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Review

Doxil® — The first FDA-approved nano-drug: Lessons learned

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ABSTRACT

Doxil®, the first FDA-approved nano-drug (1995), is based on three unrelated principles: (i) prolonged drug circulation time and avoidance of the RES due to the use of PEGylated nano-liposomes; (ii) high and stable remote loading of doxorubicin driven by a transmembrane ammonium sulfate gradient, which also allows for drug release at the tumor; and (iii) having the liposome lipid bilayer in a "liquid ordered" phase composed of the high-T_m (53 °C) phosphatidylcholine, and cholesterol. Due to the EPR effect, Doxil is "passively targeted" to tumors and its doxorubicin is released and becomes available to tumor cells by as yet unknown means. This review summarizes historical and scientific perspectives of Doxil development and lessons learned from its development and 20 years of its use. It demonstrates the obligatory need for applying an understanding of the cross talk between physicochemical, nano-technological, and biological principles. However, in spite of the large reward, ~2 years after Doxil-related patents expired, there is still no FDA-approved generic "Doxil" available.

Contents

1. What led to Doxil® development: OLV-DOX

Development of Doxil was initiated in Israel and the USA ~14 years ago when it became evident in a "first in man" (FIM) clinical trial by

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Gabizon and Barenholz that a "first generation" liposomal doxorubicin did not justify further clinical development despite an elevation of drug MTD (rev. in [1]). In this FIM trial we used negatively charged, medium-size oligolamellar liposomes (OLV) composed of two low-T_m (fluid) phospholipids [the zwitterionic egg-derived phosphatidylcholine (EPC), the negatively charged egg-derived phosphatidylglycerol (EPG)], and cholesterol. In these OLV the doxorubicin was membrane associated and passively loaded during the lipid hydration. This liposomal doxorubicin (DOX) is referred to as OLV-DOX (for more information on the OLV-DOX formulation development, characterization, performance, and clinical experience see [2–17], and reviewed in [1]).

In this FIM we also determined the patients' plasma PK of doxorubicin and of phosphatidylglycerol (PG), a phospholipid that is not normally present in human plasma and therefore was used as the liposome marker of the OLV-DOX. From the ratio between the DOX PK and the PG PK we calculated the drug release rate in human plasma in vivo [14,15]. We also determined the OLV biodistribution (BD) by imaging of ^{111}In-remotely-loaded OLV (^{111}In-OLV, [14]). These studies clearly demonstrated that the clearance of DOX when delivered as OLV-DOX is a composite of two processes: (i) clearance of liposomes containing DOX by the RES, predominantly liver and spleen, but not the liver tumor, which is avoided by these OLV; and (ii) clearance of free DOX released fast from liposomes in plasma. The analysis, which includes PK of total drug (DOX), liposome-associated DOX, and liposome markers (PG and ^{111}In-OLV), suggests that both processes operate in human patients and that factors such as the patient's liver function may affect their relative contribution [14,15].

These PK, BD, and imaging data suggest that the reduced clinical toxicity of OLV-DOX results from a somewhat lower peak level of free drug and possibly some changes in the tissue distribution of the liposomes, with a partial shift toward drug accumulation in the RES at the expense of other tissues. The main limitations of the therapeutic strategy based on OLV-DOX, as revealed by this study, are the significant drug leakage and preferential RES uptake.

These shortcomings are probably the result of the basic inferior formulation physicochemical characteristics given below.

(i) Drug location in the liposome bilayer as opposed to encapsulation in the liposome aqueous interior. Bilayer-associated drug may be more accessible to be released to the plasma upon dilution and to associate with plasma proteins [10,11,15] This process is determined by the drug membrane/medium partition coefficient, which in the case of doxorubicin is not high enough to retain the drug during the major dilution the OLV-DOX undergo as a result of intravenous slow infusion to humans [12,15,18,19]. We demonstrated that the discrepancy between the successful therapeutic efficacy in mice and the failure in the human studies is a result of the very large difference in plasma volume (compare, ~1 mL in mice with >3500 mL in humans and in mice and human size). The association of doxorubicin with liposomes is related to the liposome membrane/aqueous medium (plasma) partition coefficient (Kp). Therefore, slow infusion of the liposomes will result in an immediate very large dilution of 3500-fold for each mL that reaches the plasma, compared with only a 5-fold dilution with the i.v. bolus injection of the same liposomes to mice. The fast free drug clearance from plasma keeps this huge dilution effect active throughout all the time of the infusion [12,15,18–20]. The burst of drug leakage shortly after injection into patients (Fig. 4 in [14]) is compatible with the dilution release effect.

