Oleandrin: A cardiac glycosides with potent cytotoxicity

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ABSTRACT

Cardiac glycosides are used in the treatment of congestive heart failure and arrhythmia. Current trend shows use of some cardiac glycosides in the treatment of proliferative diseases, which includes cancer. Nerium oleander L. is an important Chinese folk medicine having well proven cardio protective and cytotoxic effect. Oleandrin (a toxic cardiac glycoside of N. oleander L.) inhibits the activity of nuclear factor kappa-light-chain-enhancer of activated B chain (NF-κB) in various cultured cell lines (U937, CaOV3, human epithelial cells and T cells) as well as it induces programmed cell death in PC3 cell line culture. The mechanism of action includes improved cellular export of fibroblast growth factor-2, induction of apoptosis through Fas gene expression in tumor cells, formation of superoxide radicals that cause tumor cell injury through mitochondrial disruption, inhibition of interleukin-8 that mediates tumorigenesis and induction of tumor cell autophagy. The present review focuses the applicability of oleandrin in cancer treatment and concerned future perspective in the area.

Key words: Cardiac glycosides, cytotoxicity, oleandrin

INTRODUCTION

Cardiac glycosides are used in the treatment of congestive heart failure (CHF) and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants and in some animals, such as the milkweed butterflies [Figure 1].[1] Their utility in CHF results from an increased cardiac output by increasing the force of contraction. By increasing intracellular calcium as described below, cardiac glycosides increase calcium induced calcium release and thus contraction.[2] Ouabain and digoxin are used as cardiac glycosides since ancient time. Digoxin from the foxglove plant is used clinically, whereas ouabain is used only experimentally due to its extremely high potency.[3] Basically, cardiac glycosides are of two types-cardenolides (C-23 steroids with methyl groups at C-10 and C-13 and a five member lact at C-17) and bufadienolide (term derives from the toad genus Bufo that contains bufadienolide glycosides, the suffix-adien-that refers to the two double bonds in the lactone ring and the ending-olide that denotes the lactone structure. Consequently, related structures with only one double bond are called bufadienolide).

Cardiac glycosides are composed of two structural features: The sugar (glycoside) and the non-sugar (aglycone-steroid) moieties. The R group at the 17-position defines the class of cardiac glycoside. Two classes have been observed in nature - the cardenolides and the bufadienolides [Figure 2]. The cardenolides have an unsaturated butyrolactone ring while the bufadienolides have a-pyrone ring.

The cardiac glycosides occur mainly in plants from which the names have been derived. Digitalis purpurea, Digitalis lanata, Strophanthus gratus and Strophanthus kombe are the major sources of the cardiac glycosides. The term “genin” at the end refers to only the aglycone portion (without the sugar). Thus, the word digitoxin refers to an agent consisting of digitoxigenin (aglycone) and three sugar moieties. The aglycone portion of cardiac glycosides is more important than the glycone portion [Table 1].

Mechanism of action of cardiac glycosides

Normally, Na⁺-K⁺ pumps in the cardiac myocytes pump the potassium ions inside and sodium ions out. Cardiac glycosides inhibit this pump by stabilizing it in the E₂-P transition state; so that sodium cannot be extruded and intracellular sodium concentration therefore increases. A 2nd membrane ion exchanger, i.e., Na⁺/Ca²⁺ exchanger,
is responsible for “pumping” calcium ions out of the cell and sodium ions in (3Na/Ca); raises intracellular sodium levels, which inhibit this pump; thus, calcium ions are not extruded and begins to build up inside the cell.\textsuperscript{[3]} Increased cytoplasmic calcium concentrations cause increased calcium uptake into the sarcoplasmic reticulum (SR) through the sarco/endoplasmic reticulum $\text{Ca}^{2+}$-ATPase transporter.

Raised calcium stores in the SR allow for greater calcium release on stimulation so that myocytes could achieve faster and more powerful contraction by cross-bridge cycling\textsuperscript{[Figure 3]}. The refractory period of the atroventricular node is increased and finally cardiac glycosides function to regulate heart rate. Binding of cardiac glycoside to Na-K-ATPase is slow, but after binding, intracellular calcium increases gradually.\textsuperscript{[4]} Thus, the action of cardiac glycosides is delayed. Raised extracellular K\textsuperscript{+} decreases binding of cardiac glycoside to Na-K-ATPase. Consequently, increased toxicity of these drugs is observed in the presence of hypokalemia. If calcium of SR stores becomes too high, some ions are released

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**Figure 1:** Plants and animal producing cardiac glycosides

**Figure 2:** Generic structure of cardiac glycosides
Table 1: Structure of some common cardiac glycosides

<table>
<thead>
<tr>
<th>Name</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digitoxigenin</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Digoxigenin</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Gitoxigenin</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Ouabagenin</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Strophanthidin</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>=O</td>
</tr>
<tr>
<td>Bufalin</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

spontaneously through SR receptors. After depolarization, this effect leads initially to bigeminy (regular ectopic beats following each ventricular contraction). If higher glycoside doses are given, rhythm is lost and ventricular tachycardia originates, followed by fibrillation.

Accumulating the in vitro and in vivo evidences highlighted the potential of anticancer properties of these compounds. Despite the fact that members of this family have advanced to clinical trial testing in cancer therapeutics, their cytotoxic mechanism is not yet elucidated [Tables 2a and b]. New findings within the past 5 years have revealed these compounds to be involved in complex cell-signal transduction mechanisms, resulting in selective control of human tumor, but not normal cellular proliferation. As such, they represent a promising form of cancer chemotherapy. New clinical studies of their anticancer potential as single or adjuvant treatments may provide insight into these potentially valuable therapeutic options.

Botanical description of Nerium oleander L. as source of oleandrin

A toxic cardiac glycoside isolated from oleander (N. oleander L.) is considered as important Chinese folk medicine [Figure 4]. N. oleander L is an evergreen shrub or small tree, family Apocynaceae. Toxicity is reported in all its part. N. oleander L is classified in the genus Nerium. It is most commonly known as oleander, from its superficial resemblance to the unrelated olive Olea. It is so widely cultivated that no precise region of origin has been identified, though Southwest Asia has been suggested. Oleander is one of the most poisonous of commonly grown garden plants.[28] Oleander grows to 2-6 m (6.6-20 feet) tall, with erect stems that splay outward as they mature; 1st year stems have a glaucous bloom while mature stems have a grayish bark.[29] The leaves are in pairs or whorls of three, thick and leathery, dark-green, narrow lanceolate, 5-21 cm (2.0-8.3 inches) long and 1-3.5 cm (0.39-1.4 inches) broad and with an entire margin. The flowers grow in clusters at the end of each branch; they are white, pink to red, 2.5-5 cm (0.98-2.0 inches) diameter, with a deeply 5-lobed fringed corolla round the central corolla tube. The fruit is a long narrow capsule 5-23 cm (2.0-9.1 inches) long, which splits open at maturity to release numerous downy seeds. Oleander grows well in warm subtropical regions, where it is extensively used as an ornamental plant in landscapes, in parks and along roadsides. N. oleander L is drought-tolerant and will tolerate occasional light frost down to −10°C (14°F). Its toxicity renders it deer-resistant. It is tolerant of poor soils and drought. Oleander can also be grown in cooler climates in greenhouses and conservatories or as indoor plants that can be kept outside in the summer. Oleander flowers are showy and fragrant and are grown for these reasons. Over 400 cultivars have been named, with several additional flower colors not found in wild plants having been selected, including red, purple, pink and orange; white and a variety of pinks are the most common.

