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Characterization of the pharmacological effects of cardiac glycosides on lung epithelial cells

PhD dissertation defense by

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Introduction

• Lung cancer alone accounts for 15% of cancer incidences and 30% of cancer related mortality.

• Lung cancer has poor prognosis due to an intrinsic wide signaling array, and limited therapeutic approaches.

• Developing new therapeutic alternatives for lung cancer is of critical need.

• Cardiac glycosides are potential candidates.
General outline

• Digitoxin, a cardiac glycoside with the potential to provide a new hope for cancer therapy.

• Digitoxin and a synthetic monosaccharide analog inhibit cell viability in lung cancer cells.
Digitoxin, a cardiac glycoside with the potential to provide a new hope for cancer therapy

• Outline

1. What are CGs?
2. Evidence for anticancer effect.
3. Potential CGs candidates.
4. Differential effects on Na+/K+ATPase.
5. Structural manipulation enhanced digitoxin’s cytotoxic activity.
6. Potential mechanism for CG’s selective cytotoxicity
7. Conclusions and future directions
What are cardiac glycosides?

- A large family of chemical compounds found in several plants and animal species.

- Known for more than 1500 years for several medical conditions as diuretics, emetics, abortifacients, antineoplastics, and heart tonics.

- Patients with congestive heart failure, “dropsy”’, improved after administering foxglove extract (*Digitalis purpurea* L.)
Cardiac glycosides share a common structural motif
Evidence for anticancer effect for cardiac glycosides

• Historical records indicated CGs extracts for treating malignant conditions.

• Shiratori et al., (1967) found anticancer potential for CGs on rodent cancer models.

• Stenkvist et al. (1979-2001), and Goldin et al., (1984) showed that women on digitalis therapy
  – developed more benign forms of breast tumors, and
  – 9.6-times lower cancer recurrence rate when compared to control patients.

• CGs mediate a significant anticancer effect.
What are potential candidates from cardiac glycosides?

• Digitoxin is an ideal candidate as an anticancer drug because:
  
  – Anticancer effect at therapeutic concentrations
  
  – Long half life (7days)
    
    • 97% bound to plasma proteins
    
    • Large $V_d$
  
  – Complete clinical profile
What are potential candidates from cardiac glycosides?

Iyer et al., 2010, Wang et al., 2010
Manipulation of the glycosidic linkage and saccharide moiety of digitoxin

(A) n=1; Digitoxin mono-MeON-saccharide
n=2; Digitoxin di-MeON-saccharide
n=3; Digitoxin tri-MeON-saccharide

(B) n=1; Digitoxin mono-O-saccharide
n=2; Digitoxin di-O-saccharide
n=3; Digitoxin

n= alfa-L-rhamnose; D6-MA
Digitoxin O-saccharides are more potent than digitoxin MeON-saccharides

Iyer et al., 2010
Digitoxin monosaccharide analogs are more potent anticancer agents than their disaccharide and trisaccharide counterparts

Iyer et al., 2010
Digitoxin affects $\text{Na}^+/\text{K}^+\text{ATPase}$ differently depending on its concentration

**Digitoxin (0.5-5μM)**

- $\text{Na}^+/\text{K}^+\text{ATPase}$ inhibition
- Intracellular $\text{Na}^+$
  - $\text{Na}^+/\text{Ca}^{2+}$ exchanger activation
  - Intracellular $\text{Ca}^{2+}$
  - Intracellular events

**Digitoxin (10-100 nM)**

- $\text{Na}^+/\text{K}^+\text{ATPase}$ signalosome (MAPK, SRC, Akt, and PLC signaling)
- Intracellular events
  - Manipulated gene expression
  - Cancer specific genes
  - Cell death
CGs reduce cell cycle regulatory proteins that are specifically overexpressed in cancer cells by blocking AP-1 and NF-κB signaling.
Conclusions and future directions

• How do digitoxin and analog cause G2/M phase arrest and apoptosis in cancer cells?

• What are potential p53-independent therapeutic target(s) that mediate cancer cell death?

• How do digitoxin and D6-MA inhibit survivin and p53 expression in cancer cells?

