Dear Editor,

The article written by Dr. Sharma and his colleagues: Increased uptake of doxorubicin by cells undergoing heat stress does not explain its synergistic cytotoxicity with hyperthermia, has been read with great interest [1].

In this study, it was found that simultaneous exposure of HCT116 cells to therapeutic hyperthermia (HT) and doxorubicin (Dox) was more effective than sequential exposure (Dox followed immediately by HT and HT followed immediately by Dox). Moreover, compared with the control group which was exposed to Dox at 37°C, HCT116 cells heated at 42°C (exposed simultaneously to the same Dox concentration) exhibited higher intracellular Dox concentration, and plotting thermal enhancement ratio (TER) against the difference in intracellular Dox showed that a large increase in Dox uptake was accompanied by only a modest increase in TER. Thus, the conclusion was drawn that the increased concentration of intracellular Dox under heat stress was an unlikely explanation for the synergistic benefit of the combination treatment. We believe this is a very meaningful and valuable work to suggest the mechanism of the increased sensitivity of Dox after HT. However, we have a few thoughts and suggestions about this study.

Cytotoxicity was determined by clonogenic survival assay in the study of Sharma et al. In the field of radiobiology, clonogenic survival assay is regarded as the gold standard and it directly reflected the ability of surviving cells to retain clonogenicity. Meanwhile, we suggest that more comprehensive and systematic experiments, such as detecting cellular apoptosis by flow cytometry, should be done to assess cytotoxicity after cell intervention. Moreover, in the study of Sharma et al. clonogenic survival assay was performed on 3 cell lines and mass spectrometry was performed on 1 cell line. The conclusion that increased uptake of doxorubicin by cells under heat stress does not explain its synergistic cytotoxicity with hyperthermia is based on the results of only 1 cell line. We suggest more cell lines should be used in the study to verify this finding.

When stimulated by heat, tumor cells undergo a series of stress reactions, such as generating heat shock proteins (HSPs), inducing apoptosis, changing cell membrane compositions, inhibiting DNA damage repair and so on [2–4]. These changes are regarded as an important biological basis for hyperthermia therapy. Studies have indicated that these biological reactions are continuously changing during a period after heat exposure. For example, maximum HSP expression occurred at 16h post-heating and diminished substantially after 72h in normal prostate cells and prostate cancer cells [5]. Heating of liver tumor cells showed that induced apoptosis could last up to 96h and peak at 24h after heat exposure [6]. In a VX2 rabbit tumor model, injection of doxorubicin hydrochloric 24h after thermal ablation could increase tumor coagulation and end-point survival compared with single treatment, and the immunohistochemical results of coagulation margin at 4h and 24h after treatment showed that the expression of cleaved caspase-3 and HSP70 was continuously changing during this period [7]. Since the biological reactions caused by heat stress can last for a relatively long time after heat exposure, the timing of adding chemotherapy may finally affect the synergistic effect. In breast cancer cells, we found that the synergistic cytotoxicity was obvious when Dox was added at 48h after thermal stimulation. This result has not been published and we will submit our final results in the near future. But in the present study, Dox was added immediately or almost simultaneously after heating, which may not achieve the strongest synergistic cytotoxicity.

Overall, time interval after heat stress is an important factor in the combination treatment. We suggest that more measurements using different methods at different times should be considered in this study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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