Oleandrin contains a central steroid nucleus with an unsaturated lactone structure on C-17 and a dideoxy arabinose group on C-3. In addition, the steroid ring has a substitute of an acetyloxy group on C-16. Oleandrin is a derivative of oleandrin and this derivative is a more potent glycoside than ouabain.[30] N. oleander is classified in Table 3.

Oleandrin has structural similarity with other glycosides. Cardiac glycosides have more or less the same characteristics as oleandrin. Oleandrin is a deglycosylated metabolite of oleandrin. Physical properties of oleandrin are presented in Table 4.
Table 2a: Some important cytotoxic active cardiac glycosides

<table>
<thead>
<tr>
<th>Cardiac glycosides</th>
<th>Plant/animal species</th>
<th>Cytotoxic effect reported on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elaeodendrosides</td>
<td>Elaeodendron sp.</td>
<td>Human ovarian carcinoma (A2780)</td>
</tr>
<tr>
<td>Acovenosigenin A 3-O-α-lramnopyranoside,</td>
<td>Euonymus alata (Thunb.)</td>
<td>Human oral epidermoid (KB), promyelocytic lymphoma (HL-60), non-small-cell lung carcinoma (A549) and cervical carcinoma (Hela)</td>
</tr>
<tr>
<td>Euonymoside A</td>
<td>Sieb. (Celastraceae)</td>
<td>Human lung carcinoma (A549) and ovarian adenocarcinoma (SK-OV-3)</td>
</tr>
<tr>
<td>Maquirosida A</td>
<td>Euonymus sieboldianus</td>
<td>Human oral epidermoid carcinoma (KB)</td>
</tr>
<tr>
<td>Oleander, Oleandrin, Cardenolide N-1, cardenolide N-4, 3 β-O-(β-d-sarmentosyl)-16 β-acetoxy-14-hydroxy-5 β, 14 β-card-20-(22)-enolide, 16 β-acetoxy-3 β, 14 dihydroxy-5 β, 14 β-card-20-(enolide)</td>
<td>Nierembergia aristata D. Don (Solanaceae)</td>
<td>Human breast carcinoma (BC1), fibrosarcoma (HT), lung cancer (LU1), melanoma (Mel2), colon carcinoma (Col2), oral epidermoid (KB), drug resistant KB with and without vinblastine, epidermoid carcinoma (A-431), prostate carcinoma (LNcap), hormone dependent breast carcinoma (ZR-75-1) and glioma (U373)</td>
</tr>
<tr>
<td>Convallatoxin</td>
<td>Ornithogalum umbellatum L. (Hyacinthaceae)</td>
<td>Human oral epidermoid carcinoma (KB)</td>
</tr>
<tr>
<td>3′-O-β-d-glucopyranosylcalactin, 12-dehydroxyghalakinoside, 6′-dehydroxyghalakinoside, ghalakinoside, calactin</td>
<td>Pergularia tormentosa L. (Asclepiadaceae)</td>
<td>Kaposi’s sarcoma</td>
</tr>
<tr>
<td>Perilocin isomers</td>
<td>Periplaca graeca L. (Asclepiadaceae)</td>
<td>Human prostate carcinoma (PC-3)</td>
</tr>
<tr>
<td>Rhodexin A</td>
<td>Rhoea japonica (Thunb.) Roth. (Liliaceae)</td>
<td>Human leukemia (K562)</td>
</tr>
<tr>
<td>Apocannoside, cymarin</td>
<td>Apocynum cannabinum L. (Asclepiadaceae)</td>
<td>Human nasopharynx carcinoma (KB)</td>
</tr>
<tr>
<td>Calotropin, 16 α-acetoxycalotropin, 15 β-hydroxycalotropin, calactin, 15 β-hydroxycalactin, asclepin, 16 α-hydroxyasclepin, uscharadin</td>
<td>Asclepias curassavica (Asclepiadaceae)</td>
<td>Human lung carcinoma (A549), breast carcinomas (MCF-7 and MDA-MB-231) and hepatoma (HepG2)</td>
</tr>
</tbody>
</table>

