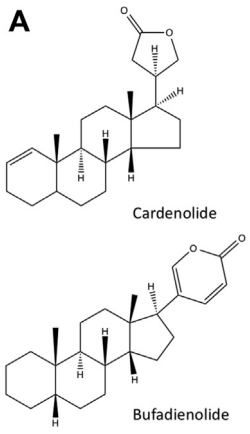

Fig. 1. (A) Main cardiac glycosides. Cardiac glycosides (CGs) present a steroidal nucleus with 4 rings (A, B, C and D). The sugar moiety on ring A, in position 3, controls the pharmacodynamics of the compound. The nature of the lactone on the D ring in position 17 divides CGs into two subgroups: cardenolides present a butyrolactone, and bufadienolides are characterized by an α-pyrone. The sugar and lactone moieties are in the *cis* conformation relative to the steroid core [1]. (B and C) The molecular structures of CGs as described in the text. Each molecule is numbered in order of its appearance in the text.