Cardiac Glycosides

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Cardiac glycosides (CG's) are naturally occurring biologically active small molecules, used to diagnose a diversity of heart diseases such as congestive heart failure and cardiac arrhythmia. The story of CG's started over 100 years ago when Sir William Withering reported the use of foxglove plant for treating "dropsy" associated with congestive heart failure and the foxglove plant (*Digitalis purpurea*) was still in the use for the extraction of Digoxin, a cardiac glycoside used to treat congestive heart failures (Johnson., 2012). The core structure of CGs comprises a steroid nucleus attached to a five-membered lactone ring (cardenolide) or six-membered lactone rings (bufadienolides) along with sugar moieties. Major plant-derived CGs were obtained from plant families of *Apocynaceae*, *Scrophulariaceae*, and *Asparagaceae* (*Thevitia neriifolia, Neerium oleander, Digitalis purpurea*, *Digitalis lanata*, *Urginea maritime*, and *Strophanthus kombe*). Structurally, all these contain a core steroid nucleus connected with sugar moiety at C_3 position and lactone moiety at C_{17} position (Figure 1.3). The pharmacological significance of all the CG's lies in the core steroid confirmation that contains A/B and C/D cis- portions and the properties such as pharmacokinetics and pharmacodynamics lie between the confined sugars molecules (Pongrakhananon., 2013). Apart from the plant sources, CG's were also isolated from several animal species such as bufadienolide was isolated from frogs, and also mammalian tissues contain a cardiac glycoside which is similar to endogenous digitalis (Melero et al., 2000). Quite a few studies have conveyed that the human body does contain a lot more CG's in different parts. For example, the plasma membrane contains Ouabain and Proscillaridin A and human urine contains digoxin and marinobufagenin whereas human lenses consist of 19-norbufalin (Schoner and Scheiner-Bobis., 2007). In the year 1785, William Withering was the first person to use a digitalis compound from *Digitalis purpurea* to treat congestive heart failures. Currently, Digoxin is used for treating congestive heart failures. The mechanism of action of Digoxin is that it can inhibit the sodium-potassium pump (Na^+/K^+-ATPase). Living organisms maintain more percentage of K^+ within the cell and less percentage of Na^+. However, the scenario at the outside of the cell is quite opposite to the intracellular conditions where a high percentage of Na^+ and less percentage of K^+ will be maintained. Hence, there is a concentration incline that exists between the outside and inside cellular environments, which will be maintained by sodium-potassium pump. The Na^+/K^+-ATPase is recognized as a transmembrane protein whose functions are to maintain ionic balance in the heart tissue. Na^+/K^+-ATPase utilizes ATP as the whole energy source, to exchange two K^+ ions inside the cell and pushes three Na+ ions outside to maintain intra cellular homeostasis. Also, Na^+/K^+-ATPase transports glucose and amino acids by keeping less concentration of Na^+ within the cell and helps in the maintenance of electrochemical incline. The increment of the Na^+ level inside the cell retort to CGs fortifies the ion exchange mechanism. This leads to the expansion of intracellular $Ca2^+$ percentage which therefore promotes organelle instances such as myocardial contractibility, and generates optimistic inotropic effects in the heart cell with CGs (Kaplan., 2002).

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1. Cardiac Glycosides as Immune Modulators

Retrospective clinical data demonstrated the use of CGs as possible anticancer drugs to treat cancer patients. However, several CGs are known to stimulate the immune response to several diseases including cancers at multiple stages. In accordance with this, CGs activate the immunogenic cell death (ICD) of various cancer cells [1]. Several CGs such as lanatoside C, digoxin, digitoxin, and ouabain particularly act as efficient inducers for ICD in vitro. Further, the mechanism of ICD was validated as the ecto-expression of calreticulin expression, HMGB1 release, and ATP secretion on human cancer cells and mouse systems. Subsequently, CGs were identified to stimulate the antitumor immune response in vivo by discovering the role against murine colon cancer cells treated in combination with digoxin and chemotherapy [2]. Furthermore, CGs intensified the anti-neoplastic effects by DNA damage in combination with mitomycin c and cisplatin in the immunocompetent mice model. Here the combination of digoxin with mitomycin c resulted in the more pronounced destruction of tumors by interferon γ-producing α/β CD4+ or CD8+ T lymphocytes compared to that in isolation [2]. Apart from this, CGs can also reduce the off-target effects where these CGs bind to the estrogen receptors (ER) because most

of the Digitalis compounds are phytoestrogens and tend to bind with ER with low affinity than that of estrogen. Additionally, CGs may also contribute to the antagonistic activity on ER, where digoxin plays a crucial role in the steroid receptors. This finding was further validated at a large scale chemical screen where digoxin particularly acted as a retinoic acid receptor inhibitor [3].

