Niclosamide Stearate Prodrug Therapeutic (NSPT) Enhances Mitochondrial Proton Leak and Induces Potent Cytotoxicity in Osteosarcoma

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Purpose

This study evaluates the bioenergetic effect of NSPT in OS mitochondria, a prodrug that increases the bioavailability of niclosamide, on murine and human OS cells.

Methods

DEVELOPMENT OF NSPT

- Rapid solvent exchange packages niclosamide into particles surrounded by lipid.
- Particle size was measured to ensure pharmaceutical quality utilizing dynamic light scattering (Figure 1).

RESULTS

NSPT can reduce tumor burden similar to that of the standard of care therapy Doxorubicin.

CONCLUSIONS

- Examination of the MOA of niclosamide established the presence of increased proton leak, which is consistent with its proposed MOA as a shuttle of protons across the membrane.
- NSPT is able to induce apoptosis across two human and two canine cell line models showing the ability to induce apoptosis is conserved and may be efficacious in most types of OS.
- An ex vivo model representing established metastatic OS was created and showed that NSPT is as efficacious as the current standard of care therapy, Doxorubicin, and that it may be efficacious in treating established metastatic disease.
- This study, in conjunction with the Ewarb Lab’s canine study, supports further in vivo testing with NSPT and supports future human trials.
- Finally, NSPT could replace or reduce the amount of doxorubicin needed for treatment of OS, which might reduce cardiotoxicity and extend life.

DISCUSSION

- OSs rely on a larger proportion of aerobic glycolysis for their energy needs than normal cells.
- Aerobic glycolysis may be advantageous by allowing OS cells to shun access into many precursors important for growth.
- However, a reliance on aerobic glycolysis may leave OS cells energetically vulnerable in that they have very little reserve if their basal energy production is disrupted.
- Therefore, a chemotherapeutic that targets the mitochondria may be efficacious in OS and other cancer cells.
- Further, the results of our ex vivo experiment may show that NSPT could be efficacious in established metastatic OS.

REFERENCES

- Mark M Cullen, BS1, BS2, Ettiene M Flamant, BS3, BS4, BS5, Philip H Khoury, BS6, Halley E Bright, BS7, PhD7, David Kerr, MD8, Michael Global, MD9, James M Wexler, MD7, PhD9, Giresse B, Reddy, MD7, Harry O. Lawton, MD10, Sarah M Plumlee, BS11, Sarah M Plumlee, BS11, Beatrice C Thomas13, BS14, BS15, Isabelle Byers15, BS6, Kathryn E Ware, PhD9, Suzanne Bartholdi Dewitt, DVM, PhD9, Juliana D Visgauss, MD8, Brian E Bigman, MD16, Jason A Somarelli, PhD16, Mark Needham, PhD17, William C Eward, DVM, MD18.

-Figure 1: A Diagram of the proposed mechanism of action of NSPT and, thus, niclosamide, on the inner mitochondrial membrane. It is thought that niclosamide acts as a shuttle, carrying protons from the matrix to the intermembrane space. B) Mitochondrial stress test after 3 hours of treatment showing that all cell lines tested demonstrated a significant increase in proton leak. This supports the niclosamide treatment used in panel A. C) ECAR, a measure of aerobic glycolysis, for each of the cell lines. The canine cell lines appeared to have an increase in aerobic glycolysis. This pattern was not observed in the human cell lines.

-Figure 2: A) Diagram of the proposed mechanism of action of NSPT and, thus, niclosamide, on the inner mitochondrial membrane. It is thought that niclosamide acts as a shuttle, carrying protons from the matrix to the intermembrane space. B) Mitochondrial stress test after 3 hours of treatment showing that all cell lines tested demonstrated a significant increase in proton leak. This supports the niclosamide treatment used in panel A. C) ECAR, a measure of aerobic glycolysis, for each of the cell lines. The canine cell lines appeared to have an increase in aerobic glycolysis. This pattern was not observed in the human cell lines.

-Figure 3: A) OS cell lines treated with either control or NSPT and a caspase 3/7 fluorescent markers in all cell lines, the level of caspase 3/7 activity was markedly increased in the treatment group compared to the control. B) Significant differences were observed in the green image mean, indicating level of apoptosis, in the cell lines over a 24-hour period. C) Left: One of the panel assays that the number of viable cells in the treatment group was significantly lower than that of the control group.

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-Figure 4: A) NSPT’s tumor burden is significantly reduced when compared to NSPT control B) NSPT can reduce tumor burden similar to that of the standard of care therapy Doxorubicin.

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