BIOCHEMICAL STUDIES ON OVINE CEROID-LIPOFUSCINOSIS

A thesis presented in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

in

Veterinary Pathology

at

Massey University

David Norris Palmer

1987

ABSTRACT

The ceroid-lipofuscinoses are a group of inherited diseases of humans and animals characterised by brain atrophy and the storage of a fluorescent lipopigment in a variety of tissues. Clinical signs include loss of vision, seizures, mental retardation and dementia, with the disease culminating in premature death. Defects in lipid metabolism or the control of lipid peroxidation have been postulated to explain their pathogenesis. Specific defects in peroxidases, fatty acid metabolism, dolichol metabolism, retinol metabolism and iron metabolism have been proposed. Evidence for these mechanisms arises from the apparent lipoid fluorescent Schiff base nature of the lipopigment.

This study reports the analysis of total tissue lipids and lipopigment isolated from tissues of sheep affected with ceroid-lipofuscinosis. Brain grey matter phospholipid fatty acids of diseased sheep were compared with those of normal sheep. Phosphatidylethanolamine of diseased sheep contained more 18:1(n-9) and less 22:6(n-3) than normal and their phosphatidylcholine less 16:0. Other differences were minor. No differences were found between the liver lipids nor the fatty acid profiles of their phosphatidylcholine, phosphatidylethanolamine or triglycerides.

Lipopigment from the liver of affected sheep was 70% proteinaceous, the rest being mainly lipids. These were only one sixth as fluorescent as total liver lipids, but contained a number of fluorophors. None were major components of the lipopigment or the postulated fluorescent product of lipid peroxidation. Lipopigment lipids included the lysosomal marker bis(monoacylglyceryl)phosphate that contained 42.9% linoleate and 16.5% linolenate. Lipopigment neutral lipids were dolichol, dolichyl esters, ubiquinone, free fatty acids and cholesterol, indicative of a lysosomal origin of the lipopigment. Phosphatidylcholine, phosphatidylinositol, phosphatidylserine and phosphatidylethanolamine were present in proportions and with fatty acid profiles typical of lysosomes.

Lipopigment isolated from liver, kidney, pancreas and brain of affected sheep without the use of proteolytic enzymes was two-thirds protein. Silver staining after sodium dodecyl sulphate polyacrylamide gel electrophoresis showed a major band of M_r 14,800, heterogeneous material between 5,000 - 9,000 M_r and a major band of M_r<3,500. These components did not stain for RNA or carbohydrate, and were digested by a nuclease free protease. They are not normal lysosomal proteins. The presence of the 3,500 M_r proteins in whole affected tissue homogenates distinguished them from homogenates of normal tissue. Lipopigment levels of dolichol, ubiquinone and cholesterol were consistent with the lipopigment being protein enriched lysosome derived cytosomes.

The concentration of metals in lipopigment from the four tissues was also analysed. Liver lipopigment had a high copper content, 1.3%, kidney lipopigment a high iron content and brain lipopigment showed an accumulation of some trace elements. These data indicate that the lipopigment cytosomes have a history of being functional lysosomes as far as metal metabolism is concerned. Metal analyses of cerebrospinal fluid revealed no indication of a defect in metal metabolism.

It is concluded that ovine ceroid-lipofuscinosis is not a lipidosis, nor does the lipopigment arise from the abnormal peroxidation of lipids. There is no evidence of any disturbance in metal metabolism. Low molecular weight proteins are stored in lysosome derived organelles and on these grounds the ceroid-lipofuscinoses should be regarded as lysosomal proteinoses. These may result from defects in lysosomal proteolysis or its control.

ACKNOWLEDGEMENTS

I would like to acknowledge my debt to my first supervisor, Professor R.D. Jolly, who made this project possible and organised the resources required to pursue it. He also provided helpful advice and encouragement throughout and educated me in the principles of biochemical pathology and storage diseases. His energy, commitment and mental acuity have been invaluable. I would also like to acknowledge the help of my second supervisor, Dr. David Husbands who encouraged my education in lipid and comparative biochemistry and ensured that facets of the work of biochemical interest were not undervalued.

Much of the work in this thesis would not have been possible without the assistance of a number of people. Mrs. Gill Barns was responsible for running the polyacrylamide gels. Her considerable professional diligence and skill ensured the success of this work in often difficult and trying circumstances. She also provided technical help with a number of other experiments as did Mr. Peter Winter, Mrs. Maureen Anderson and Miss Brenda Hobman. I would also like to thank Mr. Russell Graydon, Ms. Jayne Chapman, Mrs. Rosalind Dalefield and Dr. Susan Cooper for the post mortems and the electron micrographs. Ms. Jayne Chapman and Dr. Bruce Farquharson performed the liver biopsies and Mr. Boyd Jones collected the cerebrospinal fluids. Mr. Faris Sharpe helped with the post mortems and in obtaining control tissues. Professor Jolly oversaw this work and ensured a continued supply of accurately diagnosed animals in as good a physical condition as practicable.

I would also like to thank the numerous other people in this Faculty; and other faculties and research establishments who gave helpful advice and technical assistance on numerous occasions. Particular thanks go to Dr. Roger Reeves of the Chemistry and Biochemistry Department who organised and performed the metal estimations, Dr. Christopher Moore and Mr. Julian Reid of the same department who supplied the quantitative amino acid analyses, Professor R.E. Munford of the Department of Physiology and Anatomy who helped with the equivalent chain length calculations.

Dr. John Blunt of the Chemistry Department, University of Canterbury, ran the NMR spectra and Dr. Neville Grace of the Department of Scientific and Industrial Research provided the control tissue metal concentrations.

I would also like to thank Mr. Tom Law for the photography and Mrs. Elizabeth Wake and especially Mrs. Allain Scott for their help in typing the manuscript and ensuring its production.

The work was supported by the United States National Institute of Neurological and Communicative Disorders and Stroke Grant NS 11238 and the New Zealand Medical Research Council grant 83/1.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES