Na,K-ATPase isoform-selective cardiac glycosides-a potential anti-cancer drug?

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Over the years, several reports have suggested that cardiac glycosides may have an anticancer utilization. In vitro and ex vivo experiments have revealed that some cardiac glycosides induce potent and selective anticancer effects, which may occur at concentrations commonly found in the plasma of patients treated with these drugs.

**Na+ /K+ -ATPase could be targeted to combat chemoresistant cancers.**

### In vitro antiproliferative and/or apoptotic effects of cardiac glycosides in cancer cells

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Compounds tested</th>
<th>Cancer cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Digitoxin, digoxin, proscillaridin A, ouabain, digoxigenin, gitoxin, gitoxigenin</td>
<td>MCF-7, MDA-MD-435</td>
</tr>
<tr>
<td>Prostate</td>
<td>Oleandrin, ouabain, digoxin, bufalin, cinobufagenin</td>
<td>PC-3, LNCaP, DU145</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Digoxin, oleandrin, digoxin, proscillaridin A, ouabain, digitonin</td>
<td>UACC-62, BRO</td>
</tr>
<tr>
<td>Lung</td>
<td>Digitoxin, digoxin, ouabain, UNBS1450, oleandrin</td>
<td>A549, NCI-H-358, Calu1, Skl1, NCI-H6, H69AR</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>Bufalin, oleandrin, digoxin, proscillaridin A, ouabain</td>
<td>HL60, U-937, CCRF-CEM, CEM-VM-1</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Digoxin, ouabain</td>
<td>SH-SY5Y, Neuro-2a</td>
</tr>
<tr>
<td>Renal</td>
<td>Digitoxin, digoxin, digotoxinagenin, proscillaridin A, ouabain</td>
<td>TK-10, ACHN</td>
</tr>
<tr>
<td>Myeloma</td>
<td>Digitoxin, digoxin, proscillaridin A, digotoxinagenin, ouabain, digitonin, lanatocide C</td>
<td>8226-S, 8226-LR5, 8226-DOX-40</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Oleandrin</td>
<td>PANC-1</td>
</tr>
</tbody>
</table>
**Na,K ATPase** is a vital protein in all mammalian cells.

- Na,K -ATPase is an oligomeric transmembrane protein, localized to the basolateral plasma membrane in most epithelial cells.
- Na,K-ATPase pumps Na+ and K+ against their physiological gradients.

### β Subunit
- Targets into the membrane, 3 isoforms: β1, β2, β3

### Extracellular space

**FXYD** accessory protein, regulator

### membrane

**α subunit**
- Catalytic moiety, 4 isoforms: 
  - a1, ubiquitous, all tissues
  - a2, skeletal muscle, heart, eyes
  - a3, neurons, brain
  - a4, sperms

**Digoxin** Specific inhibitor
Na,K pump is essential for many physiological processes

- Renal function, and regulation of hypertension.
- Cardiac contraction
- Regulation of intra ocular pressure, IOP.
And many more....

Expression of isoforms in different tissues.

All of the isoforms are expressed in a tissue and functional specific manner.
Cardiac glycosides (CG)

The cardiac glycosides are an important class of naturally occurring drugs whose actions include both beneficial and toxic effects on the heart.
Cardiac glycosides, naturally occurring in plants and animals.

Withering W (1785). “An account of the foxglove and some of its medical uses: with practical remarks on dropsy and other diseases.”
Cardiac glycosides have a long history of therapeutic application.

- Plants containing cardiac steroids have been used as poisons and heart drugs at least since 1500 B.C.
- The early understanding of their positive inotropic effects facilitated their use as effective drugs for the treatment of heart-related pathologies, yet their toxicity remains a serious problem.
- More recently, considerable in vitro, in vivo and epidemiological data support novel roles for CG’s such as inducing apoptosis and inhibit the growth of cancer cell lines.

Liang-Fei Ye, et al. 2013 Oncology letters
The decrease in intracellular K+ and increase in intracellular Na+ and Ca2+ following inhibition of the Na+/K+-ATPase may induce apoptosis.
Na,K-ATPase as a versatile signal transducer?

