Morphopathological features in tissues of α-mannosidosis guinea pigs at different gestational ages

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Alpha-mannosidosis is an inherited metabolic disorder characterized by a reduction in α-D-mannosidase and intralysosomal accumulation of undegraded mannose-containing oligosaccharides. The α-mannosidosis guinea pig exhibits pathological similarities to its human counterpart, which make it a valuable animal model. To trace the progression of α-mannosidosis during foetal development, brain and visceral organs from affected and unaffected guinea pigs at 30, 36, 38, 51 and 65 days of gestation (dg) were examined by light and electron microscopy (term: approximately 68 dg). In the affected brain, distended lysosomes (vacuoles) were scarce up to 38 dg and were seen in few differentiating neuronal cells but mostly in macrophages, pericytes and endothelial cells. At 51 and 65 dg, several vacuoles were observed in some neurones, in many Purkinje cells, pericytes, endothelial and microglial cells, and in few cerebellar internal granule cells. Myelination had started by 51 dg. Non-myelinated axonal spheroids were detected in the brainstem at 65 dg. In the kidney cortex and liver, an increase in vacuolation was noticed between 36 and 65 dg. Some vacuolated cells were also noticed in the lungs and spleen at 51 and 65 dg. Altogether, these histological observations suggest that α-mannosidosis is unlikely to affect ontogenesis before the second half of gestation in guinea pigs; however, the morphopathological features recorded during the last quarter of gestation (which may roughly correspond to the period covering near term to 1–2 years of age in human) were clearly noticeable and may have had some impact.

Keywords: alpha-mannosidosis, brain, foetal development, guinea pigs, lysosomal storage diseases, vacuoles

Introduction

Alpha-mannosidosis, one of more than 45 lysosomal storage diseases (LSD), develops secondary to the loss or decrease of α-D-mannosidase (EC 3.2.1.24) within lysosomes, owing to a mutation within the gene encoding this specific enzyme. The pathological hallmark of the disease is the progressive intralysosomal accumulation of undegraded mannose-rich oligosaccharides, which leads to cell engorgement and cellular dysfunction [1,2]. In α-mannosidosis, a multitude of cell types are affected, including many cells in the central nervous system (CNS). Alpha-mannosidosis patients usually appear normal at birth but eventually develop progressive neurological deficits, mental retardation and psychiatric symptoms [3]. The estimated incidence of α-mannosidosis in humans is very low (approximately 1 in 1 056 000 livebirths in...
was also carried out.
Furthermore, identification of early signs of pathology in guinea pigs during foetal development using light microscopy (LM) and transmission electron microscopy (TEM).

guinea pigs has been partly characterized morphologically, clinically and behaviourally from birth until maturity [11,13–15]. The α-mannosidosis-affected guinea pigs display stunted growth and progressive neurological deterioration and mental dullness. Widespread pathological signs of the diseases are already observed within the CNS of 3-day-old guinea pigs, and the earliest abnormal neurological signs are usually detected around 2–3 months of age [13,15]. As for other LSD with CNS involvement, the pathogenic events leading to neurological dysfunction in α-mannosidosis still remain to be elucidated [16,17].

Characterization of α-mannosidosis during foetal life has not yet been achieved. However, valuable knowledge has recently been gained regarding morphological changes occurring during foetal development in β-mannosidosis goats [18,19]. Reports of histological observations made on human foetuses/abortuses affected with LSD and on other LSD-affected animal foetuses at various gestational ages have also been published [20–22].

The aim of this study was to characterize the nature and progression of the CNS pathology in α-mannosidosis guinea pigs during foetal development using light microscopy (LM) and transmission electron microscopy (TEM). Furthermore, identification of early signs of pathology in liver, spleen, kidneys and lungs at various gestational ages was also carried out.

Materials and methods

Test animals and husbandry

Housing facility and feeding of guinea pigs have been described previously [13]. Briefly, all animals were indoor-housed in floor-pens. Their diet contained dry guinea pigs pellets, straw, lucerne hay, fresh green vegetables and water with vitamin C supplement. All animal studies were reviewed and approved by the Children, Youth and Women’s Health Service’s Animal Ethics Committee. The original carriers introduced in the colony originated from domestic pet guinea pigs, in which the natural mutation was first detected [11]. The guinea pigs are basically ‘outbred’ of the English short-haired breed. All breeders were carriers (+/–). Of the 85 foetuses examined in this study, 17 foetuses (20%) were α-mannosidosis (–/–), 29 foetuses (34%) were unaffected (+/+), and 39 foetuses (46%) were carriers (+/–) (Table 1). Both +/+ and +/– guinea pigs have normal phenotypes [13].

