**Preparation and Characterization of Fatty-Acid Modified Pirarubicin Nanoparticles Stabilized by Albumin**

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**Background and aims.** Pirarubicin (THP) shows faster intracellular uptake, greater antitumor activity, and reduced cardiac toxicity compared to doxorubicin. However, THP is distributed indiscriminately to both tumor and normal tissues. In this study, we chemically modified THP with fatty acids and prepared optimally-sized nanoparticles to enhance tumor accumulation of THP through an anti-solvent precipitation technique. We also evaluated the antitumor activity and biodistribution of the fatty acid-modified THP nanoparticles *in vitro* and *in vivo*.

**Methods.** Fatty acid-modified THPs (FA-THPs), namely Oct-THP, Dod-THP, and Pal-THP, were synthesized by chemically modifying THP with fatty acids—octanoic acid, dodecanoic acid, and palmitic acid—via a hydrazone bond (Figure 1). The nanoparticles were prepared by adding ethanolic solutions of these FA-THPs to an aqueous solution of bovine serum albumin (BSA). Their cytotoxicity in Colon 26 cells, as well as antitumor effects and biodistribution in Colon 26-bearing mice, were then evaluated.



**Figure 1.** Scheme of the synthesis of fatty acid-modified THPs (Oct, Dod, and Pal-THPs) using THP and fatty acid hydrazide.

**Results.** Among the FA-THPs, the most efficiently drug-loaded nanoparticles were obtained from Pal-THP using an aqueous anti-solvent containing BSA as a stabilizer. The Pal-THP nanoparticles were confirmed to be of optimal size (100–125 nm) for delivery to tumor tissues. The nanoparticles disintegrated upon exposure to lecithin, a component of cell membranes, and the hydrazone bond was cleaved in an acidic environment, allowing for the release of THP. The Pal-THP nanoparticles exhibited cytotoxic effects on Colon 26 cells (1). When administered to Colon 26 tumor-bearing mice, these nanoparticles demonstrated improved blood retention, greater tumor accumulation, and enhanced antitumor effects compared to free THP. Additionally, it was confirmed that Pal-THP was converted into THP within the tumor tissue.

**Conclusion/Discussion.** It is therefore proposed that Pal-THP nanoparticles, upon reaching tumor cells after intravenous administration, would exert antitumor effect by liberating pal-THP (i.e., disintegration of the nanoparticles after interaction with the cell membrane), followed by the release of free THP in the acidic milieu of tumor cells. These findings indicate that fatty acid-modified THP nanoparticles, particularly Pal-THP nanoparticles, stabilized by albumin, hold promise as a candidate for cancer treatment.

**References:**

(1) Hasegawa T. et al. (2024) Chem Pharm Bull (Tokyo) 72:21-27