Urease-mediated alkalization of tumor microenvironment and its effects on T cell proliferation, cytokine release, and PD-1/PD-L1 interactions

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INTRODUCTION

Solid tumors become ischemic due to a reduced blood supply and abnormal metabolic processes. As a consequence, lactate levels tend to be higher than normal within and around tumors and the pH tends to be low. The acidic microenvironment is key for cancer progression as it promotes the invasiveness and metastatic behaviors of cancer cells. In addition, it protects cancer cells from immunotherapy by supressing the proliferation and cytotoxic activities of local immune effector cells. Thus, treatments that raise the pH of the tumor microenvironment are predicted to reactivate anti-tumor immune responses. This has been tested by others, who have previously reported that treatments with bicarbonate or bases that neutralize the tumor microenvironment can in fact help improve responses to immunotherapy. In this study, we present a novel method to raise the pH of the tumor microenvironment, using the previously described antibody-urease conjugate, L-DOS47.

L-DOS47 is currently in Phase I/II testing for treatment of non-small cell lung cancer. It is prepared by conjugating urease to the camelid single domain antibody specific for human CEACAM6. The immunoconjugate specifically targets and delivers urease to CEACAM6-expressing cancer cells, where the urease enzyme converts urea into cytotoxic ammonia. The ammonia also increases the pH of the tumor microenvironment *in situ*.

In this study, L-DOS47 was used to augment the extracellular pH of acidified culture media that mimics the tumor microenvironment *in vitro*, and the effects on the human T lymphoblastoid cell line, Jurkat Clone E6-1 were examined.

RESULTS

L-DOS47: A urease-based alkalization reagent

Table 1: Hydrolysis of urea by the urease moiety of L-DOS47 produces ammonia [$NH_2CONH_2 + H_2O \rightarrow CO_2 + 2NH_3$], which converts into ammonium ions and augments pH in aqueous medium [$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$]. The data show that in the presence of 1µg/mL L-DOS47, 2-4mM of urea is sufficient to restore the pH of lactic acid-treated RMPI 1640 medium (supplemented with 5% heat-inactivated FBS and GlutaMax) to physiological levels after an 18-hour incubation at 37°C and 5% CO_2.

Lactic acid (mM)	pH					
	Time 0	Time 18h	1µg/mL L-DOS47 (Time 18h)			
			No urea	2mM urea	4mM urea	8mM urea
0	7.58	7.22	7.39	7.45	7.55	8.16
6	7.03	7.24	7.32	7.43	7.52	7.66
12	6.53	6.99	7.21	7.34	7.45	7.60

Effects of lactic acid on Jurkat cell proliferation

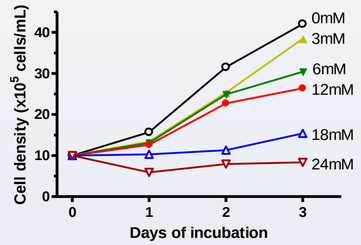


Figure 1: Jurkat cells (1x10^6 cells/mL) were incubated in complete RPMI 1640 medium containing various amounts of lactic acid (3 to 24mM) for 1-3 days. Cell count was performed using a hemocytometer after Trypan Blue staining. The results show that lactic acid prohibits Jurkat cell proliferation at concentrations ≥3mM. A similar growth inhibition profile was observed when lactic acid was replaced with the same concentrations of HCl (data not shown).

Effects of lactic acid on IL-2 release in activated Jurkat cells

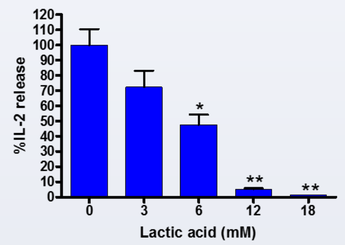


Figure 2: Jurkat cells (5x10^6 cells/mL) were activated by incubation in complete RPMI medium containing 2.5µg/mL PHA, 50ng/mL PMA, and 0.75µg/mL Ionomycin at 37°C for 24 hours. IL-2 released by the activated Jurkat cells was measured using a sandwich ELISA. It was found that lactic acid at concentrations ≥6mM caused a significant decrease in IL-2 production (* $p < 0.05$ and ** $p < 0.01$ as compared to the control).

