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NO_2^- and NO_3^- enhance cold atmospheric plasma induced cancer cell death by generation of $ONOO^-$

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Cold atmospheric plasma (CAP) is a rapidly developed technology that has been widely applied in biomedicine especially in cancer treatment[1]. Due to the generation of various active species in plasma, CAP could induce various tumor cells death and showed a promising potential in cancer therapy. By far it is considered that the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in plasma are the main factors[2]. Among these species, hydroxyl radical (OH), hydrogen peroxide (H_2O_2), ozone (O_3), superoxide anion (O_2^-), nitric oxide (NO), and peroxynitrite anion ($ONOO^-$) are the main components related to biological effects induced by CAPs[3]. $ONOO^-$ is a potent oxidizing and nitrating specie formed from a diffusion-controlled reaction between O_2^- and NO, which could penetrate bilayer lipid membrane and disturb the function of mitochondrion and consequently influence cell metabolism and cause DNA damage leading to cell death[4]. Our previous study also demonstrated the involvement of $ONOO^-$ in the induction of apoptosis by N_2/O_2 plasma jet[5].To enhance the biological effects of gas plasma, changing the discharging parameters is the most commonly used method, yet increasing discharging power will lead to a higher possibility of simultaneously damage surrounding tissues.

In this study, by adding nontoxic concentration of additional nitrite and nitrate, we found that more nitrogen supplies benefited for the production of RNS especially $ONOO^-$ and resulted in a better killing effect to cancer cells. We found both NO_2^- and NO_3^- (at two different concentration, 10 μM and 50 μM) could significantly enhance cell viability reduction that was induced by He plasma in myeloma LP-1 cells and leukemia Molm-13 tumor cells. Furthermore, 50 μM of NO_2^- and NO_3^- showed a better synergistic effect with He plasma than that of 10 μM. As demonstrated in our previous study, NO and O_2^- are two of the main substrate to produce $ONOO^-$, so we monitored intracellular and extracellular NO and O_2^- level and found that He plasma treatment could significantly increase extracellular NO and O_2^- level. Our results showed that NO_2^- and NO_3^- could enhance the cytotoxity of He plasma treatment on myeloma and leukemia tumor cells by the accumulation of $ONOO^-$.Our results provided a new strategy to enhance the killing effect by plasma jet treatment without changing the discharging conditions.

References

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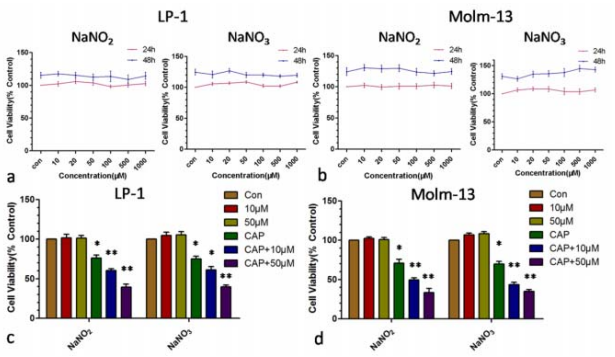

Figure 1: Cytotoxicity of $NaNO_2$, $NaNO_3$ and their synergies with plasma on tumor cells. NO_2^- and NO_3^- could enhance the cytotoxity of plasma treatment and higher concentration of NO_2^- and NO_3^- showed a better synergistic effect with plasma treatment.