Na+/K+-ATPase may also act as a signal transducer. When intact cells are exposed to digitalis drugs (e.g., ouabain and digoxin) specific inhibitors of this enzyme various cell signaling pathways are activated leading to highly cell-specific down-stream consequences.
Breast cancer cells are not more sensitive to CG’s cytotoxicity than are normal cells.

Clifford RJ, and Kaplan JH 2013 PLOS ONE 8,
Purification and stabilization of isoforms of human Na,K-ATPase expressed in *Pichia pastoris*
Isoforms of human Na,K-ATPase expressed in *Pichia pastoris*

**Functional, stable, detergent-soluble αβFXYD complex**

- **Detergent, C12E8**
- **Phosphatidyl serine, SOPS**
- **Phosphatidyl choline, PC**
- **Cholesterol**

**Enables different combinations of isoforms**
Isoform selectivity is determined by the sugar component!

Digoxin, is partially $\alpha_2$-selective CG, while Ouabain shows very low selectivity.
## Isoform selectivity of Cardiac glycosides

<table>
<thead>
<tr>
<th>CG</th>
<th>Calculated Kd±SEM, nM</th>
<th>Ratio of Kd’ s, ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α1</td>
<td>α2</td>
</tr>
<tr>
<td>Ouabain</td>
<td>9.8 ± 0.3</td>
<td>21.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Digoxin</td>
<td>87 ± 6.0</td>
<td>25.6 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>P=0.001</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Digoxigenin</td>
<td>270 ± 21</td>
<td>332 ± 11</td>
</tr>
<tr>
<td>β-Methyl digoxin</td>
<td>129 ± 20</td>
<td>43 ± 7</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>38 ± 3</td>
<td>18.3 ± 4</td>
</tr>
<tr>
<td>Digitoxigenin</td>
<td>101 ± 13.4</td>
<td>125 ± 13.6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Bufaline</td>
<td>42.5 ± 6.5</td>
<td>45 ± 8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Marinobufagenin</td>
<td>2240 ± 13</td>
<td>2470 ± 45</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
The residues common to $\alpha_2$ and $\alpha_3$ and different in $\alpha_1$ are all located on the extracellular loops at the entrance to the Ouabain binding site, close to the sugar moiety.
In vitro growth inhibitory concentrations at 50% (IC50) in human cancer cells after three days of culture in the presence of the drug of interest.
2.

SF-268 (central nervous system)

- Digoxin
- DM
- Ouabain
- GBR
- D.EA
- Ol
A549 (non small cell lung carcinoma)

HFF (Human foreskin fibroblast)
*In vitro* growth inhibitory concentrations at 50% (IC$_{50}$) in human cancer cells after two days of culture in presence of GBR, the most effective CG.

Correlation of Ki for inhibition of human α1β1 by cardiac glycosides and the growth inhibition effects

IC50 of CG for cancer cell lines

Ki of CG's on purified Na,K-ATPase

GBR  Oleandrin  Hellebrin  Digoxin
Hellebrig.  Ouabain

ACNH  SF 268  MCF 7  MDA-MB-23  Sk-Mel-5  HCT 116  A549  TK 10  ovcar 3
Correlation of Ki for inhibition of human α1β1 by cardiac glycosides and the growth inhibition effects

Cell growth is linearly correlated to the affinity of individual CG to the Na,K-ATPases

4 repetitions with 14 different cancer cells.

1. Digoxin
2. Hellebrin
3. Ouabain
4. Oleandrin
5. Hellebrigenin
6. Gamabuf. Rhamnoside
Viability is linearly correlated with number of active pumps in partially silenced H1299 cells.
Viability is linearly correlated with number of active pumps in a panel of cells

A

B

C

Viability (% of NT)

Total \( \alpha_1 \) (a.u.)

Bmax (pmol oub*mg protein\(^{-1} \))
Viability is linearly correlated with number of active pumps—CG affect cancer cell viability only through binding and inhibition of NaK ATPase transport.

Although cardiac glycosides can inhibit the proliferation of cancer cells at very low concentrations (nM), they inhibit the proliferation of human nonmalignant cells at similar concentrations; this strongly suggests that their potential for cancer therapy is low.

More experimental data are needed to further decipher the structure-activity relationship between CG’s and cancer cell cytotoxicity.
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Thank you for your attention.