Hypoxia Potentiates the Cytotoxic Effects of Nitrogen-Containing Bisphosphonates in Human Esophageal Cancer Cell Line

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Purpose

Tumor hypoxia, an important obstacle to effective cancer therapy, is related to angiogenesis, metastasis, and therapeutic resistance in cancer cells, and contributes to poor prognosis. Therefore, it is necessary to develop hypoxia-targeted cancer treatments. Bisphosphonates (BPs) have been widely used to treat osteoporosis and cancer metastasis to the bone. Nitrogen-containing BPs (N-BPs), namely second- and third-generation BPs, inhibit enzymes of the mevalonate signaling pathway such as farnesyl pyrophosphate synthase and geranylgeranyl pyrophosphate (GGPP) synthase. Recently, BPs, especially N-BPs, were found to show anticancer activity in various cancer cell types in vitro and in vivo under normoxia. However, it is unknown if N-BPs can inhibit the growth of cancer cells, including esophageal cancer (EC) cells, under hypoxic conditions. In this study, to clarify the efficacy of N-BPs under hypoxic conditions, we investigated the effect of hypoxia on the growth inhibitory effects of N-BPs and other mevalonate pathway inhibitors such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, in a human EC cell line.

Methods

We used KYSE150 cell line. Two anti-cancer drugs (5-fluorouracil; 5-FU and cisplatin; CDDP), four N-BPs (alendronate; ALE, pamidronate; PAM, risedronate; RIS, and zoledronate; ZOL), and two HMG-CoA reductase inhibitors (atorvastatin; ATO, simvastatin; SIM) were used in this study. Hypoxia (1% O2) was induced by a multi-gas incubator. Cell viability was detected by means of CellQuanti-Blue™ Cell Viability Assay Kit, and the concentration producing 50% growth inhibition (IC50) was calculated. ZOL-induced apoptosis was measured by annexin V-FITC and propidium iodide staining. Hypoxia-inducible factor-1α (HIF-1α) protein expression levels were determined by western blotting.

Results

5-FU, CDDP, and N-BPs showed concentration-dependent growth inhibitory effects under normoxic and hypoxic conditions. 5-FU and CDDP sensitivity was not different under both conditions, but N-BPs sensitivity under hypoxia was significantly higher than in normoxia. The IC50 values for N-BPs were significantly lower in hypoxic conditions than in normoxic conditions. Sensitivity to ALE, PAM, RIS, and ZOL (Fig. 1) was 3.80-, 2.57-, 2.99-, and 1.95-fold higher, respectively, under hypoxia than under normoxia. Similar results were observed for the HMG-CoA reductase inhibitors, ATO and SIM. Hence, it is suggested that mevalonate pathway inhibitors show cytotoxicity in EC cells in hypoxic conditions via a unique mechanism that chemotherapeutic drugs do not possess. HIF-1α protein was not observed in normoxia and was induced in hypoxia. ZOL treatment did not affect the observed levels of HIF-1α protein in hypoxia and significantly increased the number of annexin V-positive cells in both normoxia and hypoxia. The number of annexin V-positive cells after ZOL treatment was significantly higher in hypoxia (54.2 ± 2.36%) than in normoxia (42.1 ± 3.68%) (Fig. 2). The growth inhibitory effects of ZOL and SIM were completely abolished by co-treatment with GGOH, a precursor of GGPP, in both normoxia and hypoxia. Therefore, the enhanced cell growth inhibition by the mevalonate pathway inhibitors in hypoxia can be explained by the augmentation of apoptosis induction and mevalonate pathway inhibition.

Conclusion

The cytotoxic effects of N-BPs and HMG-CoA reductase inhibitors are related to GGPP inhibition in the mevalonate pathway, and are potentiated under hypoxia. Therefore, mevalonate pathway inhibitors are promising anticancer agents in both normoxia and hypoxia.