2. Role of Cardiac Glycosides on Signaling Pathways for Their Anticancer Mechanism

CGs have been used for decades to treat congestive heart failures and cardiac arrhythmia. Because of the mutations in the Na^+/k^+-ATPase, it has been linked with several diseases including diabetes and Alzheimer's disease and other bipolar diseases. Recent reports have suggested that the mutation in the sodium-potassium pump could lead to cancer cell proliferation. Several signaling pathways were involved in the process of these diseases such as epithelial-to-mesenchymal transition (EMT), p38 mitogen-activated protein kinase (MAPK) cascade, PI3K/Akt/mTOR (PAM) signaling, p21 Cip, and cholesterol homeostasis. Interestingly, all these pathways are known for cancer promotion and are linked to α and β subunits of Na^+/k^+-ATPase [4]. Out of these, the β subunit plays a crucial role in cancer suppression by tumorigenesis and cancer metastasis. On the other hand, methylation of ATP1B1 inhibits the activity of the β subunit and encourages cancer growth in renal cell carcinoma [5].

2.1. Effect on EMT

Epithelial-to-mesenchymal transition is a process, where the epithelial cell changes their phenotype to acquire mesenchymal properties to increase the migrative ability required for cancer progression and invasion all over the body [6]. The β subunit of sodium–potassium pump plays a major role in this process and regulates the integrity of cell polarization [5]. During this process, β-subunit dimerizes with nearby β subunit to increase the cell-to-cell adhesion by forming β-β subunit bridges. During this course of time, the expression of the β subunit decreases along with E-cadherin, which is responsible for the EMT and in the process of cell invasion. This decreased E-cadherin results in the increased activity of β-catenin, which ultimately promotes cancer metastasis [7]. Snail, Zinc finger protein SNAI1, plays a major role in the inhibition of E-cadherin and this snai1 is also responsible for the suppression of β-subunit of the Na^+/k^+-ATPase in cancer cells [8].

2.2. Effects on p38 MAPK/ERK Signaling Pathway

The MAPK pathway proteins are known to play a crucial role in cell survival, cell cycle, and cell death. Proteins such as c-Jun, JNK, MEK1/2, ERK1/2, and p38MAPK play a major role in these cellular events [9][10]. Reports have suggested that the inhibition of Na^+/k^+-ATPase regulates the MAPK pathway and leads to cell death and cell cycle arrest. Ye et al. has shown that the inhibition of Na^+/k^+-ATPase leads to the interaction between v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (Src) kinase and ultimately attenuates MAPK/ERK pathway [11]. In addition, the ATP/ADP ratio is responsible for the autophosphorylation of Src kinase [12]. ouabain activates p38MAPK by inhibiting the activity of Na^+/k^+-ATPase [13] and this activation promotes the transcription of p53 and NF-kB. Thus, activated NF-kB can trigger the Fas-mediated apoptosis of cancer cells.

2.3. Effects on Src Kinase Signaling

Src is a non-receptor protein tyrosine kinase that functions to promote cancer cell proliferation and invasion [14]. Inhibition of Na^+/k^+-ATPase with CGs leads to the activation of Src which in turn interrelates with EGFR to promote a signaling cascade of Ras to MAPK [15]. The increased activity of Src promotes cell survival and enhances ROS production. Furthermore, this activity of Src could be suppressed by the tyrosine residue on the α-subunit of the Na^+/k^+-ATPase [12]. This hindering effect promotes the activation of Src and helps in the proliferation [16].

