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DEVELOPMENT OF A TUMOR-SPECIFIC PHOTOACTIVATABLE DOXORUBICIN PRODRUG

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Abstract

This is a retrospective highlight on the publication by Ibsen and co-workers: Localized In Vivo Activation of a Photoactivatable Doxorubicin Prodrug in Deep Tumor Tissue, which appeared in a preceding issue of *Photochem. Photobiol.* (2013, **89**:698–708). The authors describe the synthesis and properties of a novel doxorubicin (DOX) prodrug, DOX-PCB, which contains a photocleavable linker group. Systemic administration of the prodrug to a tumor-bearing animal followed by LED/fiber optic 365 nm light delivery allowed active DOX to be released site-specifically in the tumor area. This elegant and timely study provides compelling evidence that photocleavable DOX-PCB can eliminate many of the toxic side-effects of DOX that have plagued clinical use of this highly effective antitumor drug for many years.

COMMENTARY

The anthracyclines doxorubicin (DOX, Fig. 1), also known as Adriamycin®, and its close analogue daunorubicin (DNR) are considered to be two of the most effective anticancer drugs ever developed (1,2), DOX being used for breast, bladder, liver, and lymphoid cancers, and DNR for various leukemias (3,4). DOX and DNR were originally isolated in the early 1960s from a strain of pigment-producing *Streptomyces* and since then, numerous derivatives have been generated in an effort to improve therapeutic action and reduce adverse side effects (2). DOX and DNR both contain a quinone moiety (C-ring), which is converted to a semiquinone radical upon one-electron reduction. For some time, it was believed that toxic oxyradical production from DOX/DNR redox cycling in tumor cell mitochondria or endoplasmic reticulum was the mechanism by which these anthracyclines kill tumor cells. However, the consensus now is that in rapidly dividing tumor cells, interference with DNA replication via inhibition of topoisomerase-II (or in some instances direct intercalation into DNA) is the primary means by which these drugs suppress tumors (1,5,6). Like other cancer chemotherapeutic agents, DOX and DNR are inherently cytotoxic and this cytotoxicity is often not limited to the tumor per se when these drugs are administered systemically. Adverse effects on normal tissue of the gastrointestinal tract, skin, and hematopoietic system have been reported in connection with typical DOX dosing for cancer patients or DOX in combination with other therapeutics (7). However, damage to heart tissue is the most widely recognized and perhaps most serious side effect of DOX/DNR (2,8,9). Such damage can lead to mitochondrial swelling and dysfunction, dilation of the sarcoplasmic reticulum, and overall loss of myofibrils. The molecular basis of DOX cardiotoxicity is well established and relates primarily to its redox cycling activity in mitochondria (10,11). Biochemical studies have shown that DOX can intercept electrons one at a time emerging from Complex 1 and then be re-oxidized by O₂ to give reactive oxygen

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species (ROS), i.e. superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO\cdot$), the latter being a powerful oxidant which can indiscriminately damage mitochondrial proteins and DNA (12). Modifying the C-ring of DNR with a 5-imino group dramatically reduced oxyradical formation (10,12), but subsequent studies revealed that 5-imino-DNR was too myelotoxic for clinical development (2). Anthracyclines might also cause cardiotoxicity by forming a ternary complex with topoisomerase-II, which is present in adult cardiomyocytes and other quiescent cells. This process is associated with DNA double strand breaks and transcriptome changes that eventually result in defective mitochondrial biogenesis and ROS formation (13). A more recently recognized side effect of DOX, which may prove to be as serious as cardiotoxicity, is long-term cognitive impairment, also referred to as “chemo-brain” or “chemo-fog” (14). A mechanism has been proposed in which systemic cell injury due to DOX redox cycling elevates levels of TNF- α , a pro-inflammatory cytokine (14,15). The latter enters the brain and can activate inflammatory signaling pathways, leading to brain damage.

In the highlighted study (16) and a preceding one (17), Ibsen *et al.* developed a remarkable new DOX prodrug with high tumor specificity upon activation and minimal toxicity to normal tissue outside the activation area. The prodrug, referred to as DOX-PCB, consists of a photocleavable o-nitrophenyl group (18) attached to the sugar amino group of DOX and conjugated to a biotinylated polyethylene glycol linker. The rationale behind this modification was to reduce DOX systemic cytotoxicity while increasing lifetime in the circulation (pegylation) and intratumoral delivery of the prodrug (biotinylation). *Ex vivo* and *in vivo* tumor experiments were carried out using human lung A549 tumor-bearing mice. Photoactivation of DOX-PCB *in vivo* (administered in Captisol® cyclodextrin) was accomplished with a custom LED/fiber optic system delivering high intensity 365 nm light. Whereas this light was sufficiently penetrating in tumor tissue to activate the pro-drug *in situ*, activation by ambient light was found to be negligible. Other key findings were as follows: (i) DOX-PCB was ~200-times less cytotoxic *in vitro* than DOX itself; however, interaction with DNA and cytotoxicity increased dramatically after photoactivation; (ii) LC/MS analysis of serum samples from DOX-PCB-treated mice indicated that its circulation half-life was ~10 min, which is comparable to DOX itself; (iii) After DOX-PCB injection, no DOX was detected in urine over at least 24 h, ruling out any spontaneous or metabolic release of DOX from the prodrug *in vivo*; (iv) After DOX-PCB injection and in-tumor photoactivation, LC/MS/MS analysis of tumor tissue revealed that liberated DOX was localized predominantly in the tumor i.e. any leakage into the systemic circulation was negligible. Thus, most of the desired criteria for a photocheavable prodrug were satisfied, including minimal toxicity in the uncleaved state, minimal spontaneous or metabolic activation during delivery, and maximal tumor retention of liberated DOX. An added advantage of DOX-PCB is that its low non-specific cytotoxicity would allow much higher doses to be administered than permitted with DOX itself.

Over the past 12 years, numerous other strategies have been used in an attempt to limit the harmful side effects of DOX on the heart and other non-tumor tissues. These include (i) introduction of DOX in uncoated or pegylated liposomal form in order to slow delivery to normal tissues while favoring delivery to tumors with leaky vasculatures (2,19); (ii) design of prodrugs that liberate DOX in response to environmental differences between tumor and normal tissues, e.g. local pH and degree of oxygenation (20,21); and (iii) prodrugs that are activated by enzymes expressed at much higher levels in tumors than in normal tissues (22). However, certain inadequacies have become apparent with each of these strategies, e.g. suboptimal tumor targeting of DOX in the case of liposomal delivery, and insufficient specificity with formulations based on low pH, low oxygenation, or enzymatic cleavage.

The elegant and highly innovative study described in this highlight represents a conceptual breakthrough in the development of DOX prodrugs. However, the antitumor efficacy (cure rate) of photocleavable DOX-PCB still needs to be tested in an animal tumor model and possible drawbacks related, for example, to insufficient tumor vascularization and/or limited light penetration in rapidly growing tumors need to be elucidated. Yet, the pharmacological and photochemical prospects described in this initial investigation appear to be excellent, and we await further reports on this very promising prodrug.

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Figure 1.
Chemical structure of doxorubicin (DOX).