Protective effects of L-DOS47/urea on Jurkat cells cultivated in lactic acid-treated medium

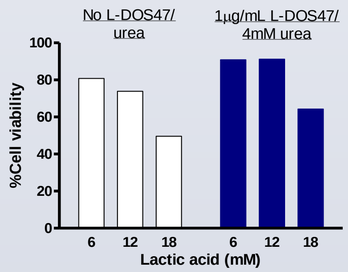


Figure 3: Jurkat cells (3x10^6 cells/mL) were incubated in complete RPMI 1640 medium containing 6 to 18mM of lactic acid for 1 day. Cell count was performed using a hemocytometer after Trypan Blue staining. Cell proliferation was found to be reduced by 20% to 50% (open bars). Addition of L-DOS47 (1µg/mL) and urea (4mM) suppressed the growth inhibitory effects of lactic acid and increased cell viability (solid bars).

Restoration of IL-2 production in lactic acid-treated Jurkat cells

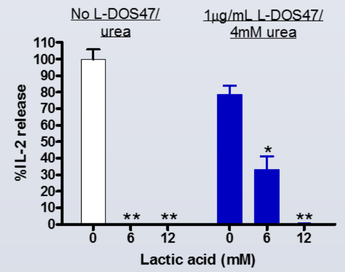
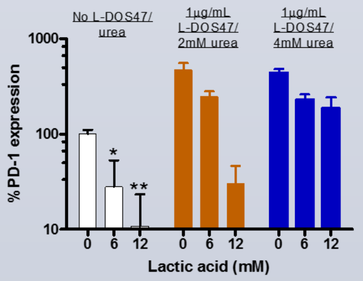


Figure 4: Lactic acid inhibited IL-2 production in Jurkat cells stimulated with 2µg/mL PHA and 50ng/mL PMA, which was partially restored by addition of 1 µg/mL L-DOS47 and 4mM urea in medium containing 6mM lactic acid. At a higher acid concentration (12mM), the tested L-DOS47 and urea combination are insufficient to restore IL-2 release. (* $p < 0.05$ and ** $p < 0.005$ as compared to the blank medium).

Restoration of PD-1 expression in lactic acid-treated Jurkat cells

Figure 5: Expression of PD-1 on Jurkat cells was evaluated by whole-cell ELISA. The cells were stimulated to express PD-1 receptor by immobilized anti-CD3 antibody and soluble anti-CD28 antibody (data not shown). Addition of lactic acid significantly reduced PD-1 expression (open bars, * $p < 0.05$ and ** $p < 0.005$), while addition of L-DOS47/urea greatly enhanced PD-1 expression (solid bars).



Interferon gamma-stimulated tumors and their effects on IL-2 release from activated Jurkat cells

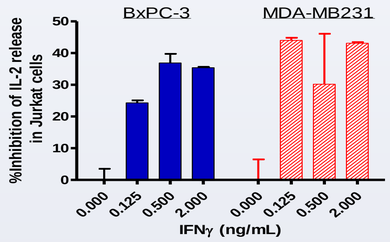


Figure 6: BxPC-3 and MDA-MB231 tumor cells were stimulated with various concentrations of IFNγ for 2 days. After removal of the original media, activated Jurkat cells were added and co-cultured with the tumor cells for 24 hours at 37°C. The results show that IFNγ stimulated tumor cells inhibit IL-2 release in Jurkat cells by as much as 40%.

CONCLUSIONS

- An acidic microenvironment has several immuno-inhibitory effects on activated Jurkat cells, including the inhibition of cell proliferation, IL-2 production, and expression of PD-1 at the cell surface
- Addition of L-DOS47 and urea to the media raises the extracellular pH, and also partially restores levels of Jurkat cell proliferation, IL-2 production, and surface expression of PD-1
- Tumor cells pre-treated with IFNγ also inhibit production of IL-2 from activated Jurkat cells, possibly due to activation of PD-L1 expression that binds to the immune checkpoint receptor PD-1 on Jurkat cells. Future work will focus on understanding the mechanism of this inhibition.

REFERENCES

- Ibrahim Hashim A, Zhang X, Wojtkowiak JW, Martinez GV, Gillies RJ. Imaging pH and metastasis. NMR Biomed 2011;24: 582–91.
- Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004; 4:891–9.
- Tian B, Wong WY, Hegmann E, Gaspar K, Kumar P, Chao H. Production and characterization of a camelid single domain antibody-urease enzyme conjugate for the treatment of cancer. Bioconjug Chem. 2015; 26(6):1144-55.
- Pilon-Thomas S, Kodumudi KN, El-Kenawi AE, Russell S, Weber AM, Luddy K, Damaghi M, Wojtkowiak JW, Mulé JJ, Ibrahim-Hashim A, Gillies RJ. Neutralization of Tumor Acidity Improves Antitumor Responses to Immunotherapy. Cancer Res. 2016;76(6):1381-90.

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