• Would digitoxin and D6-MA induce mitotic catastrophe in cancer cells? if so, how?
Digitoxin and a synthetic monosaccharide analog inhibit cell viability in lung cancer cells

• Outline

  – Introduction

  – Objectives and hypothesis

  – Results

  – Discussion and conclusions
Introduction

• Appropriate cell cycle progression is crucial for cell viability.

• *In vitro* CGs studies showed apoptosis, autophagy, and cell cycle arrest; however, mechanism is unclear.

• Wang et al. synthesized and compared several digitoxin monosaccharide analogs for lethal and growth inhibitory effects.

• Three monosaccharide analogues showed at least a 5-fold increase in antineoplastic potency in NSCLC.

• Understanding the cytotoxic mechanism of CGs in NSCLC will help in developing safer and more effective anti-cancer drugs.
Objective and Hypothesis

• NCI-H460 cells were chosen as a model for NSCLC because
  – NSCLC cells are more sensitive to digitoxin and D6-MA
  – Recalcitrance of NSCLC cells to chemotherapy

• Objective:
  – compare digitoxin with D6-MA with respect to their cytotoxic mechanisms

• Hypothesis:
  – therapeutically relevant doses of digitoxin and D6-MA would decrease cell viability due to G2/M arrest and induce apoptosis in NCI-H460 cells, with D6-MA being more potent.
Digitoxin and D6-MA causes inhibition of NCI-H460 cell viability and Na⁺/K⁺ATPase enzyme activity
Digitoxin and D6-MA induces apoptosis in NCI-H460 cells

A

Apoptosis (%) vs. log[Dose], M

B

0 nM

Digitoxin 50 nM

D6-MA 10 nM
D6-MA exhibits selective cytotoxicity to NSCLC cells

A

B

C

D

IC₅₀ (nM)

Apoptosis (%)

Apoptosis (%)

Cell type

Cell type

Control

Digitoxin at 50 nM

D6-MA at 10 nM

SAEC cells

tERT SAEC cells

BEAS-2B cells

NCI-H460 cells

Digitoxin

D6-MA

hTERT SAEC cells

BEAS-2B cells

NCI-H460 cells

hTERT SAEC cells

BEAS-2B cells

NCI-H460 cells

hTERT SAEC cells

BEAS-2B cells

NCI-H460 cells
Digitoxin and D6-MA induces extensive caspase-9 cleavage
Digitoxin and D6-MA induces expression of cytochrome c

A

Digitoxin (nM)  
0  10  20  50

D6-MA (nM)  
0  10  20  50

Cytochrome C  
β-actin

B

Fold change in cytochrome c

Concentration (nM)

0  10  20  50

*  +  *  *

Digitoxin  
D6-MA

*  +  *  *

0  10  20  50
Digitoxin and D6-MA induce G2/M phase arrest
Digitoxin and D6-MA induced down-regulation of cyclin B, cdc2, and survivin
Digitoxin and D6-MA-mediated G2/M phase arrest does not correlate with up-regulation of p53-related signaling.
Digitoxin and D6-MA-mediated G2/M phase arrest does not correlate with up-regulation of Chk1/2.
Discussion and Conclusions

- Na+/K+ ATPase inhibition by either digitoxin or D6-MA does not account for drug or analog cytotoxic effects.

- Na+/K+ ATPase signalsome activation is a viable possibility.

- Digitoxin and D6-MA are selective to NSCLC cells.

- Digitoxin and D6-MA induced differential caspase-9 cleavage, but not caspase-8.
Discussion and Conclusions

- Digitoxin and D6-MA induce cytochrome c expression which contrasts previous claims of general inhibition of protein synthesis.

- Inhibiting the expression of cyclin B1, cdc2, survivin, and Chk1/2 explain the potent and selective cytotoxic effect of digitoxin and D6-MA at therapeutic concentrations.

- Digitoxin and D6-MA induce G2/M phase arrest and cyclinB1 and cdc2 down-regulation.

- G2/M phase arrest and down regulation of cyclinB1 and cdc2 are not directly controlled by up-regulation of p53 signaling or checkpoint kinase signaling.
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