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BERBERINE-INDUCED ANTICANCER EFFECTS IN SQUAMOUS CELL CARCINOMA OF ORAL TONGUE CELLS

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Squamous cell carcinoma of oral tongue belongs to highly invasive malignancies of head and neck area. The natural isoquinoline alkaloid berberine has demonstrated anti-tumor potential in various cancer types. This research was aimed to study *in vitro* effects of berberine in squamous cell carcinoma of oral tongue. Berberine reduced the proliferation of CAL-27 cells in a dose-dependent manner (IC50 = 36μM). Furthermore, berberine strongly inhibited invasion of squamous cell carcinoma of oral tongue cells (EC50 = 0,9μM). Anti-proliferative and anti-invasive actions displayed by berberine in CAL-27 cell line suggest the *in vitro* anticancer efficacy of the natural isoquinoline alkaloid.

Key words: Squamous cell carcinoma of oral tongue, berberine, CAL-27, invasion

Oral cancer is a serious problem in many parts of the globe, which remains a significant cause of morbidity and mortality, accounting annual estimated incidence around 300,000 [1]. Two-thirds of these cases occur in developing countries where smoking and alcohol abuse are considered to be the major etiological factors [2,3]. Squamous cell carcinoma of oral tongue (SCCOT) is a highly invasive and metastatic malignancy in head and neck area [4]. In spite of improvements in diagnostic and therapeutic techniques, the 5-year-survival rate of patients with this diagnosis has not improved for the last decades [5]. For this reason, application of novel therapeutic agents that could eliminate progression of SCCOT is strongly required.

Berberine is a natural isoquinoline alkaloid, whose biological effects in treating diabetes, hypertension, arrhythmias, digestive and inflammatory diseases have been widely investigated [6-8]. To date, an increasing amount of research has involved studies of berberine's anticancer properties [9-12]. In our previous research we have clarified that berberine possesses anticancer effects in hepatocellular carcinoma via activation of pyroptosis [13]. However, the impact of berberine on cancer cell viability and invasion of SCCOT has not been clearly determined. Therefore, this study was carried out to explore anti-tumor potential of the natural isoquinoline alkaloid in SCCOT.

Material and methods

Drug preparation and treatments.

The Berberine Chloride (Figure 1) was purchased from SIGMA (PHR1502, LOT LRAA9232). The stock solution of berberine was prepared by dissolving isoquinoline alkaloid in DMSO, following by autoclaving. For experimental needs three dilutions have been used in low (1μM), medium (10μM) and high concentrations (100μM).

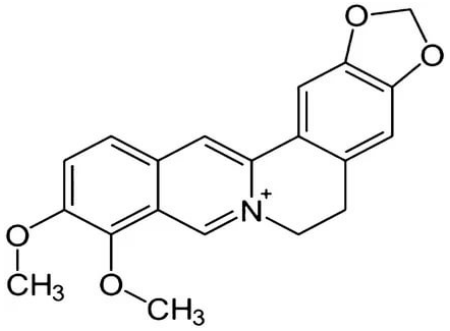


Fig. 1. Chemical structure of berberine

Cell culture. The human SCCOT cell line, CAL-27, was purchased from the American Type Culture Collection (ATCC) and maintained in DMEM (GE Healthcare HyClone, USA) containing 10% fetal bovine serum (Biowest, USA) at 37℃ in an atmosphere containing 5% CO2.

Cell viability assay by cell counting kit-8. CAL-27 cells were seeded at a 1x105 cells/well in 96-well plates, and incubated overnight. After attachment to the well, cells were then treated with indicated concentrations of berberine (1, 10, 100 μg/ml) for 48 h. Consequently, the medium was replaced by 100μL of fresh medium containing 10% Cell counting kit-8 (CCK-8, WST-8, Dojindo Laboratories, Tokyo, Japan), and the cells were incubated at 37℃ for another 1 hour. The optical density (OD) was measured by microplate reader at 450 nm wavelength. The experiment was performed three times.

