Bisphosphonate induces the mitophagy of osteoblastic cells by forming the chelate with intracellular metal ions

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Extracting teeth of patients treated with bisphosphonate (BP) occasionally induces the necrosis of jaw, but the cause of disease is still unclear. I have proved that the BPs taken into osteoblastic cells were gradually accumulated in lysosomes. In the present study, I investigated the mechanism of BP-induced cytotoxicity in osteoblast, focusing on mitochondria. MC3T3-E1 cells were used as osteoblastic cells. The uptake of BP into cells was observed by fluorescent BP. The intracellular reactive oxygenspecies (ROS) were evaluated by CM-H_2-DCFDA. Detection of autophagy and mitophagy was used DALGreen and mtphagy dye, respectively. The intracellular Ca^{2+} and mitochondrial Fe^{2+} were measured by Fluo 4-AM and Mito-FerroGreen, respectively. Zoledronate (ZD) impaired cells dose-dependently. BP taken into cells was accumulated into lysosomes. MC3T3-E1 cells were always occurred autophagy flux, but bafilomycin A1(BM), a lysosome inhibitor induced cell death, by inhibiting autophagy flux. ZD slightly suppressed the autophagy flux, however the combination of BM and ZD strongly enhanced cell death. ZD decreased intracellular Ca^{2+} and mitochondrial Fe^{2+}, and inhibited the response of intracellular ROS generation by oxidative stress, resulting in promotion of mitophagy. These results suggest that BP may form the chelate with Ca^{2+} and Fe^{2+}, and promote mitophagy of damaged mitochondria. Furthermore, the accumulation of BP into lysosomes indicates to induce cell death by inhibiting the autophagy flux.