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Trial watch
Cardiac glycosides and cancer therapy

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Abbreviations: CG, cardiac glycoside; CI, 95% confidence interval; CRT, calreticulin; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HMGB1, high mobility group box 1; ICD, immunogenic cell death; IFN, interferon; IL, interleukin; NSCLC, non-small cell lung carcinoma; NCX, Na^+/Ca^{2+}-exchanger; RR, relative risk

Cardiac glycosides (CGs) are natural compounds sharing the ability to operate as potent inhibitors of the plasma membrane Na^+/K^+-ATPase, hence promoting—via an indirect mechanism—the intracellular accumulation of Ca^{2+} ions. In cardiomyocytes, increased intracellular Ca^{2+} concentrations exert prominent positive inotropic effects, that is, they increase myocardial contractility. Owing to this feature, two CGs, namely digoxin and digitoxin, have extensively been used in the past for the treatment of several cardiac conditions, including distinct types of arrhythmia as well as contractility disorders. Nowadays, digoxin is approved by the FDA and indicated for the treatment of congestive heart failure, atrial fibrillation and atrial flutter with rapid ventricular response, whereas the use of digitoxin has been discontinued in several Western countries. Recently, CGs have been suggested to exert potent antineoplastic effects, notably as they appear to increase the immunogenicity of dying cancer cells. In this Trial Watch, we summarize the mechanisms that underpin the unsuspected anticancer potential of CGs and discuss the progress of clinical studies that have evaluated/are evaluating the safety and efficacy of CGs for oncological indications.

Introduction

The term cardiac glycosides (CGs) refers to a large family of natural (mostly plant-derived) compounds that are best known for their prominent cardiovascular effects. Although historical records indicate that extracts from the common foxglove *Digitalis purpurea* were used (mainly as poisonous preparations) as early as in Egyptian and Roman times, the first scientific reports on the medical application of CGs date back to 1785, stemming from the work of the English botanist William Withering.[1] Since then, ever more purified preparations of CGs have been employed worldwide to treat a large panel of cardiac disorders, including various types of arrhythmia as well as cases of cardiac insufficiency of variable etiology.[2] One of the major issues related to the medical use of CGs originates from their rather narrow therapeutic index, with most prominent adverse effects including anorexia, nausea, vomiting, diarrhea and life-threatening alterations of cardiac rhythm (either bradycardia or tachycardia).[2,3] Still, the prototypic CGs digoxin and digitoxin have been approved by the FDA for the therapy of atrial fibrillation, atrial flutter and paroxysmal atrial tachycardia prior to 1982 (www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/default.htm). In 1998, the FDA has extended the indications of digoxin to congestive heart failure. Nowadays, while the approval status of both digoxin and digitoxin has not been revised, the use of the latter is being discontinued in several Western countries.[4] Of note, at least 10 distinct CGs have been identified so far, including the *Strophanthus gratus*-derivative ouabain.[5,6] Intriguingly, a circulating variant of ouabain is also produced by the adrenal glands of several mammals, and operates as an endogenous regulator of cardiovascular functions.[7]

CGs potently inhibit the transport activity of the plasma membrane Na^+/K^+-ATPase, resulting in the intracellular accumulation of Na^+ ions.[8] In this setting, increased intracellular Na^+ concentrations drive the antiporter activity of the Na^+/Ca^{2+}-exchanger (NCX) and hence promote a conspicuous Ca^{2+} uptake. In cardiomyocytes, Ca^{2+} ions enhance the contractility of troponin, thus exerting prominent positive inotropic effects.[9] This

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said, the pharmacological activity of CGs is not restricted to cardiomyocytes, as (1) the Na⁺/K⁺-ATPase is expressed ubiquitously, and (2) several CGs including digitoxin and digoxin exhibit a high bioavailability (> 75%) and readily cross both the blood-brain and the placental barriers.[10] In line with this notion, most of the adverse effects of CGs de facto constitute "on-target" toxicities, stemming from the inhibition of Na^+/K^+-ATPase in extra-cardiac tissues.[11–13]

Along the lines of our Trial Watch series,[14–23] here we briefly discuss the mechanisms underlying the unsuspected anticancer potential of CGs and summarize the progress of clinical studies that have evaluated/are evaluating the safety and efficacy of CGs for oncological indications.