Pairing time and gestational age

In order to take advantage of the postpartum oestrus, each dam was paired with one male for at least 24 h after parturition [24]. The day following parturition was therefore considered the first day of gestation (1 dg). For one dam (D15), the timing of pairing was not related to parturition, and mating was estimated to have occurred around the time when the vaginal membrane was completely perforated [24]. The average age of the dams used in this study was 427 days (± 156 SD). The average number of pregnancies per dam was 3.8 (± 1.4 SD). The duration of gestation in guinea pigs is approximately 68 dg [24].

The term ‘foetus’ is used indiscriminately in the text, for all guinea pigs, irrespective of the stage of development reached (age range: 20–65 dg).

Anaesthesia, caesarean section and euthanasia of female breeders

At various gestational ages, induction and maintenance of general anaesthesia was achieved using Isoflurane. Lignocaine HCl 1% (Pharmacia & Upjohn, NSW, Australia) was also injected subcutaneously before the skin incision was made along the midline. Each caesarean was performed rapidly in order to quickly remove all foetuses from the uterus. Whenever possible, the younger foetuses (≤ 51 dg) were kept inside their own placenta and placed on ice until further processing. The older and larger foetuses (> 51 dg) were decapitated immediately on removal from the placenta before the appearance of gasping reflexes. Immediately after removal of the foetuses, the
dam was sacrificed using an overdose of pentobarbitone sodium (Lethabarb; 325 mg/mL, Virbac Pty Limited, NSW, Australia) by intracardiac injection.

Bodyweight, crown–rump length and statistical analysis

Bodyweight (BW) and crown–rump length (CRL) of each foetus were measured as described by Kaufmann [25] before further dissection was carried out (Figure 1A). ANOVA was used to compare maturational changes in BW and CRL between disease groups. A linear regression was used to estimate CRL and BW for any age for each disease groups. Six unaffected foetuses obtained at 20 dg were not separated from their placenta before measurement of weight and length; therefore, these overestimated values were not included in the statistical analysis.

Light microscopy

For the foetuses older than 35 dg, pieces of liver, spleen, kidneys and lungs were immersed in 10% (v/v) formaldehyde, and half brain was immersed into 18.5% (v/v) formaldehyde before further processing for LM (Figure 1B) [21]. For the younger foetus (≤35 dg), the whole brain was immersed into 18.5% (v/v) formaldehyde before further processing for LM (if not kept for TEM, see below). Paraaffin-embedded sagittal sections were stained with cresyl violet and were assessed qualitatively for suggestive signs of accumulation of storage material within the cells, such as vacuolated or foamy cytoplasmic appearance.

Transmission electron microscopy

For the foetuses older than 35 dg, small randomly selected tissues samples (approximately 2 × 2 × 1 mm) from six brain segments (Figure 1B,D) and from liver, spleen, kidney cortex and lungs were immersed in 4% (v/v) glutaraldehyde/1.2% (w/v) paraformaldehyde plus 4% (w/v) sucrose in PBS buffer (pH 7.2), fixed overnight at 4°C, then transferred in PBS buffer (pH 7.2) until further processing. These samples were post-fixed in 1% (v/v) osmium tetroxide for 1 h, dehydrated through 70%, 90%, 99% and 100% ethanol and embedded in epoxy resin. Randomly selected samples from four segments of the...
brain of younger foetuses (≤ 35 dg) were also processed for TEM as above (if not kept for LM) (Figure 1C). Semi-thin survey sections (1 μm thick) were stained with 1% (w/v) toluidine blue in 1% borax and were evaluated for presence of intracytoplasmic storage vacuoles and abnormal staining. Ultra-thin sections (60–80 nm) of selected areas were stained with 2% (w/v) uranyl acetate/1% (w/v) lead citrate and viewed on a Philips CM100 electron microscope (Eindhoven, the Netherlands).

