Hyperthermia and Liposomal Encapsulated Doxorubicin

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Abstract

Background: Several *in vitro* studies have reported on the efficacy of combined liposomal encapsulated doxorubicin (Doxil or Caelyx, MedEquip, UK) and hyperthermia over Doxil alone.

Objectives: To document the beneficial effect of Doxil-HT over Doxil alone in mice and to investigate the length of time HT should be delivered.

Methods: M/109 lung tumor cells were injected into both leg pads of Balb/c female mice at age of 6–7 weeks. Two weeks later i.v. Doxil in a dose of 8 mg/kg (20–25 μg per mouse) was given and 4 HT sessions (2–3 days apart) were delivered during the subsequent 2 weeks at 2–3 days apart. HT was given to the left pad only for either 5 or 30 minutes (HT5 and HT30 respectively). Five weeks after tumor injection the mice were sacrificed and tumor volume and weight in both pads were measured. Internal comparisons between mice in the same treatment group and comparisons between different treatment cohorts were performed.

Results: In the combined Doxil-HT5 and Doxil-HT30 cohorts the tumor volume and weight in both pads were similar and did not differ from those achieved by Doxil alone. In the Doxil-HT30 cohort the tumor weight, but not the tumor volume, were smaller than those in Doxil-HT5 and Doxil alone (P = 0.006 and 0.01 respectively).

Conclusions: The combined Doxil-HT30 treatment is more effective then Doxil-HT5 or Doxil alone. Additional studies with different time scheduling and different temperatures are warranted.

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Liposomal encapsulated doxorubicin, also known as Doxil or Caelyx (MedEquip, UK), is a formulation of long-circulating, polyethylene (glycol)-coated liposomes containing doxorubicin. It consists of a liquid suspension of single lamellar vesicles with an approximate mean size in the range of 80–90 nm. In contrast to the short distribution half-life of doxorubicin (5 minutes), Doxil is cleared from the plasma with a half-life of 2–3 days [1–3]. Circulating liposomes appear to cross the leaky tumor vasculature and accumulate at high drug concentrations in the interstitial fluid of tumor tissue [3]. Several-fold enhancement of tumor exposure to Doxil has been observed in a variety of syngeneic and human xenograft murine tumor models and in malignant lymphomatous ascites [4,5]. The evidence of Doxil localization in human tumors includes: higher drug levels in skin biopsies of patients receiving Doxil as compared to equal doses of doxorubicin in AIDS-related sarcoma [6], greater drug accumulation in malignant effusions after Doxil treatment [3], imaging of radiolabeled liposomes pointing to concentration in various cancer types [7], and drug levels in breast cancer bone metastases showing a tenfold greater concentration than in adjacent muscle after Doxil injection [8].

Thermosensitive liposomes have been investigated in *in vivo* studies, showing enhancement of liposomal delivery to the tumor site, release of their contents in the target organs, and higher uptake by tumor cells [9–11]. Kong et al. [12] found that extravasation of 100 nm liposomes increased with temperature reaching maximal extravasation at 42°C. Enhanced nanoparticle extravasation was observed several hours after heating and returning back to baseline 6 hours post-heating. Re-heating 8 hours after an initial heating (42°C for 1 hr) did not result in any increased extravasation. These researchers described various types of liposomes based on their heat sensitivity [13,14], and reported on a new lipid formulation containing doxorubicin that had a rapid release of its content following mild hyperthermia. Among the chemotherapy agents that are encapsulated by liposomes are cisplatin, taxol and even tumor necrosis factor [15–17]. The treatment time needed for delivery of hyperthermia is usually 30–60 minutes, based on *in vivo* and clinical studies in which hyperthermia was combined with radiation therapy.

The aim of the present study was to investigate whether treatment time of 5 minutes HT is as effective as 30 minutes when delivered with Doxil.

Materials and Methods

M/109 lung tumor cells in the amount of $1x10^6$ were injected into each of both pads of Balb/c female mice at age 6–7 weeks. Two weeks later when the tumor reached a size of 1–2 cm^3 the mice were grouped into four cohorts (four to six mice in each) according to their treatment assignment.

- Group I (control group): tumor injection only
- Group II (Doxil group): tumor injection and i.v. Doxil at a dose of 8 mg/kg (20–25 μg per mouse) given through the tail vein.
- Group III (Doxil-HT5): tumor and Doxil were given as in the previous groups. HT sessions were delivered for 5 minutes, 2–3 days apart in the subsequent 2 weeks. HT was given to the left pad only.
- Group IV (Doxil-HT30): treatment as in group III except that the HT treatment time continued for 30 minutes.

HT equipment and thermometry

HT was given through a 915 MHz operated system (Medispec, Israel). The applicator size was 2 cm and attached to it, 1 cm apart,

HT = hyperthermia

were two thermometers. This rather small complex was inserted into a thin hollow tube that was attached to the mouse pad. The maximal temperature was set to 44°C. When this maximal temperature was reached the power supply was automatically cut off to be restarted within several seconds to maintain the setting temperature. Quality control of this setting was confirmed in a phantom model (unpublished data).

Tumor measurement

Three-dimensional tumor size was measured before treatment, at every HT session, and at the end of the study. Tumor volume was calculated by simple multiplication of x, y and z. The maximal tumor reduction was usually seen at the end of study and that was the one used for comparison. Tumor weight was measured once at the end of study after dissecting the tumor-bearing pad. Intentionally the study was completed 5 weeks after tumor injection (a week after the last HT session).

Statistical analysis

Two-sided Student's *t*-test was used for comparison between the groups.