Antineoplastic Effects of Cardiac Glycosides

Interest in the links between CGs and cancer began to raise in the late 1970s, owing to the discoveries that (1) malignant cells exhibit alterations in the activity of the Na^+/K^+-ATPase,[24] and that (2) some CGs constitute bona fide phytoestrogens and hence—at least theoretically—can affect the development and progression of hormone-sensitive cancers like breast carcinoma.[25] Since then, the potential antineoplastic activity of CGs and the underlying molecular mechanisms have intensively been investigated. Thus, a large panel of cancer cells of different histological origin have been shown to express peculiar combinations of Na^+/K^+-ATPase subunits,[26] yielding functional differences[24,27] and rendering them much more sensitive to CGs than their non-transformed counterparts. Accordingly, CGs have been demonstrated to exert antiproliferative as well as pro-apoptotic effects against neoplastic (but not normal, even when highly proliferating) cells in vitro, both as standalone interventions[10,28–30] and in combination with chemo- and radiotherapy.[31–34] Of note, the anticancer potential of CGs cannot be properly assessed in vivo in standard rodent models, as murine cells express CG-insensitive variants of the Na^+/K^+-ATPase.[35–37]

Whether the antineoplastic effects of CGs entirely stem from ionic imbalances or also involve (1) the activation of signal transduction cascades organized around the Na^+/K^+-ATPase[38] and/or (2) the inhibition of glycolysis[39] remains obscure. Nevertheless, alterations in the expression levels/pattern of Na^+/K^+-ATPase subunits have also been detected in subjects affected by a variety of neoplasms including glioblastoma,[40] non-small cell lung carcinoma (NSCLC),[41] melanoma,[42] colorectal carcinoma[43] and urothelial cancer.[44] These observations indicate that Na^+/K^+-ATPase may influence oncogenesis and/or tumor progression and hence perhaps constitutes a viable target for the development of antineoplastic therapies.

Recently, a drug repositioning screen has lead to the discovery that CGs are capable of eliciting the emission all the hallmarks of immunogenic cell death (ICD), a functionally peculiar instance of apoptosis that stimulate cognate immune responses.[45–48] Thus, cancer cells challenged with CGs expose the endoplasmic reticulum chaperon calreticulin (CRT) on outer leaflet of the plasma membrane,[49] actively secrete ATP via an autophagy-dependent mechanism,[50] and release the non-histone chromatin-binding protein high mobility group box 1 (HMGB1).[51] In addition, CGs exacerbate the antineoplastic efficacy of otherwise non-immunogenic chemotherapeutic agent (e.g., cisplatin, mitomycin C) in vivo, in immunocompetent but not in immunodeficient mice.[46] Finally, malignant cells dying in response to cisplatin or mitomycin C coupled to CGs acquire the ability to protect syngenic mice against a subsequent challenge with live cells of the same type.[46] Of note, the immunogenic effects of CGs appear to stem from the on-target inhibition of Na^+/K^+-ATPase and the consequent alterations of intracellular Ca^{2+} homeostasis.[46,52] Taken together, these observations suggest that CGs may exert antineoplastic effects not only as they preferentially inhibit the growth of malignant cells, but also as they promote the activation of tumor-specific immune responses.

Retrospective Studies

Over the last three decades, several retrospective, mostly epidemiological, clinical studies have investigated whether the use of CGs would influence the incidence and/or clinical outcome of several malignancies (**Table 1**). Early studies reported that, five years after mastectomy, the recurrence rate of breast carcinoma among patients who did receive CGs was 9.6-times lower than that of subjects who did not. Moreover, neoplastic cells isolated from breast carcinoma patients treated with CGs exhibited more benign features as compared with cells obtained from patients not receiving CGs.[53–55] In the context of a long-term follow-up (22.3 y) study involving a total of 175 breast carcinoma patients (of which 32 were on CG therapy), the same authors reported a significantly lower death rate (6%) among patients who did receive CGs along with conventional cancer therapy as compared with those who did not (34%).[56] In 2001, a study involving more than 9,000 patients evidenced an inverse correlation between the plasma levels of digitoxin and the risk to develop leukemia, lymphoma, renal cancer and tumors of the urinary tract, although the patient sub-cohort using CGs had a priori a higher risk of cancer than age- and sex-matched individuals not affected by cardiac disorders.[4] More recently, the incidence of prostate cancer has been correlated with CG use in 47,884 men followed up from 1986 through 2006.[57] This analysis revealed that, as compared with individuals who do not receive CGs, regular digoxin users have a lower relative risk (RR) of developing prostate carcinoma (RR = 0.76; 95% confidence interval, CI = 0.61–0.95), which is further decreased for long-term (> 10 y) users (RR = 0.54; CI = 0.37–0.79; p trend = 0.001).[57] Along similar lines, digoxin exposure has been associated with a (slightly sub-significant) reduction in the risk of prostate cancer-related mortality (RR = 0.69; CI = 0.47–1.01; p trend = 0.059) in a cohort of 786 patients (of which 395 received digoxin in the 90 d pre-diagnosis).[58] Conversely, prostate cancer patients using digoxin or verapamil (an L-type calcium channel blocker commonly used in therapy of various cardiac conditions)[59] along with chemotherapy have been reported to survive shorter ($p = 0.046$ and $p = 0.011$, respectively) than matched patients not receiving co-medications for cardiac diseases.[60,61]

