final report

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‘Biologically Active Glycoconjugate Product from Red Meat Processing – Phase III’

Development of a Pet Food Joint Care Nutraceutical

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Background

The aim of this project was to develop a next generation nutraceutical for the treatment of inflammatory diseases such as osteoarthritis (OA), and rheumatoid arthritis (RA). The initial target market is a veterinary nutraceutical as time to market is short due to the lack of regulatory hurdles (relative to the human therapeutic market). Further, links are already established between pet food manufacturers and meat processors which should help to facilitate rapid adoption. However, whilst this project focused on the development of veterinary nutraceutical, it should be appreciated that this has been adopted as a lower risk strategy providing a 'stepping stone' to the larger and more lucrative human nutraceutical and therapeutic markets.

Inflammatory diseases include various forms of Arthritis, Lupus, Gout and Scleroderma. There is currently no cure for an inflammatory disease and the worldwide market for treatments is currently worth in excess of $50Bn. Arthritis represents a group of inflammatory diseases that are a major cause of chronic pain and disability affecting the quality of life of millions of people in worldwide. It has been estimated that 3.1 million Australian's (June 2000) suffer from this debilitating disease [1] which correlates to approximately 16.5% of the population. This represents a major cost to the Australian economy in terms of direct (medical expenses) and indirect costs (loss of income, early retirement). The overall economic impact of arthritis costs the Australian economy approximately 9 billion dollars per year (as of 2000). The market for arthritis treatments alone has been estimated at $21bn (2010) and is growing rapidly concurrent with an aging population. The number of arthritis cases is steadily increasing and US data shows that in 1985 - 35 million had arthritis, in 1990 - 37.9 million, 1998 - nearly 43 million (1 in 6 people) and now 2006 – 66 million (nearly 1 in 3 adults).

The market availability of nutraceuticals for veterinary use for the treatment of osteoarthritis includes: glucosamine, chondroitin sulphate, pentosan polysulphate. There is considerable opportunity for new products targeting inflammatory disease as currently no products provide an ideal solution. Most current products on the market target the repositioning of the equilibrium between cartilage synthesis and destruction mediated by proteases and do so by providing precursor molecules for cartilage synthesis (namely glucosamine and chondroitin sulphate). The precursors do not directly address the problems associated with the immune system.

The initial triggers responsible for the development of cartilage diseases are yet to be elaborated but continued immune system involvement in cartilage destruction is a given. New products targeting immune regulation have been problematic due to the complications associated with the immune system. Products that alter the immune function may give a competitive advantage compared to entrenched products in the market place and give a point of difference that could be exploited.

The project intends to exploit the existing relationship between meat processors and the pet food industry to gain access to the veterinary nutraceutical market. Subsequently for the human

nutraceutical market it was intended to work with local companies, who are focused on joint care nutraceuticals and are interested in extending their product range. They have a good distribution network throughout New Zealand and Australia. It was intended eventually to partner to gain access to customers for a human joint health nutraceutical. The key objective was to develop new products that might complement / add value to their current joint care range.

Outcomes and Conclusion

Evaluation of a specific hydrolyzed bovine tissue extract prepared by extraction with 1M NaOH was undertaken using five assay systems to demonstrate an osteoarthritis protective function and anti-inflammatory activity. This included:

1) Elastase inhibition assay.

2) Cartilage explant assay, where a number of factors effecting GAG (Glycosaminoglycan) release and protease activity were evaluated including: iodoacetate treatment, addition of LPS (Lipopolysaccharide) and sheep spleen cells, protease addition and analysis of TNF alpha and IL-1_ cytokine levels in the explants conditioned media.

A commercial enzyme was tested and shown to be suitable for producing the inhibitory activity from the NaOH tissue extract. The extract prepared by 1 M NaOH extraction and enzymically hydrolyzed had moderate inhibitory activity toward elastase. It was discovered that the enzyme itself became inhibited during the hydrolysis reaction and this lead to a reduction in proteolytic activity and a reduction in the level of elastase inhibition. This had previously not been observed for chymotrypsin hydrolysis of the tissue extract prepared by urea extraction.

Treatment of the hydrolysis reaction with either heat or pH modification resulted in an extract having a higher level of elastase inhibitory activity. When the enzyme hydrolysate reaction was denatured by heat and then tested for elastase inhibitory activity, inhibition levels increased 2 fold.

Acidification of the NaOH extract produced an insoluble protein pellet which was collected by centrifugation. The majority of the work was performed using the pellet material. However, it was found that the supernatant material also contained elastase inhibitory activity. It would therefore appear that the acidification step to recover the protein by centrifugation is not required for purification. The addition of the supernatant (without prior hydrolysis) to the cartilage explants enhanced the rate of GAG release. The reason for this is currently unknown.

Iodoacetate treatment of the articular cartilage explants resulted in a reduction in endogenous protease activity, leading to a reduction in the rate of GAG release. The use of iodoacetate therefore appeared to be detrimental in studying the effect of the hydrolysed tissue extract on GAG release from the explants.

The addition of ovine spleen cells to the explants with and without LPS appeared to increase the levels of pro-inflammatory cytokines but the presence of spleen cells made it difficult to analyse GAGs

released, due to the presence of GAG in the spleen cell extract. Protease levels were also difficult to analyse due to the presence of particulate material leading to light scattering. The addition of proteases such as elastase did not result in increased rates of GAG release, whereas the addition of chymotrypsin or the commercial protease did increase GAG release rates which caused problems as the level of GAG release was higher in the treatments than in the control. Several methods were tested but considered not suitable for evaluation of the enzymic tissue hydrolysate extract's ability to inhibit GAG release from the explants due to interference with the analysis methods.

The most suitable approach for evaluation of the extract's ability to inhibit GAG release required prior removal of the protease used to hydrolyze the extract (inhibitor). Heating or pH adjustment appeared to be the most suitable method, as this still retained the majority of the protease inhibitory activity of the hydrolysate. This was directly added to the explant and samples from the conditioned media were recovered after overnight incubation at room temperature. Under these conditions the tissue hydrolysate was shown to inhibit GAG release from the explants in a dose dependent manner.

Inhibition of the endogenous proteases produced by the explant was also observed. This would appear to be the most physiologically relevant model system to evaluate the nutraceutical properties of the extract without having to go to the added time and expense of doing another live animal study.

The next step envisaged in this project was to further develop a supporting data package required for regulatory approval and scale up of the process, leading to a joint health nutraceutical for use as an ingredient in animal feed, which may provide greater benefit to animals suffering from osteoarthritis.

The project did not however proceed to this next step, due to a change in research priorities for the research funds and the project was terminated at this point. The results to date confirm the potential to extract bioactive fractions from bovine tissue with relatively simple technology, for use in the pet food industry.