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Autofluorescence Emission Spectra of Neuronal Lipopigment in Animal and Human Ceroidoses (Ceroid-Lipofuscinoses)

J. H. Dowson[1]*, D. Armstrong[2]**, N. Koppang[3], B. D. Lake[4], and R. D. Jolly[5]

[1] Department of Psychiatry, University of Cambridge Clinical School, Addenbrooke's Hospital, Hill Road, Cambridge CB2 2QQ, U.K.
[2] Department of Ophthalmology, University of Florida College of Medicine, Gainesville, FL, USA
[3] Veterinaerinstituttet, Oslo, Norway
[4] Department of Histopathology, The Hospital for Sick Children, London, U.K.
[5] Faculty of Veterinary Science, Massey University, Palmerston North, New Zealand

Summary. A method for the measurement of autofluorescence emission spectra of intraneuronal lipopigment in tissue sections has been applied to specimens from dogs and sheep with forms of neuronal ceroid-lipofuscinosis (NCL). The characteristics of an emission spectrum probably reflect the composition of the lipopigment, and the results are compared with those previously reported from human NCLs and lipofuscin in non-diseased elderly human brains. Lipopigment in the animal NCLs differed from lipofuscin in the non-diseased human brains, but no differences could be demonstrated between the spectra from animal and human NCLs. These findings support the use of the animal diseases as models for research into the pathogenesis and treatment of human NCLs. Both human and animal NCLs would be more accurately designated as the "ceroidoses".

Key words: Animal diseases – Ceroid – Lipofuscin

Introduction

The term "lipofuscin" has been used to denote the lipopigment which can be found in many organs and cell-types, including neurones, in non-diseased animals and man; as the degree of pigment accumulation is related to previous functional activity, and therefore to age, lipofuscin has also been known as the "old-age" or "wear and tear" pigment (Dowson and Harris 1981).

Lillie, in 1941, designated lipopigment in the liver of rats with nutritional cirrhosis as "ceroid" (Bourne 1973), and this term has also been applied to lipopigment in the neuronal ceroid-lipofuscinoses (NCLs) as well as to pigment in macrophages associated with brain tissue necrosis (Schröder 1980). However, both these terms have no clear definition; in the present study, lipopigment is defined as a constituent of tissue which exhibits yellow autofluorescence under conditions which have been specified (Dowson and Harris 1981).

In man, the neuronal ceroid-lipofuscinoses (NCLs) are a clinically heterogeneous group of disorders which are characterized by progressive blindness and neurological deficits including dementia (Williams et al. 1977). They have been classified (on the basis of age of onset, clinical presentation and pathological findings), into 4 main groups: infantile, late infantile, juvenile and adult forms, but the differences between these sub-types are quantitative rather than qualitative (Greenwood and Nelson 1978; Lake and Cavanagh 1978; Goebel and Schulz 1979; Goebel et al. 1979). In all these disorders there is an abnormal degree of accumulation of lipopigment in neurones, but despite the involvement of other cell types (Gadoth et al. 1975), the clinical effects result from neuronal dysfunction and eventual cell death. It has been suggested that while the accumulation of lipopigment may result from an underlying enzymic deficiency (Siakotos et al. 1978), it is also a pathogenic mechanism in itself, which may act by causing a blockage of cell processes or by disrupting cellular constituents (Williams et al. 1977; Dowson 1982a). There is also evidence that lipopigment can be cytotoxic (Armstrong et al. 1980).

Various physical, chemical and ultrastructural differences have been reported between lipopigment extracted from NCL brains and from non-diseased elderly brains; these findings have led to the classification of neuronal lipopigment into "ceroid" which has been considered to signify the presence of a pathological process (usually NCL) and "lipofuscin" which is a

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Offprint requests to: J. H. Dowson, PhD, Dept. of Psychiatry, New Addenbrooke's Hospital, Hill Rd., Cambridge CB2 2QQ, U.K.

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normal cellular constituent (Siakotos 1974). However, extraction methods examine pigment derived from many cell populations, and the results are affected by variables in the extraction procedure. A recent study (Dowson 1982b) has reported the evaluation of lipopigment autofluorescence emission spectra in individual cells, and this technique was able to distinguish the lipopigment in (human) NCLs from that in elderly brains with no evidence of disease, thus supporting the distinction between "ceroid" and "lipofuscin".

However, "neuronal ceroid-lipofuscinoses" appears to be an inappropriate designation, as the differences between lipopigment in these diseases and that in normal elderly brains have been used to define the properties of "ceroid". Although lipofuscin may be present in NCL brains, there is no evidence that the massive intraneuronal accumulations of lipopigment in the NCLs are a mixture of two clearly defined substances, as has been suggested (Vandervelde and Fatzer 1980); thus the NCLs would be more accurately described as the "ceroidoses".

There have been several reports of progressive neurological disease associated with intraneuronal lipopigment accumulation in dogs, cats and sheep (Cummings and de Lahunta 1977; Armstrong et al. 1978; Jolly et al. 1980; Vandervelde and Fatzer 1980), and Koppang (1973) has identified and bred a reproducible form of hereditary canine ceroid-lipofuscinosis (CCL) in English Setters. The validity of this form of CCL as a model for human disease is of importance to current and future research, and the present study involved an investigation of the properties of the CCL lipopigment autofluorescence emission spectra, which probably reflect the composition of the lipopigment. Tissue from a sheep with a similar inherited disease was also studied; a flock of sheep with this disorder is also maintained as a model for studying the pathogenesis and treatment of NCL (Jolly et al. 1980).

Methods

Post-mortem specimens were obtained from 4 English Setter dogs affected with hereditary canine neuronal ceroid-lipofuscinosis (CCL) who died at 20, 23, 23 and 24 months of age. The following cell populations were studied: spinal cord neurones, neurones of the inferior olivary nucleus, Purkinje cells and neurones of the cerebral cortex. Specimens were also examined from a 24 month old (heterozygote) English Setter which was clinically unaffected by the disease. The tissue had been stored in formalin before preparation for varying periods between 2–4 months.

Neurones of the cerebral cortex, spinal cord neurones and spinal ganglion neurones were examined in specimens from an 18 month old South Hampshire ram with a form of NCL which has been previously reported (Jolly et al. 1980). Specimens were also examined from the cerebral cortex of an unaffected sheep of similar age. The tissue had been stored in formalin before preparation for about 2 months.

Unstained sections were examined with a Leitz Ortholux II microscope with photometric accessories which were used to measure the intensity of 24 narrow bands of each emission spectrum, each band being centred on a selected wavelength. A series of such readings indicated the characteristics of the emission spectrum from each region of lipopigment. The spectrum of lipopigment autofluorescence is distorted by various factors, so that the spectrum derived from a series of measurements is designated as "uncorrected". It is important to keep the distortions constant so that the various uncorrected spectra can be compared. Details of the equipment, preparation of tissue and the method for the measurement of emission spectra have been previously reported (Dowson 1982b). Each emission spectrum was derived from the means of 6 sets of 24 measurements, each set corresponding to one region of lipopigment from 1 neurone. Thus, for each emission spectrum, 6 neurones were examined from a cell population which was assumed to be homogenous. It has been reported (Dowson 1982b) that a major difference between the various spectra from lipopigment in human NCL and the lipofuscin in elderly human brains without NCL could be expressed by a "spectral ratio", which comprises the sum of the measurements at the narrow bands of each emission spectrum centred on 581 nm, 615 nm and 648 nm, divided by the measurement at the narrow band centred on 548 nm. Thus, the "spectral ratio" reflects the shape of an emission spectrum above 548 nm.

The characteristics of the emission spectra from the animal NCL tissue were compared with previously reported results from lipopigment in infantile, late-infantile and juvenile human NCL (Batten's disease), and from lipofuscin in elderly human brains without evidence of NCL (Dowson 1982b).

Results

All the neurones which were examined from diseased tissue showed a marked accumulation of yellow-white autofluorescent pigment, in contrast to the neurones in tissue from the non-diseased dog which contained relatively small amounts of yellow autofluorescent material whose "spectral ratios" were in the region of 1.7, i.e. within the range characteristic of "lipofuscin" in the non-diseased elderly human brain (Dowson 1982b). The neurones of the specimen of cerebral cortex from the non-diseased sheep contained insufficient lipopigment for an emission spectrum to be obtained.

Table 1 shows the "spectral ratios" of the animal NCL specimens, together with the means of the "spectral ratios" for the Batten's NCLs and elderly non-diseased human brains which have been previously reported (Dowson 1982b).

While a clear distinction has been previously demonstrated between the lipopigment in human NCL and non-NCL tissue, the present study did not detect any significant differences between the lipopigment in human and animal NCL tissue, with respect to their emission spectra.

There were also no consistent differences between the spectra from the dogs and the sheep, or between the various cell populations examined in the animal tissue; Fig. 1 shows the uncorrected emission spectrum (to-

Table 1. The "spectral ratios" (SRs) derived from the (uncorrected) emission spectra of intraneuronal lipopigment autofluorescence

Origin of tissue	Region	"Spectral ratio" (SR)	Mean SR (animals 1–5)
A. Animal NCLs			
1. Sheep with NCL	Cerebral cortex	1.18	
	Spinal cord	1.14	
	Spinal ganglion	1.25	
2. English Setter with CCL (20 months old)	Purkinje cells	1.19	
	Cerebral cortex	1.21	
3. English Setter with CCL (23 months old)	Spinal cord	1.23	1.22 (S.D. 0.05)
4. English Setter with CCL (23 months old)	Purkinje cells	1.28	
	Olivary nucleus	1.27	
5. English Setter with CCL (24 months old)	Olivary nucleus	1.24	

	Number of SRs	Number of brains	Mean SR
B. Human NCLs (Batten's disease)	8	8	1.15 (S.D. 0.08)
C. Non-NCL human elderly brains (Olivary neurones)	14	14	1.83 (S.D. 0.14)
D. Non-NCL human elderly brains (Parietal neurones)	14	14	2.05 (S.D. 0.16)