Boyden chamber cell invasion assay. Following pretreatment with different concentrations of berberine, CAL cells were harvested, seeded into Corning Matrigel Invasion Chambers (Corning, Bedford, MA, USA) with 8 μm pore-sized filters at 1x105 cells/well in FBS-free medium, and incubated for 24 hours at 37℃ in humidified atmosphere. 10% serum-medium was used as a chemoattractant in the bottom chamber. Afterwards, the invaded cells were fixed with 4% par-

aformaldehyde and stained with 0.1% crystal violet. Non-invaded cells on upper surface of Transwell membranes were removed by cotton swab. The images of invaded cells were obtained under phase-contrast microscope at the five random fields and proceeded to Image-J software for cell quantification. Each experiment was conducted in triplicate.

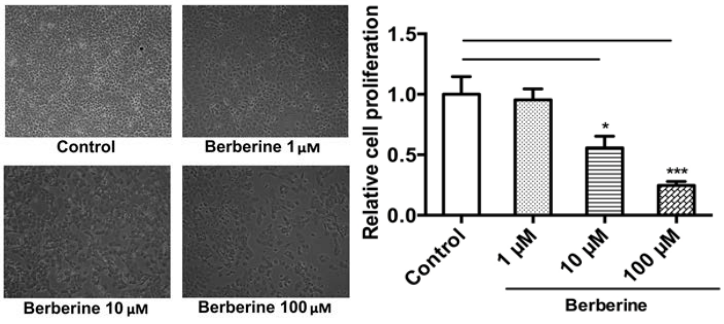
Statistical analysis. The results are presented as mean ± standard deviation (SD) from at least three independent experiments and analyzed by analysis of variance (ANOVA), two-tailed P<0,05 was considered statistically significant. All data in treatment groups were normalized to the results in control cells.

Results

Berberine-induced inhibition of oral cancer cell proliferation. We first studied the berberine's effect on cell proliferation. When the berberine-treated CAL-27 cells were examined under phase-contrast microscopy, it was observed that in treatment groups the cell density was decreased compared to control cells (Figure 2A). In order to confirm whether berberine possess anti-proliferative effect in CAL-27 cells, the CCK-8 kit was applied. The results shown in Figure 2B demonstrated that natural isoquinoline caused a dose-dependent suppression of cell proliferation.

The results of cell viability assay have led us to construct the growth inhibition curve and identify the IC_{50}, inhibitory concentration for berberine in CAL-27 cells. As it is illustrated on the Figure 3, the IC_{50} value for berberine was 36μM.

Suppression of CAL-27 cells invasion by berberine treatment. Invasive capacities of cancer cell define the aggressiveness of malignancy. Therefore, we next examined the behavior of berberine-pretreated cells in Matrigel coated Transwell chambers. It was found that invasion of CAL-27 cells was inhibited by more than 50% in treatment groups, indicating a strong anti-invasive effect of natural isoquinoline (P<0.001).



A B

Fig. 2: Inhibition of cell proliferation by berberine in CAL 27 cells. A. Phase contrast images of CAL-27 cells treated with different concentrations of berberine for 48 hours at 100X magnification. B. Oral cancer cells treated with berberine (1μM, 10μM and 100μM) for 48h. Cell proliferation was measured by CCK-8 assay. *P < 0,5 versus Control, ***P < 0,001 versus Control, n=6

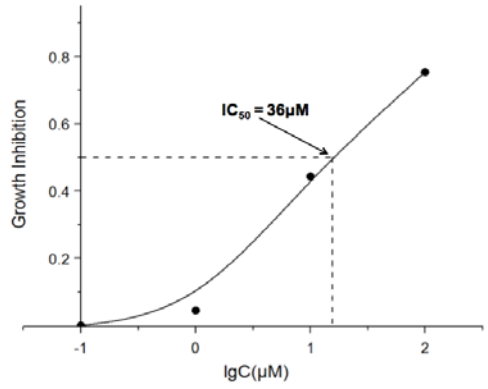


Fig. 3. IC_{50} of berberine on CAL-27 cells viability inhibition.

We next determined the EC_{50}, efficient concentration, for berberine in invasion inhibition of CAL-27 cells. Figure 5 demonstrates that EC_{50} of berberine, required to achieve the half of the maximal invasion inhibition level, hardly exceeded 1μM.