(ii) The presence of a high mole fraction of PG in the liposome bilayer may accelerate uptake by the RES [13]; it may also induce complement activation [21–23].

(iii) The liposome size is too large to allow for extravasation in extrahepatic tissues [24] and to take advantage of the enhanced permeability and retention (EPR) effect that was first described by Matsumura and Maeda [25] and reviewed by Maeda et al. [26]. This effect may allow for selective accumulation of nanoparticluates in tumors due to tumor (but not normal healthy tissue) being rich in porous blood capillaries that are permeable to particles of 100 nm and smaller. In addition, the tumor tissue is poor in lymphatic drainage, which enables prolonged retention of the nanoparticles there, followed by local (tumor) drug release and/or for the liposomes to be taken up by the tumor cells. Therefore, the fact that the same dose-limiting bone marrow toxicity was observed with OLV-DOX and with doxorubicin administrated as is (standard care) is not surprising and can be assigned to the large extent of fast drug leakage from circulating liposomes.

In view of the OLV-DOX fast plasma drug release and the changes in tissue distribution and bioavailability, it is uncertain whether the somewhat increased tolerated dosage of OLV-DOX (over free, non-liposomal DOX) will result in an enhanced antitumor activity. The liposomes used in this clinical study are cleared fast by the RES of liver and spleen and to a lesser extent by the bone marrow. These human studies suggest that the mechanism of antitumor activity of OLV-DOX is complex, and presumably results from exposure of tumor cells to drug leaking from circulating liposomes and drug released from the RES. Obviously, drug leakage from circulating liposomes is undesirable since it resulted in unwanted cardiotoxicity. Regarding drug release from the RES, the clinical conditions most likely to benefit from this approach are limited. This approach should not work for treatment of solid tumors, as in most solid tumors drug exposure in relation to dosage may be suboptimal. The OLV-DOX is expected to be highly sensitive to factors such as RES/liver function, site of tumor involvement, and proximity of tumor cells to RES cells.

The failure of this OLV-DOX used in humans had some basic "take home lessons" that led us to the development of a liposomal doxorubicin formulation that should be less toxic and more efficacious than free DOX in humans. The failure of OLV-DOX served as the main driving force and as the basis for Doxil® development.

Our failure with OLV-DOX supported the 1980s' overall low expectation of liposomes as a broad spectrum drug delivery system. This disappointment was summarized in an almost "lethal" paper (to the medical application of liposomes) in *Cancer Research* by Poste et al. [27], which states categorically that: "The inability of liposomes to escape from continuous capillaries and their rapid uptake by circulating and fixed phagocytic cells calls into question the feasibility of using liposomes to 'target' drugs to cells in extravascular tissues".

This and Poste's 1983 publication [28] were "catastrophic" to the medical application of liposomes as it led the scientific community as well as the major grant agencies, the pharmaceutical industry, and the venture capital community to lose interest in this field. It took 10 more years and a few real clinical successes for the field to recover and gain back some trust that enabled the development of more than a dozen FDA-approved liposomal drugs from 1995 to the present.

In planning our advanced liposomal anticancer drug, Liposome Technology Inc. (LTI), Gabizon, and I decided to stay with doxorubicin as the cancer chemotherapeutic agent of choice as most of our considerations (medical, scientific, and practical) for the selection of this drug [1] were still valid. Doxorubicin, like many other anthracyclines, is produced by one of the *Streptomyces* bacteria (*Streptomyces peucetius* var. *caesius*). It was discovered in the 1960s near the Adriatic Sea, which explains the source of the brand name Adriamycin, and showed significant anticancer activity [29–31]. Doxorubicin acts on the nucleic acids of dividing cells by two main mechanisms of action. Firstly, it inhibits DNA and RNA synthesis by intercalating between base pairs of the DNA strands, thus preventing the replication and transcription in rapidly-growing cancer cells. This mechanism is based on the chemistry and physics of the doxorubicin molecule (its positively charged mannose amine that binds efficiently to the negatively charged nucleic acid phosphate diester groups and the excellent fit of the drug anthroquinone planar ring structure for intercalation into the double-stranded DNA). All together, these structural features lead to high affinity of the drug to double stranded nucleic acids in a way that is not dependent on cell metabolism. The high affinity to