NCL neuronal ceroid-lipofuscinosis; CCL canine ceroid-lipofuscinosis

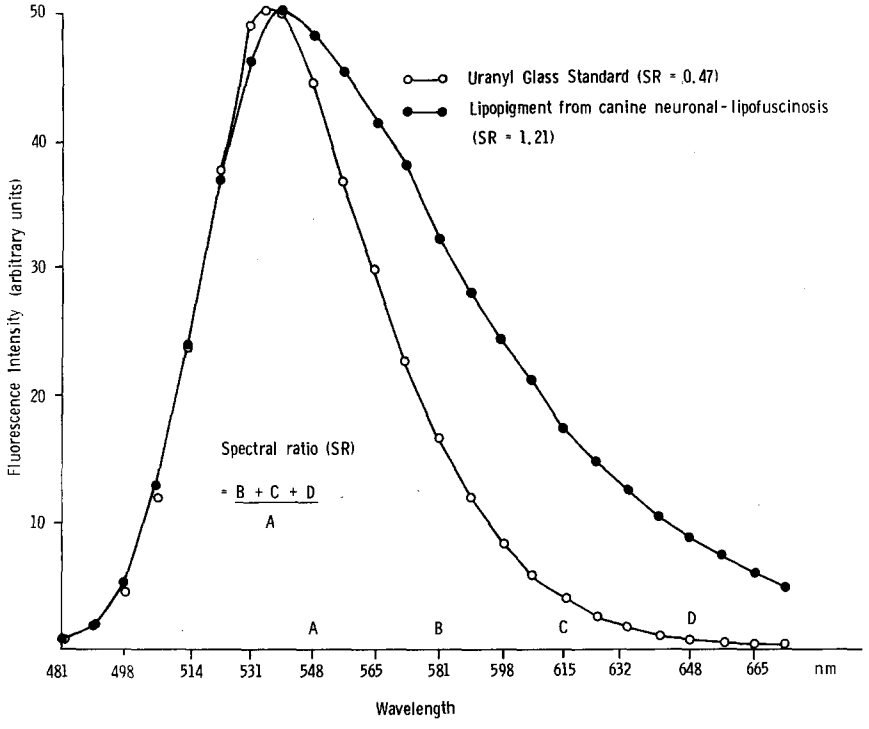

Fig. 1. Uncorrected emission spectra of autofluorescence from an Uranyl Glass Autofluorescent Standard (GG17 Leitz) and from lipopigment in cerebral cortex neurones of a dog with hereditary canine neuronal ceroid-lipofuscinosis. Each spectrum was obtained from the means of 6 sets of measurements

gether with that of the Uranyl Glass autofluorescence standard, GG17, Leitz) from neurones of the cerebral cortex of the 20 month old English Setter with CCL.

Lipopigments in non-NCL elderly human brains (i.e. lipofuscin) and in human NCLs (i.e. ceroid) have also been distinguished by their uncorrected emission maxima and by their fading rates of autofluorescence intensity during illumination (Dowson 1982b). In the present study, the uncorrected emission maximum was the same from all affected animals and was identical to

the uncorrected value (using the same exciter and barrier filter combination) for all the Batten's NCL tissue previously reported, namely 539 nm (± 5 mm). The mean fading rate of human NCL lipopigment autofluorescence intensity over a constant time, due to illumination by a constant intensity of exciting light, has been previously reported to be 13.8% of the original reading, in contrast to a considerably lower rate of fading for lipofuscin (Dowson 1982b). In the present study, the equivalent mean value for the animal NCL tissue was 16.7%, but the differences between animal and human NCL fading rates were not of statistical significance.

Discussion

The characteristics of the emission spectra of lipopigment probably reflect the composition of the fluorophores (Dowson 1982b). A previous study has demonstrated differences between lipofuscin in the elderly human brain and ceroid in various forms of Batten's NCL, and the present results indicate that the lipopigment in canine and sheep NCLs can also be distinguished from lipofuscin associated with normal ageing. Furthermore, no significant differences could be demonstrated between the lipopigment autofluorescence emission spectra of the animal and human NCLs.

A previous study has described the various possible factors which might influence the characteristics of autofluorescence emission spectra other than the composition of the pigment before death (Dowson 1982b); these include the duration of storage of the tissue in formalin, and the time between the preparation of sections and examination with the fluorescence microscope. Although it was not possible to keep these potential variables constant, the period of storage in formalin is unlikely to have had a significant effect on the results, as the tissue from the Batten's NCLs (Dowson 1982b) consisted of cryostat sections (which had not been stored in formalin) and specimens which had been stored in formalin for varying periods of up to several years; despite these variables, the tissues yielded similar lipopigment emission spectra. In the present study the possible effects of storage as mounted slides on the characteristics of the emission spectra were investigated by comparing spectra from slides stored for less than 2 weeks, with spectra from the same slides after a year's storage; no changes were apparent.

It has been previously reported that the composition of lipopigments as indicated by their emission spectra show considerable variations within the two broad divisions of lipofuscin and ceroid, and it was suggested that the classification of lipopigments should not be restricted to just two categories (Dowson 1982b). The application of the technique for evaluating lipopigment emission spectra to a larger number of samples may be able to detect any consistent difference between sub-groups of NCL, between different cell populations, or between different stages of these diseases. Such information could aid the study of the pathogenesis of the NCLs and the effects of attempts to modify the course of these disorders.

In conclusion, the present results give further support to the use of the diseased dogs and sheep as models for research into the human NCLs.

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