Contd...
CHEMICAL CONSTITUENTS

Apart from cardiostimulatory action, oleander is diuretic also. Cardenolides gentiobiosyl oleandrin and odoroside also are present in Nerium. The seeds contain glucosides (oleandrine, odorosides, adigoside). The bark contains glucosides (rosaginoside, neroside, corteneroside). The roots of Nerium contain steroids. Its lymph contains minerals and α-tocopherol. There are also weakly active cardenolides (heterosides of uzarigenine) and inactive cardenolides (heteroside of adynergenine, of digitalose), triterpenoids, a resin, tannins, glucose, paraffin, ursolic acid, vitamin C are found in oleandrin. Some common structures are presented in Figure 5.

DOSAGE

Route of administration varies with type and location of disease.
- Parenteral: 0.3-0.7 ml intramuscular daily or every other day. Therapy continues until remission of disease. Doses up to 1.2 ml/m²/d have been used in pharmacokinetic study
- Oral: 0.3-0.7 ml 3 times a day after meals (maximum of 2 ml per dose reported in patent)
- Gargle: 5% mouthwash used daily.

In a recent study, oleandrin, in Anvirzel™ has exhibited anti-cancer properties, but its efficacy against human immunodeficiency virus is still under investigation. The principal active constituent of the botanical drug candidate PBI-05204, a supercritical CO₂ extract of N. oleander L., is the cardiac glycoside oleandrin and it exhibits potent anticancer activity as well as it is currently in phase I clinical trial as a treatment for patients with solid tumors.

Oleandrin and oleandrinogen are able to inhibit proliferation...
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<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae (angiosperms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Unranked)</td>
<td>Eudicots</td>
</tr>
<tr>
<td>(Unranked)</td>
<td>Asterids</td>
</tr>
<tr>
<td>Order:</td>
<td>Gentianales</td>
</tr>
<tr>
<td>Family:</td>
<td>Apocynaceae</td>
</tr>
<tr>
<td>Subfamily:</td>
<td>Apocynoideae</td>
</tr>
<tr>
<td>Tribe:</td>
<td>Wrightieae</td>
</tr>
<tr>
<td>Genus:</td>
<td>Nerium L.</td>
</tr>
<tr>
<td>Species:</td>
<td>N. oleander</td>
</tr>
</tbody>
</table>

Table 3: Classification of N. oleander

Table 4: Physical properties of oleandrin

<table>
<thead>
<tr>
<th>Properties</th>
<th>Detail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C32H48O9</td>
</tr>
<tr>
<td>Molar mass</td>
<td>576.72 g/mol</td>
</tr>
<tr>
<td>Appearance</td>
<td>Oleandrin forms colorless, odorless, acicular crystals that are very bitter</td>
</tr>
<tr>
<td>Melting point</td>
<td>250.0°C</td>
</tr>
</tbody>
</table>

of tumor cells and stimulate their apoptosis as a result of the high concentration of intracellular Ca++. In addition, it inhibits the excretion of fibroblast growth factor-2 (FGF-2) through membrane interaction and through inhibition of the Na, K-ATPase pump.\(^{36}\)

In PANC-1 cells (human pancreatic cancer cell line), cell death occurs not through apoptosis, but rather through autophagy and it has been noticed that oleandrin at low nanomolar concentrations potently inhibited cell proliferation associated with induction of a profound G(2)/M cell cycle arrest. Inhibition of cell cycle is not accompanied by any significant sub G1 accumulation of cells, suggesting a non-apoptotic mechanism of oleandrin.\(^{37}\)

In a recent study, the effect of oleandrin on the growth of human and mouse cancer cells in relation to Na, K-ATPase subunits were investigated. Oleandrin treatment resulted in selective inhibition of human cancer cell growth, but not rodent cell proliferation, which corresponded to the relative level of Na, K-ATPase alpha3 subunit protein expression. In a recent study, human pancreatic cancer cell lines has been observed for differentially expressing varying levels of alpha3 protein, but rodent cancer cells lacks discernable expression of this Na, K-ATPase isoform.\(^{38}\)