Qualitative assessment of ultra-thin tissue sections at 30, 36, 38, 51 and 65 dg included: overall degree of vacuolation over whole tissue section, degree of vacuolation per affected cell, and general description of storage vacuoles (e.g. shape/size/content).

**Results**

**Bodyweight and CRL of foetuses vs. gestational age**

The scatterplot of the association between CRL and age is shown in Figure 2A. The scatterplot of the association between BW and age is shown in Figure 2B. Natural logarithm transformation of BW and age values had to be applied before a linear correlation between the two variables was established (Figure 2C), while a linear correlation between CRL and age was established without transformation of data (Figure 2A). Statistically, there was a significant age effect on BW and CRL ($P < 0.001$ for both); however, no significant disease group-by-age inter-
action effect nor overall disease group main effect were found.

Gross pathology

All dams used in this study were found to be in excellent body condition at the time of euthanasia. Apart from two resorbing dead foetuses (one +/− at 32 dg and one +/− at 36 dg), all foetuses seemed to have reached expected developmental stages when recovered from the uterus. The presence of resorbing foetuses did not appear to affect the development of the adjacent foetuses recovered at the time. During gross examination of the foetuses, no obvious abnormalities were detected, and the α-mannosidosis foetuses could not be differentiated from the unaffected foetuses (+/+ or +/−).

Brain pathology: light microscopy

The paraffin-embedded sections of α-mannosidosis brains stained with cresyl violet did not show any abnormality at younger gestational ages (≤ 38 dg), while at 51 and 65 dg the largest neurones exhibited abnormal staining conferring a foamy appearance to the cytoplasm. The accumulation of storage material reflected by the foamy appearance of the cytoplasm was more pronounced in the pyramidal cells of the hippocampal formation (Figure 3 A,B), in the neurones of the thalamus

![Image](https://via.placeholder.com/150)

**Figure 3.** Appearance of the neurones (A) in the hippocampal formation of the 65-dg unaffected guinea pig and (B) of the 65-dg α-mannosidosis guinea pig and (C) in the thalamus of the 65-dg unaffected guinea pig and (D) of the 65-dg α-mannosidosis guinea pig. Appearance of the largest neurones (E) in the brainstem of the 51-dg unaffected guinea pig and (F) of the 51-dg α-mannosidosis guinea pig. (A–F) Paraffin-embedded sections stained with cresyl violet. Scale bars: 10 μm.
(Figure 3C,D) and in the largest neurones located near or within the brainstem (Figure 3E,F). Except for some slightly foamy cerebrocortical neurones and Purkinje cells (not shown), the amount of storage material accumulated in the other cells was not sufficient to produce detectable changes.

On 1-μm resin-embedded sections, more details about the extent of vacuolation within different cell types were obtained; however, retracing the approximate location of each randomly selected sample was only possible if landmarks such as pia mater, ependymal layer and Purkinje cells were identified. In younger α-mannosidosis brains (≤38 dg), the only abnormality detected was the presence of rare vacuoles occasionally observed in cells located near small blood vessels (not shown). At 51 and 65 dg, vacuolated cells were unevenly distributed throughout all sections obtained from the six segments of the brain of α-mannosidosis foetuses, while vacuolated cells were very rarely observed in unaffected brains. A small number of intracytoplasmic vacuoles were present in some neurones located in the grey or white matter; however, the majority of neurones appeared unvacuolated. In the cerebral cortex of 51- and 65-dg α-mannosidosis foetuses, some elongated pyramidal neurones stained darkly and contained many well-demarcated vacuoles, while the majority of adjacent neurones appeared rounder and paler and contained only 1 or 2 vacuoles or no vacuole at all (Figure 4A). In contrast, in age-matched unaffected cerebral cortex, the cytoplasm of a few darkly stained neurones appeared slightly irregular or uneven rather than clearly vacuolated (Figure 4B). In 51- and 65-dg α-mannosidosis brains, several glial, endothelial and perivascular cells, as well as several cerebellar Purkinje cells, also contained well-defined intracytoplasmic vacuoles (not shown).