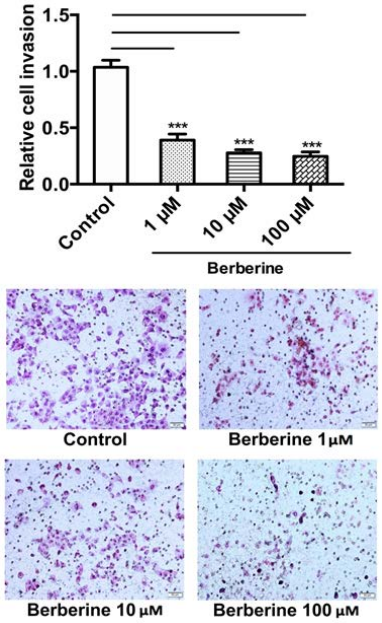


Fig. 4. Berberine-induced suppression of oral cancer cell invasion. Phase contrast images of invaded CAL-27 cells pretreated with berberine for 48h at 200X magnification. The number of invaded cells was analyzed by the software Image-J. *** P< 0,001 versus Control, n=3.

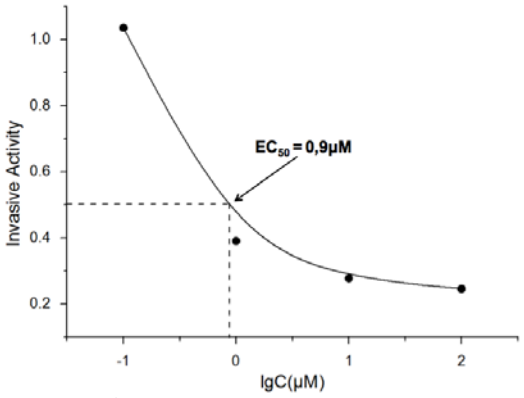


Fig. 5. EC_{50} of berberine on CAL-27 cells invasion inhibition.

Discussion

The present study aimed to explore the anti-cancer effects of berberine in SCCOT. At first, the anti-proliferative potential of the natural isoquinoline alkaloid in SCCOT cell line was examined. We have demonstrated that CAL-27 cells were susceptible to the berberine's treatment resulting in suppression of cancer cell viability with IC_{50} value 36μM. These in vitro findings indicate that the isoquinoline alkaloid could produce anti-SCCOT effects by mitigating the cancer proliferation.

Numerous reports showed that the ability of SCCOT to invade in surrounding tissues is an essential indicator that determines the prognosis of malignant process [14]. In clinical aspect for improvement of patients' survival it is highly important to eliminate bone resorption caused by invasion of SCCOT. Therefore, we have focused our research on anti-invasive characteristics of berberine. We have figured out that invasive capacities of CAL-27 cell were repressed in berberine-treatment groups. Although the results of Transwell invasion assay were limited due to obtained strong anti-invasive effects, we could identify that less than 1μM of berberine is enough to obtain the half of the maximal drug's effect.

Growing evidence suggests the involvement of various microRNAs in the pathogenesis of cancer development. MicroRNAs are short noncording RNA molecules that could interfere mRNA translation [15]. Moreover, it was demonstrated that berberine's antitumor effects could be explained by its interaction with cancer-related micro-RNAs. In one study berberine was shown to upregulate miR-22-3p to suppress hepatocellular carcinoma proliferation [16]. Another research disclosed microRNA-21 as a mediator of berberine-induced apoptotic functions in myeloma cells [17]. Therefore, we hypothesize that berberine-induced anticancer effects in SCCOT could be ascribed to regulation of cancer-related micro-RNAs. For better understanding the underlying mechanisms of berberine's antitumor effect in SCCOT, further investigations should be carried out.

Conclusions

This study shows an inhibitory effect of berberine on proliferation and invasion of SCCOT cells, suggesting that the use of the natural isoquinoline can be a potential therapy for this malignancy. However, it is essential to confirm the appropriate dosage of berberine treatment for conducting further in vivo and clinical trials.