Table 1. Retrospective clinical studies assessing the impact of CGs on oncogenesis, tumor progression and response to therapy

Agent	Setting	N*	HR, OR or RR, (95% CI), p value	Notes	Ref.
Digitoxin	Multiple types of cancer	9,271	HMs: 0.57, (0.30–1.08), p = 0.008 KC/UTC: 0.45, (0.24–0.83), p = 0.05	Inverse correlation between plasma levels of digitoxin and the risk to develop HMs, KC and UTC	4
Digitoxin Digoxin Other CGs	Breast carcinoma	33	n.a.	Use decreased relapse rate	55
Digoxin	Breast carcinoma	28	n.a.	Tumor cells from treated patients had comparatively more benign features	54
		175	n.a.	Use decreased death rate	56
		324	1.30, (1.14–1.48)	Use increased risk among postmenopausal women	63
		104,648	1.39, (1.32–1.46) ER^+: 1.35, (1.26 - 1.45)	Use increased risk, mainly of developing ER^+ lesions	65
	Carcinoma	145	Global: 0.62, (0.46–0.84), p = 0.002 BC p = 0.04 CRC p = 0.03 HCC p = 0.04 HNC p = 0.02	Use increased OS	46
	Prostate cancer	786	0.69, (0.47–1.01) p = 0.059	Use decreased cancer-related death rate	58
		1,006	p = 0.046	Inverse correlation between use and survival	61
		47,884	Regular users: 0.54, (0.37–0.79) p < 0.001 Users for > 10 y: 0.76, (0.61–0.95)	Use (in particular ≥ 10 y) decreased risk	57
	Reproductive tract cancer	638	CC, users: 1, (0.79–1.25) OC, users: 1.06, (0.92–1.22) UC, current users: 1.48, (1.32–1.65) UC, users for ≥ 36 mo: 1.91, (1.51–2.41) UC, former users: 1.20, (0.99–1.45)	Current (and possibly former) use decreased risk of developing UC, but not CC and OC	64

BC, breast cancer; CC, cervical cancer; CG, cardiac glycoside; CI, 95% confidence interval; CRC, colorectal cancer; ER, estrogen receptor; HCC, hepatocellular carcinoma; HNC, head and neck cancer; HM, hematological malignancy; HR, hazard ratio; KC, kidney cancer; n.a., not available; OC, ovarian cancer; OR, odds ratio; OS, overall survival; RR, relative risk; UC, uterine cancer; UTC, urinary tract cancer. *n° of patients.

A few months ago, we have observed that the overall survival of 145 carcinoma patients who received CGs along with chemotherapy due to an underlying cardiac disorder was significantly longer (survival rate at 5 y = 65%) than that of 290 age-, sex-, tumor type-, treatment- and main prognostics factor-matched control patients (survival rate at 5 y = 52%).[46] Further subgroup analyses demonstrated that digoxin was particularly efficient at increasing the overall survival of carcinoma patients treated with agents other than ICD inducers (i.e., anthracyclines and oxaliplatin), which suggests that digoxin may ameliorate the efficacy of non-immunogenic anticancer therapies.[46]

As CGs constitute bona fide phytoestrogens, multiple retrospective studies have investigated whether CG-based therapy may affect the incidence of hormone-sensitive tumors. Early studies concluded that the use of CGs is not associated with an increased risk of breast cancer,[4,55] and some authors actually suggested that CGs may exert antineoplastic effects not only by virtue of their capacity to inhibit the Na^+/K^+-ATPase, but also as they would operate as estrogen receptor (ER) antagonists.[62] More recently, evidence arguing against these conclusions has been accumulated. In particular, the use of digoxin for at least 1 y has been associated with an increased risk for invasive breast cancer among post-menopausal women (RR = 1.30; CI = 1.14–1.48).[63] Moreover, the current (but not the former) use of digoxin has been linked with an augmented risk of developing (mainly ER^+) breast cancers (RR = 1.39; CI = 1.32–1.46) and uterine tumors (RR = 1.48; CI = 1.32–1.65), but not ovarian (RR = 1.06; CI = 0.92–1.22) and cervical (RR = 1.00; CI = 0.79–1.25) carcinomas.[64,65]

