Adenylosuccinic acid therapy for the treatment of Duchenne muscular dystrophy: a pre-clinical evaluation of safety and efficacy

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Introduction: Duchenne muscular dystrophy (DMD) is a fatal genetic disease involving the progressive damage and and wasting of skeletal muscles, including the diaphragm, as well as cardiac muscle. There is no curative therapy, and the only approved drug treatment is corticosteroids which have serious side effects. In a pre-clinical evaluation using the mdx mouse model of DMD, we have previously demonstrated that oral adenylosuccinic acid (ASA) administration attenuates skeletal muscle damage and the deposition of non-functional fat and fibrotic tissue, which are characteristic features of the disease. The aim of this study is to establish acute safety data to enable the translation of ASA therapy into human clinical trials within the next 5 years.

Methods: Animal experimentation was approved by the Victoria University Animal Ethics Committee and performed in accordance with the Australian Code of Practice for the Care and use of Animal for Scientific Purposes. Using the Organisation for Economic Co-operation & Development (OECD)'s "up and down" method of acute toxicity testing, we have evaluated the safety of oral ASA administration in female mice. Adult female mice were dosed with scaled dosages of ASA (175, 550, 1750 and 5000mg/kg) by oral gavage and monitored daily for observable signs of toxicity for 14 days. The dose was only increased when no signs of toxicity were observed in the first 48 hours. An untreated mouse was included in the analyses as a phenotype and environmental control to validate reference values. Blood was removed from live mice *via* tail venipuncture and urine was collected where possible for biochemistry analysis. Thereafter, mice were humanely culled *via* CO_2 asphyxiation and a full necropsy, histopathology of the gastrointestinal tract, liver and kidneys was performed. All data was certified by a veterinary pathologist.

Results: All mice survived up to the maximum testable dosage of 5000mg/kg stipulated by the OECD. We observed no adverse effects of ASA, save for mild diarrhoea in the first 12 hours following administration of the 5000mg/kg dose. Necropsy, tissue histopathology and blood biochemistry results revealed no signs of toxicity induced by ASA.

Conclusions: Since a dosage of 5000mg/kg of was well tolerated in mice, ASA can be considered a non-toxic compound. Together with our pre-clinical efficacy data, this study enables leverage into the pharmaceutical sector for thorough GLP-certified testing and optimal dosage evaluation prior to human trials.