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REFERENCES

1. Parkin D.M., Bray F., Ferlay J., Pisani P. Global cancer statistics, 2002 // CA Cancer journal for clinicians. 2005. Vol. 55. P. 74–108.
2. Argiris A., Karamouzis M.V., Raben D., Ferris R.L. Head and neck cancer // Labcet. 2008. Vol. 371. P. 1695-1709.
3. Brennan, J., Boyle, J., Koch, W., Goodman, S., Hruban, R., Eby, Y., Couch, M., Forastiere, A., and Sidransky, D. (1995). Association between cigarette smoking and mutation of the p53 gene in head and neck squamous carcinoma // New England Journal of Medicine. 1995. Vol. 332. P. 712–717.
4. Mao L., Hong W.K., Papadimitrakopoulou V.A. Focus on head and neck cancer // Cancer Cell. 2004. Vol. 5. P. 311–316.
5. Pereira M, Oliveira D, Landman G and Kowalski L. Histologic subtypes of oral squamous cell carcinoma: prognostic relevance // J Can Dent Assoc. 2007. Vol. 73. P. 339-344.
6. Ni W.J., Ding H.H. and Tang L.Q. Berberine as a promising anti-diabetic nephropathy drug: An analysis of its effects and mechanisms // Eur J Pharmacol. 2015. Vol. 760. P. 103-112.
7. Tang J., Feng Y., Tsao S., Wang N., Curtain R. and Wang Y. Berberine and Coptidis rhizoma as novel antineoplastic agents: a review of traditional use and biomedical investigations // J Ethnopharmacol. 2009. Vol.126. P. 5-17.
8. Lau C.W., Yao X.Q. and Chen Z.Y., Ko W.H. and Huang Y. Cardiovascular actions of berberine // Cardiovasc Drug Rev. 2001. Vol. 19. P. 234-244.
9. Ortiz L.M., Lombardi P., Tillhon M. and Scovassi A.I. Berberine, an epiphany against cancer // Molecules. 2014. Vol. 19. P. 12349-12367.

10. Lin C.C., Kao S.T., Chen G.W., Ho H.C. and Chung J-G. Apoptosis of human leukemia HL-60 cells and murine leukemia WEHI-3 cells induced by berberine through the activation of caspase-3 // Anticancer Res. 2006. Vol. 26(1A). P. 227-242.
11. Jabbarzadeh Kaboli P., Rahmat A., Ismail P., Ling K.H. Targets and mechanisms of berberine, a natural drug with potential to treat cancer with special focus on breast cancer // Eur. J. Pharmacol. 2014. Vol. 740. P. 584–595.
12. Qi H.W., Xin L.Y., Xu X., Ji X.X., Fan L.H. Epithelial-to-mesenchymal transition markers to predict response of Berberine in suppressing lung cancer invasion and metastasis // J Transl Med. 2014. Vol. 12. P. 22
13. Chu Q., Jiang Y., Zhang W., Xu C., Du W., Tuguzbaeva G., Qin Y., Li A., Zhang L., Sun G., Cai Y., Feng Q., Li G., Li Y., Du Z., Bai Y., Yanf B. Pyroptosis is involved in the pathogenesis of human hepatocellular carcinoma // Oncotarget. 2016. Vol.7(51). P.84658-84665.
14. Haddad R.I., Shin D.M. Recent advances in head and neck cancer // New England Journal of Medicine. 2008. Vol. 359. P. 1143–1154.
15. Pasquinelli A.E. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship // Nature Review Genetics. 2012. Vol.13. P. 271-282.
16. Chen J., Wu F.X., Luo H.L., Liu J.J., Luo T., Bai T., Li L.Q., Fan X.H. Berberine upregulates miR-22-3p to suppress hepatocellular carcinoma cell proliferation by targeting Sp1 // Am J Transl Res. 2016. Vol. 8(11). P. 4932-4941.
17. Hu H.Y., Li K.P., Wang X.J., Liu Y., Lu Z.G., Dong R.H., Guo H.B., Zhang M.X. Set9, NK-kB, and microRNA-21 mediate berberine-induced apoptosis of human multiple myeloma cells // Acta Pharmacol Sin. 2013. Vol. 34(1). P. 157-166.