Hence, the antineoplastic potential of digoxin and perhaps other CGs may be limited by the fact that these agents appear to operate as ER agonists and hence stimulate the proliferation

Table 2. Prospective clinical studies assessing the impact of CGs on oncogenesis, tumor progression and response to therapy

Agent	Setting	N*	Phase	Status	Dose	Co-therapy	Notes	Ref.
Anvirzel™	NSCLC	30	I	Recruiting	n.a.	Combined with carboplatin and docetaxel	Primary outcome: MTD and pharmacokinetics	NCT01562301
		18	I	n.a.	0.1–1.2 mL/m^2/day	As single agent	Primary outcome: MTD and safety	74
	Solid tumors	52	I	Active, not recruiting	0.0083 mg/Kg/day	As single agent	Primary outcome: MTD	NCT00554268
Digoxin	Breast carcinoma	17	I	Completed	0.5 mg/day	Combined with lapatinib	Primary outcome: pharmacokinetics	NCT00650910
	Melanoma	47	II	n.a.	0.25 mg/day	Combined with cisplatin, IL-2, IFNα2b and vinblastine	Increased overall response rate from 19.5% to 55.3%	72,73
	NSCLC	24	II	Terminated	n.a.	Combined with erlotinib	Failure to increase overall response rate	NCT00281021
	Prostate cancer	16	II	Recruiting	0.125 or 0.25 mg/day	As single agent	Primary outcome: rate of positive PSADT outcomes	NCT01162135
	Solid tumors	30	I	Recruiting	0.25 mg/day	Combined with caffeine, midazolam, omeprazole, s-warfarin, vitamin K and tivantinib	Primary outcome: pharmacokinetics	NCT01517399
HuaChanSu	HCC NSCLC Pancreatic cancer	15	I	n.a.	10–90 mL/m^2/day	As single agent	Absence of DLTs Six patients experienced disease stabilization	77
	Pancreatic cancer	80	II	Completed	20 mL/m^2/day	Combined with gemcitabine	Primary outcome: PFS at 4 mo	NCT00837239

DLT, dose-limiting toxicity; HCC, hepatocellular carcinoma; IFN, interferon; IL, interleukin; MTD, maximum tolerated dose; n.a., not available; PSADT, prostate-specific antigen (PSA) doubling time; NSCLC, non-small cell lung carcinoma; PFS, progression-free survival. *n° of patients or estimated enrollment.

of nascent hormone-sensitive malignancies. If confirmed in large, prospective clinical trials, this possibility will have to be attentively considered for the identification of subsets of cancer patients who may actually benefit from the use of CGs.

Prospective Studies

Preclinical and epidemiological data suggested that CGs might be employed as antineoplastic agents, at least in a subset of cancer patients.[66] In spite of these encouraging premises, compelling evidence from randomized prospective clinical studies that would support this notion is still missing (**Table 2**).

In a Phase II clinical trial, digoxin failed to increase the response rate of 24 unresectable Stage III/IV NSCLC patients to the epidermal growth factor receptor (EGFR) inhibitor erlotinib[67,68] employed as a second-line therapy (NCT00281021). In particular, 1 patient manifested a partial response, 9 exhibited stable disease and 14 progressed in spite of chemotherapy, overall leading to the premature termination of the study. This said, the co-administration of digoxin appeared not to increase the rate of adverse effects, which were similar to those generally observed when erlotinib is used as a standalone intervention.[69] In another Phase II study involving 47 Stage IV melanoma patients, the addition of digoxin to an immunochemotherapeutic regimen including the DNA-damaging agent cisplatin,[70] the microtubular poison vinblastine,[71] as well as the immunostimulatory cytokines interleukin (IL)-2 and interferon (IFN)α2b,[21] increased the global response rate from 19.5% (as observed in a parallel Phase III study involving 200 patients)[72] to 55.3%.[73] Nowadays, one single Phase II clinical trial is ongoing to test the safety and preliminary efficacy of digoxin, employed as a standalone agent, in subjects affected by recurrent prostate carcinoma (NCT01162135). Two additional Phase I studies on the use of digoxin by cancer patients are registered at www.clinicaltrials.gov (NCT00650910, status: completed; NCT01517399, status: recruiting), yet mainly aim at investigating putative pharmacokinetic interactions between digoxin and antineoplastic agents (i.e., the dual tyrosine kinase inhibitor lapatinib and c-MET inhibitor tivantinib) *sensu stricto*.