Results

The mean tumor volume and weight in the right and left pads in the control group of mice were 1.73 cm^3, 1.95 cm^3, 1.52 g and 1.43 g respectively. As expected, there was no difference between the right and left pads [Table 1]. Measurements of the same parameters in the Doxil group of mice revealed 0.57 cm^3, 0.62 cm^3, 0.84 g and 0.9 g in left and right pads. No difference was noted between the right and left pads (P = 0.28 and 0.37 respectively). Tumor volume and tumor weight in the right and left pads were significantly smaller in the Doxil group than in the control group (P = 0.007, 0.004, 0.002 and 0.003 respectively) [Table 2]. In the combined Doxil-HT5 group the tumor volume and weight in the left and right pads were 0.68 cm^3, 0.59 cm^3, 0.92 g and 0.84 g respectively. No difference was noted between the heated left pad Doxil-HT5 mice and the non-heated left pad Doxil-alone mice (P = 0.06 and 0.24 respectively). In the longer delivered HT (Doxil-HT30) group of mice the tumor volume and weight were 0.59 cm^3, 0.76 cm^3, 0.475 g and 0.55 g in the left and right pads respectively. The tumor reduction in both volume and weight of the left pad did not reach statistical significance (P = 0.29 and 0.32 respectively). Tumor weight in the heated left pads and in the non-heated right pads of mice in the Doxil-HT30 group was remarkably low. Comparison between tumor weight in the left heated pads of the Doxil-HT30 mice and in both Doxil-HT5 and Doxil-alone mice showed a significant tumor reduction in favor of the longer HT treatment (P = 0.01 and 0.006 respectively). Similar comparison between tumor weights of the right pads did not reach statistical significance (0.06 and 0.07, respectively). A summary of tumor volume and weight as well as comparisons within a treatment group and between various groups is presented in Tables 1 and 2.

Discussion

Several randomized studies have confirmed the effectiveness of HT combined with radiation therapy over XRT alone in various tumors [18–22]. The combination of HT and chemotherapy with or without XRT has been evaluated in several phase II-III studies and reports of its effectiveness are pending [23]. Adding HT to heat-sensitive liposomes, mainly Doxil, is appealing. This drug, given alone, is active against various tumors including breast, ovary and sarcoma. The combination of both Doxil and HT was more effective than Doxil alone in several *in vitro* and *in vivo* studies. To date there are few reports on the combination of these two modalities in cancer patients.

The complexity of delivering HT, especially in deep-seated tumors, prevents its wider use. In addition, the treatment time, which is usually 45–60 minutes, makes it even harder to institute in daily practice. This long treatment time is based on preclinical and clinical experience gained in the setting of HT combined with XRT. Since the enhancing effect of HT is quite different in a Doxil-HT setting, the assumption for a longer treatment time is questionable.

In this work there was no difference between Doxil alone and Doxil-HT5 in terms of tumor volume and tumor weight. There was a significant difference in tumor weight, but not in tumor volume, in favor of mice treated with the longer duration HT sessions (Doxil-HT30) [Table 2]. In addition, no difference was noted between the weights of the left heated pad and the right non-heated pad in the

XRT = radiation therapy

Table 1. Tumor volume and weight in right and left pads of mice treated with either Doxil alone or in combination with 5 minute or 30 minute hyperthermia sessions

Treatment type	Mean tumor volume (cm^3)			Mean tumor weight (g)		
	Left pad	Right pad	(*P* value)	Left pad	Right pad	(*P* value)
Control	1.73	1.95	(0.54)	1.52	143	(0.46)
Doxil	0.57	0.62	(0.28)	0.84	0.9	(0.37)
Doxil and hyperthermia (5 min)	0.68	0.59	(0.95)	0.92	0.84	(0.24)
Doxil and hyperthermia (30 min)	0.59	0.76	(0.29)	0.475	0.55	(0.32)

Table 2. Comparisons of volume and weight of right and left pads between control and Doxil-containing regimen

Treatment groups	Volume of left pads *P* value	Volume of right pads *P* value	Weight of left pads *P* value	Weight of right pads *P* value
Doxil vs. control	0.007	0.004	0.002	0.003
Doxil/HT5 vs. Doxil alone	0.06	0.76	0.24	0.46
Doxil/HT30 vs. Doxil alone	0.75	0.64	0.01	0.06
Doxil/HT30 vs. Doxil/HT5	0.23	0.60	0.006	0.07

Doxil-HT30 mice [Table 1]. These findings may suggest a systemic effect of HT (enhancing blood flow to both pads) and possibly more necrosis in the tumor (initiating weight but not volume reduction).

Although this study has its own limitations, the results indicate the need for a longer HT treatment time. Needham and colleagues [14] claimed that various types of liposomes have different times for releasing their contents. They reported on a new temperature-sensitive liposome, tested in a human tumor xenograft model, that needs only seconds to discharge its content to the tumor. Their work is somewhat limited since the HT effect is confined to the release of the liposomal content and will not have an impact on uptake into the tumor cells. Other studies in this interesting field of HT and liposomes did not focus on duration of HT treatment, leaving room for additional studies on the subject.

In summary, Doxil with long duration HT seems to be more effective than Doxil with short duration HT or Doxil alone in this tumor model. Additional studies with different time scheduling and different temperatures are warranted.

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We simultaneously disdain and covet American culture, condemning it as junk food, even as we reach for another helping – a kind of binge-and-puke bulimia

Jonathan Freedland (1967-), British journalist and leader writer for the Guardian, he also worked for the Washington Post