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Chemical screening identifies the β-Carboline alkaloid harmine to be synergistically lethal with doxorubicin

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ABSTRACT

Despite being an invaluable chemotherapeutic agent for several types of cancer, the clinical utility of doxorubicin is hampered by its age-related and dose-dependent cardiotoxicity. Co-administration of dexrazoxane as a cardioprotective agent has been proposed, however recent studies suggest that it attenuates doxorubicin-induced antitumor activity. Since compounds of natural origin present a rich territory for drug discovery, we set out to identify putative natural compounds with the view to mitigate or minimize doxorubicin cardiotoxicity. We identify the DYRK1A kinase inhibitor harmine, which phosphorylates Tau that is deregulated in Alzheimer's disease, as a potentiator of cell death induced by non-toxic doses of doxorubicin. These observations suggest that harmine or other compounds that target the DYRK1A kinase my offer a new therapeutic opportunity to suppress doxorubicin age-related and dose-dependent cardiotoxicity.

1. Introduction

Anthracyclines are a class of cancer chemotherapeutics considered to be one of the most efficacious anti-tumor drugs ever developed (Minotti et al., 2004). Doxorubicin is one of the first anthracyclines to be discovered and is currently used clinically for several solid and liquid tumors (Arcamone et al., 1969). It is the first line treatment for breast cancer and also used for soft tissue sarcomas and aggressive lymphomas (Minotti et al., 2004; Carvalho et al, 2014). The anti-tumorogenic properties of doxorubicin are attributed at least in part due to targeting Topoisomerase II (TOP2); an essential cellular enzyme that is required for regulating DNA topology during various cellular processes such as transcription, replication and recombination (Tewey et al., 1984; Wang 2002; Corbett and Berger, 2004; Vos et al., 2011). The mammalian genome encodes two isoenzymes of TOP2: TOP2α which is highly expressed in rapidly dividing cells and TOP2β which resides in quiescent cells such as cardiac cells (Pommier et al., 2010; Vos et al., 2011). Notably, doxorubicin possesses other TOP2-independent tumoricidal mechanisms such as DNA intercalation, mitochondrial targetting, and the induction of histone eviction from open chromatin (Nitiss, 2009; Pang et al., 2013). Doxorubicin exhibits its antitumor activity primarily by targetting TOP2α in cancer cells which leads to an increase in DNA double-stranded breaks (Tewey et al., 1984).

Nevertheless, age-related cardiotoxicity in the form of chronic cardiomyopathy and congestive heart failure has been strongly associated with doxorubicin treatment (Lefrak et al., 1973; Steinherz et al., 1991; Hequet et al., 2004). Intially, doxorubicin-associated cardiac damage was attributed to the induction of mitochondrial dysfunction (Wallace, 2003) and increased ROS production in cardiac cells (Tokarska-Schlattner et al., 2006). Accumulation of mitochondiral DNA damage is an established cause for age-related degenerative diseases Doroshow, (1983); Keizer et al., (1990) (El-Khamisy, 2011; Akbari et al., 2015). However, a recent study demonstrated that these effects are secondary to TOP2β isoenzyme inhibition in the heart muscle (Zhang et al., 2012).

Development of doxorubicin-induced cardiotoxicity is largely dose-dependent (Lefrak et al., 1973), but it may also occur at low doses in the presence of other risk factors especially increasing age (Von Hoff et al., 1979; Carvalho et al., 2014). It has been shown that 5%, 26% and 48% of patients developed congestive heart failure after receiving cumulative doses of 400 mg/m^2, 550 mg/m^2 and 700 mg/m^2 of doxorubicin respectively (Swain et al., 2003). Several