Anvirzel™ is a *Nerium oleander* extract containing the two CGs oleandrin and oleandrigenin that exerts antineoplastic effects against human melanoma BRO cells in vitro.[36] Patients affected by advanced solid tumors failed to exhibit objective responses to Anvirzel^{TM} in a Phase I trial, though the preparation was shown to be well tolerated at daily doses < 1.2 mL/m^2.[74] The safety profile and antineoplastic potential of Anvirzel^{TM}, combined with the DNA-damaging agent carboplatin and the microtubular poison docetaxel, are currently being evaluated in a Phase I trial enrolling advanced NSCLC patients (NCT01562301). Along similar lines, the tolerability of a more concentrated *Nerium oleander* extract (PBI-05204) is under evaluation in a Phase I study that involves individuals bearing advanced solid malignancies (NCT00554268). Although the final data collection date for the primary endpoint measure of this trial (i.e., maximum tolerated dose) is set to October 2012, preliminary reports indicate that PBI-05204 is tolerated at doses < 10.2 mg/day, inducing very little cardiotoxicity, and that 7 out of 45 evaluable patients treated with PBI-05204 achieved stable disease for > 4 mo.[75]

Bufalin is a CG contained in the Chinese medicine HuaChanSu (also known as ChanSu) that is known to exert antineoplastic effects in vitro by targeting the α3 subunit of the Na^+/K^+-ATPase.[76] In a pilot study (based on a Phase I design) involving 2 NSCLC, 11 hepatocellular carcinoma and 2 pancreatic cancer patients, HuaChanSu appeared to be well tolerated and promoted disease stabilization (mean duration = 6 mo) in 40% of the cohort (6 individuals, of which 1 exhibited a partial regression lasting 11 mo).[77] More recently, the safety profile and therapeutic potential of HuaChanSu, combined with the nucleoside analog gemcitabine (which per se does not promote ICD), have been investigated in a Phase II clinical trial enrolling 80 individuals affected by locally advanced or metastatic pancreatic cancer (NCT00837239). In this setting, HuaChanSu was well tolerated but failed to improve objective radiographic response rates, time to progression, quality of life and overall survival.[78]

Concluding Remarks

In spite of a rather narrow therapeutic window,[11,12,79] digoxin and digitoxin have been used for a long time (and are currently approved by FDA) for the therapy of cardiac disorders including arrhythmias and congestive heart failure. Recently, CGs have attracted great attention as they appear to (1) mediate direct and selective antineoplastic effects, owing to the fact that malignant cells often differ from their normal counterparts relative to subunit composition and expression levels of the pharmacological target of CGs, the plasma membrane Na^+/K^+-ATPase;[80–82] and (2) render immunogenic the demise of cancer cells as triggered by conventional chemotherapy.[45,46] So far, only a few clinical studies (encompassing both retrospective and prospective approaches) have attempted to investigate the putative impact of CGs on oncogenesis, tumor progression and response to therapy, reporting rather heterogeneous findings. In this respect, it should be noted that the results of retrospective studies assessing patient survival (but not of those assessing tumor incidence) are potentially biased by the fact that individuals receiving CGs were invariably affected by cancer plus an underlying cardiac disorder. Hence, the actual antineoplastic potential of CGs, either as standalone interventions or combined with conventional therapeutic regimens, remains to be fully elucidated. In the attempt to get further insights into this issue, we will soon initiate a Phase I clinical trial to investigate the safety and efficacy of digitalization (0.25 mg/day digoxin for 7 d) followed by a conventional chemotherapeutic regimen including cisplatin, docetaxel and 5-fluorouracil (a pyrimidine analog) in subjects affected by locally invasive, unresectable head and neck carcinoma. We specifically chose this clinical setting for three reasons: (1) the prognosis of these patients is generally very poor, calling for the urgent development of novel therapeutic strategies; (2) in our retrospective clinical study,[46] CGs were found to ameliorate the survival of head and neck carcinoma patients with the most significant p value (0.02), as compared with other patient sub-cohorts; and (3) individuals affected by unresectable head and neck carcinoma are usually treated with a neoadjuvant therapeutic regimen including cisplatin, docetaxel and 5-fluorouracil (which per se do not promote ICD),[83,84] a scenario that may be ideal for highlighting the capacity of CGs to stimulate the immunogenicity of cancer cell death in humans. Future will tell whether CGs constitute or not a valuable approach to anticancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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