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Camptothecin enhances c-Myc-mediated endoplasmic reticulum stress, leading to cytoprotective autophagy

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Camptothecin (CPT) is known to selectively inhibit the nuclear enzyme DNA topoisomerase I which catalyzes the relaxation of negatively supercoiled DNA and thus leads to cell cycle arrest and apoptosis through DNA damages. However, whether CPT induces endoplasmic reticulum (ER) stress and autophagy has not been clearly understood. The LNCaP cells were cultured at 37°C in a 5% CO_2-humidified incubator and maintained in RPMI 1640 culture medium containing 10% FBS and antibiotics mixtures. Total RNA was isolated from LNCaP cells using Easy-Blue total RNA extraction kit (iNtRON Biotechnology, Sungnam, Republic of Korea.) according to the manufacturer′s instruction. Total cell extracts were separated on polyacrylamide gels and standard procedures were used to transfer them to the nitrocellulose membranes. Cells were seeded on a 24-well plate at a density of 1×10^5 cells/ml and transfected *c-Myc*-, *JNK*-, and *eIF2α*-specific silencing RNA (siRNA, Santa Cruz Biotechnology) for 24 h. Present study first reported that CPT enhanced DNA-binding activity of c-Myc in LNCaP cells according to electronic mobility shift assay and transient knockdown of c-Myc completely abrogated reactive oxygen species (ROS) generation which was resulting in accumulation of ER stress-regulating proteins such as PERK, eIF2α, ATF4, and CHOP. These observations suggests that CPT-induced c-Myc triggered ER stress along with the PERK-eIF2α-ATF4-CHOP pathway by increasing ROS generation. Moreover, CPT promoted formation of autophagy accompanied by increasing autophagic proteins such as beclin-1 and Atg7. Transfection of eIF2α-targeted siRNA attenuated CPT-induced beclin-1 and Atg7 expression. Treatment with autophagy inhibitors such as 3-methyladenine and bafilomycin A1 downregulated relative cell viability in response to CPT, which indicate that CPT induces ER stress-mediated cytoprotective autophagy. Additionally, CPT significantly induced AMPK phosphorylation as a result of intracellular Ca^{2+} release. Moreover, CPT phosphorylated JNK and activated DNA-binding activity of AP-1, and knockdown of JNK abolished the expression level of beclin-1 and Atg7, implying that the JNK-AP-1 pathway is a potent mediator on CPT-induced autophagy. Our findings indicate that CPT promotes ROS-mediated ER stress through the PERK-eIF2α-ATF-4-CHOP pathway, which enhances cytoprotective autophagy, resulting from the Ca^{2+}-AMPK pathway and the JNK-AP-1 pathway.

Keywords*:* Camptothecin, Endoplasmic reticulum (ER), Autophagy