2.4. Effects on PI3K/Akt/mTOR Pathway

PAM signaling is known for its diverse cellular functions such as cell survival, cell death, and autophagy [14]. This cascade consists of three important proteins phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/the mammalian target of rapamycin (mTOR). Among these, PI3K acts as the regulator protein that activates with the α-subunit of Na^+/k^+-ATPase. Binding of the proline-rich domain on the Na^+/k^+-ATPase with the regulatory subunit p85 promotes the activity of PI3K [17]. This modulation on Na^+/k^+-ATPase leads to the autophagic pathway activation and ultimately promotes cancer cell survival [18]. Activated PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol (3,4,5)-trisphosphate and is responsible for the phosphorylation/activation of Akt. Activated Akt can stimulate the activity of mTOR

and promotes the processes of cell survival, proliferation, and evasion of autophagy. The mTOR is typical serine/threonine kinase having two subdomains, namely mTOR complex 1 and mTOR complex 2 which is responsible for autophagic cell death and cellular homeostasis [19].

2.5. Effects on Autophagy

Autophagy is a process where non-essential or abnormal cells will be engulfed to maintain cell clearance through the process of programmed cell death [20]. It has been reported that CGs can either induce or inhibit autophagy by suppressing Na^+/k^+-ATPase in cancer cells. Apart from this, CGs (especially ouabain) can also inhibit autosis (a form of autophagy without apoptosis and necrotic features) in cancer cells [21]. Moreover, ouabain also sensitizes drug-resistant glioblastoma cells to necroptosis by upregulating ATP1A2 and ATP1A3 [22]. Additionally, ouabain also induces autophagy by JNK dependent decrease of Bcl-2 in A549 and H1975 cells [23]. Another study found that digoxin and ouabain induce autophagy by altering mTOR and ERK1/2 through pathway crosstalk mechanism in NSCLC cells [20].

3. Effects of Cardiac Glycosides on Gene Expression and Other Pathways

Apart from this, CGs can also trigger several other genes that are responsible for diverse cellular functions. For instance, digoxin and proscillardin A inhibit DNA topoisomerase I whereas bufalin and digoxin inhibit DNA topoisomerase II as well [24][25][26] to induce cell death in cancer cells. Moreover, some CGs inhibits TNF-α along with NF-kB and c-Myc to induce cell cycle arrest [27]. Furthermore, inhibition of Na^+/k^+-ATPase also leads to the suppression of several resistant proteins, which allows cancer cells to resist chemotherapeutic drugs [28]. peruvoside, strophanthidin, and lanatoside C show caspase-dependent apoptosis in human breast, lung, and liver cancer cells to induce mitochondrial cell death [29][30][31].

4.Conclusions and Future Perspectives

Cardiac glycosides have a long history in treating heart diseases, but recent studies on cancer cell lines and animal systems have demonstrated the anticancer and antiviral activities of several CGs. Depending on these findings CGs have been identified as potential anticancer and antiviral agents that should be assessed in clinical studies. Primarily CGs act as targets for Na^+/K^+ ATPase, which has a role in attenuating several signaling pathways linked to cell proliferation, apoptosis, and autophagy. One interesting fact is that CGs acts on the membrane targets due to their nature in adapting the membrane fluidity. However, there was no clear evidence yet stating the lipid permeability and direct contact with the targets by CGs which needs to be discovered. The key feature of any drug compound is that it should act in a target-specific manner and should be active at very less concentration that is usually nontoxic to other cells and has the chance of being used in the clinical studies. Based on this principle, CGs have shown the anticancer activity at nanomolar concentration against various cancer cells and antiviral activity on several viral diseases.

Within a narrow time window, several CGs have been developed for clinical trials (Anvirzel, UNBS1450, PBI05204, and digitoxin) for their anticancer activities against solid tumors and some of them were FDA approved for their activities against heart diseases (digitoxin, digoxin, and lanatoside C). Regarding their anticancer activities, several recommendations have been made for evaluating their anticancer potential. The anticancer and antiviral activities of CGs and their molecular targets have been discussed in an increasing number of publications in the past decade. Because of their primary target, CGs have been promising in their antiviral activities, as the strong activity of these compounds occurs at different stages of the virus species. The main finding on the antiviral activities has stated that these compounds inhibit viral mRNA or protein synthesis, signifying that these drugs target host developments that are important for the viruses to complete an efficacious replication.

Conversely, these mechanisms need to be explored to develop effective drugs with several important advantages such as less risk of resistance and a comprehensive range of action. Here in the current review we mainly focused on identifying the anticancer and antiviral activities of several CGs and we hope that this research may help the researchers to evaluate the anticancer and antiviral potential of CGs in preclinical studies for developing effective drugs.

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