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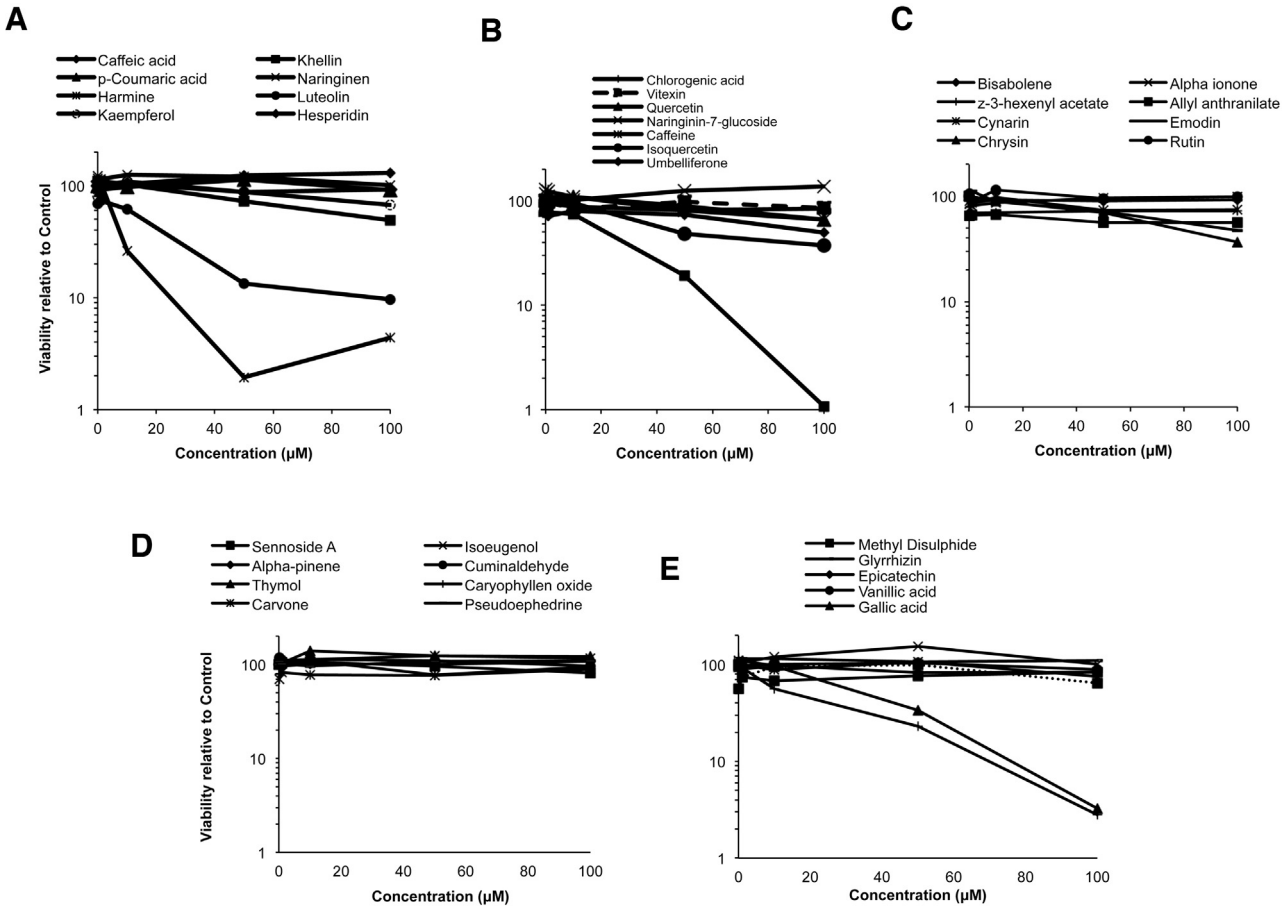

Fig. 1. Determination of the cytotoxicity of natural compounds on the breast cancer cell line, MCF-7. MCF-7 cells were treated with the indicated compounds at 0.1, 1, 10, 50 and 100 μM for 48 h followed by the determination of cell viability using the CellTiter-Blue® Cell Viability Assay (Promega). Viability at a given concentration is determined as a percentage relative to that of a DMSO control.

studies pointed towards age being one of the most significant risk factors in developing doxorubicin-mediated cardiac damage with children and elderly patients being the most suceptible to developing this side effect (Von Hoff et al., 1979; Kremer et al., 2002; Swain et al., 2003; Doyle et al., 2005; Lipshultz, 2006). For elderly patients, reduced doxorubicin clearance caused by a decline in the regional blood flow may be the primary reason for their increased susceptibility to cardiotoxicity (Robert and Hoerni, 1983; Li and Gwilt, 2003). Interestingly, postmortem analysis of patients treated with doxorubicin exhibited high accumulation of the drug in the cardiac muscle (Hong et al., 2002).

One proposed strategy to minimize this deleterious side effect is the co-administration of dexrazoxane, an FDA-approved cardioprotective agent (Speyer et al., 1988; Speyer et al., 1992). Dexrazoxane acts by complexing the TOP2β ATPase domain and thus blocking doxorubicin binding to the cardiac TOP2. However, the ATPase domain is identical in both TOP2α and TOP2β which means that dexrazoxane administration could diminish doxorubicin's tumoricidal activity (Lyu et al., 2007; Vejpongsa and Yeh, 2014). Moreover, studies regarding the combined anticancer activity of doxorubicin and dexrazoxane have reported contradictory results (Wadler et al., 1986; Hasinoff et al., 1996; Pearlman et al., 2003). The problem of doxorubicin-induced cardiotoxicity still poses a serious unmet challenge limiting its clinical utility.

Natural products have been historically the most rich source of drug leads owing to the fact that they exhibit unparalleled structural diversity compared to combinatorial chemistry (Mishra and Tiwari, 2011; Dias et al., 2012). Indeed, nature presents an untapped reservoir of chemical structures with potential biological activities (Dixon, 2001). Plant extracts such as that of *Taxus baccata* (European yew) tree and *Catharanthus roseus*, also known as *Vinca rosea*, were found to exhibit cytotoxic effects (Mantle et al., 2000). Current regimens for the treatment of breast cancer also include that of vinblastine, another indole alkaloid from *vinca* plant and a known component of a number of chemotherapies (Ospovat et al., 2009). The hunt for other potential cytotixic drug entities from natural origin will continue to grow. In the current study, we aimed to identify a synthetic lethal interaction between doxorubicin and a natural product using the breast cancer cell line MCF-7 with the view to obtain preliminary evidence to supress cardiotoxicity. For this purpose, compounds from a library of natural product isolates derived mostly from plant origin were assessed in combination with doxorubicin at sub-toxic concentrations. We identify harmine, a β-carboline alkaloid, to be synergistically toxic in short-term viability assays and additively toxic in long-term clonogenic survival assays, with doxorubicin. We propose that co-adminisstration of harmine and doxorubicin will permit the use of lower doses of the latter and thus help reduce its associated dose-dependent cardiac damage.

2. Materials and methods

2.1. Chemicals

Caffeic acid, p-coumaric acid, naringinen, chlorogenic acid, quercetin, isoquercetin, umbelliferone, harmine, luteolin, caf-