Oleandrin has shown to induce apoptosis in malignant cells through various in vitro tests on various cell lines, while human tumor cells are very sensitive to growth inhibition by oleandrin. Using human BRO and mouse B16 melanoma cell lines, several possible determinants of cell sensitivity to oleandrin and compared with ouabain were explored. This study included Na+, K+-ATPase activity and its isoforms as well as the cellular uptake of these cardiac glycosides. Oleandrin and ouabain induced apoptosis was detected in...
BRO cells while no evidence of cell death was observed in B16 cells even at concentrations 1000-fold higher than that used for BRO cells.\cite{35}

Oleandrin poisoning by eating oleander leaves can be lethal at low dosages. Cases of sheep lethality have been reported to only one leaf of oleander. The symptoms present in poisoned animals include bloody diarrhea and colic, the latter especially in horses. Because the leaf itself is quite bitter, only starving animals will be likely to eat the plant. The lethal dosage for animals is estimated to be about 0.5 mg/kg.

**PHARMACOKINETICS**

Oleandrin has lipophilic property and it can be easily absorbed in the gastrointestinal tract after oral dosing. Oleandrin is metabolized into oleandrigenin in mice. Although oleandrigenin is not formed in human plasma, it was found in the volunteers injected with oleandrin, suggesting the fact that it is formed in other human tissues. The clearance is slow. Pharmacokinetic studies of (\(3^\text{H}\)) oleandrin, a cardiac glycoside component of Anvirzel™, were conducted in mice after either an intravenous (i.v.) dose (40 \(\mu\)g/kg) or a p.o. dose (80 \(\mu\)g/kg). Oleandrin was rapidly absorbed after oral dosing (\(C_{\text{max}}\) at 20 min) although the elimination half-life was longer (2.3 ± 0.5 h) than that after i.v. dosing (0.4 ± 0.1 h). The AUC\(_{0-\infty}\) values obtained after i.v. and p.o. dosing were 24.6 ± 11.1 and 14.4 ± 4.3 (ng h/ml), respectively, resulting in an oral bioavailability of approximately 30%. After intra-venous administration of oleandrin in mice, the active metabolite is found to concentrate in the liver.\cite{39}

It is excreted mostly in feces, but also in urine. Because the main route of excretion is through biliary excretion into the feces, it is mainly the liver that is exposed to oleandrin. As excretion in urine is only a smaller route, the kidneys are less exposed. There is also accumulation in the cardiac tissue, which explains its potential for cardiac toxicity. In mouse studies, it also appeared that oleandrin rapidly accumulates in brain tissue as it can pass through the blood-brain barrier. The data suggest that other components within oleander extract may enhance transport of oleandrin across the blood-brain barrier.\cite{34}

**ANTI-CANCER THERAPY AND MECHANISM OF ACTION**

Oleandrin has potent anticancer activity and it is used for treatment of variety of cancers such as colon cancer,
CONCLUSION

Pharmacological activities of cardiac glycosides have increased significantly since the discovery of their effectiveness for treatment of CHF and also in proliferative disease. Development of clinically targeted, antiproliferative cardiac glycosides could be helped by systematic evaluations of several formulations and chemical variants in compound such as Oleandrin (a lipid soluble cardiac glycoside). Further development of synthetic, semi-synthetic 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. Oleandrin, inhibits the proliferation of various cancer cells. Human melanoma and leukemia cells are more sensitive to oleandrin than murine tumor cells, normal human epithelial cells, peripheral blood mononuclear cells and neutrophils etc. Therefore, the role of oleandrin as antiproliferative agent cannot be ignored in cancer therapy.

REFERENCES


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