Brain pathology: transmission electron microscopy

The number of intracytoplasmic membrane-bound vacuoles, believed to be distended lysosomes, detected on ultrathin tissue sections obtained from all segments of the brain of α-mannosidosis foetuses, varied considerably, depending on gestational age. Lysosomal vacuoles were relatively scarce during the first half of gestation in α-mannosidosis guinea pigs, with only a small subjective increase in overall lysosomal vacuolation noticed between 30 and 38 dg. A definite increase in overall lysosomal vacuolation was noticed thereafter with clear histopathological signs of lysosomal engorgement present in many cell types during the last quarter of gestation (i.e. 51–65 dg). At any given age, but particularly in the older α-mannosidosis foetuses, the number of vacuoles per cell varied considerably among and between cell types. Vacuole size varied to a lesser extent.

At 30 dg, rare undifferentiated neuronal cells were seen with 4–7 abnormal lysosomal vacuoles (Figure 5A), while a few vacuoles were occasionally detected in endothelium, pericytes and macrophages (not shown). At 36 and 38 dg, a few distended lysosomes were seen in the occasional differentiating neuronal cells (usually ≤6 vacuoles per cell) (Figure 5B), but primarily in perivascular cells (Figure 5C) and in macrophages/microglial cells (Figure 5D).

In the cerebrum of 51- and 65-dg affected guinea pigs, lysosomal vacuoles were seen in a minority of cerebral neurones (Figures 4C,E and 6A), in some pial cells (Figure 6B) and in many microglial cells (Figure 6C,D), as well as in many endothelial and perivascular cells (not shown). Confirming our observations made on 1-μm sections of the cerebral cortex of the older α-mannosidosis foetuses, the most severely vacuolated cells were the darkly stained pyramidal neurones that also exhibited a granular nucleus (Figure 4C,E). Dark cerebrocortical neurones were also occasionally seen on sections of unaffected brain (Figure 4D,F); however, the irregular appearance of the cytoplasm was mainly due to the presence of swollen mitochondria and minor tissue processing artefacts (also present in α-mannosidosis dark neurones) (Figure 4E,F).

In the cerebellum of 51- and 65-dg affected foetuses, most Purkinje cells (Figure 7A) and few internal granule cells (Figure 7B) were vacuolated. Subjectively, the cerebellar Purkinje cells of one of the two 51-dg affected foetuses appeared to contain more vacuoles than the Purkinje cells of the 65-dg affected foetus.

In the brainstem of 51- and 65-dg affected foetuses, a minority of neurones and many microglial cells (Figure 7C), as well as many endothelial and perivascular cells (not shown), contained distended lysosomes. In the 65-dg affected foetus, small non-myelinated axonal spheroids, appearing to contain degenerated mitochondria, were detected among myelinated axons (Figure 7C,D). Few oligodendrocytes were identified, but these cells did not appear to contain intracytoplasmic vacuoles (Figure 7D). A few myelinated axons were already seen at 51 dg (not shown).
The membrane limiting the lysosomal vacuoles was visualized by TEM at high magnification. Most lysosomal vacuoles had an oval or round shape and usually appeared to contain finely granular semilucent material (as in Figure 4E). The density of the storage material accumulated within most lysosomes varied slightly from cell to cell. The denser lysosomes were usually seen within microglial cells (as in Figure 6C). Electron-dense aggregates forming fibrillar or lamellar structures were not observed in characteristic storage vacuoles of α-mannosidosis foetuses. A small amount of granular electron-dense material, believed to result from general phagocytic activity, was occasionally seen inside lysosomes of affected macrophages (as in unaffected macrophages, see below) (Figure 5D). Most vacuoles were relatively small (approximately 0.3–2.0 μm in diameter), but large coalescent vacuoles reaching up to approximately 7 μm in diameter were occasionally seen inside some macrophages/microglial cells (e.g. Figure 6D). In general, the distended lysosomes present inside individual

Figures 4. Appearance of neurocortical neurones. (A, B) Resin-embedded sections (1 μm thick) stained with toluidine blue. Darkly stained neurones in the cerebrocortical pyramidal layer (A) of the 51-dg α-mannosidosis brain containing lysosomal vacuolation (B) of the 51-dg unaffected brain containing small artefacts. The adjacent pale neurones appear normal in all foetuses irrespective of genotype. (C, E) Electron micrographs of dark cerebrocortical neurones of the 51-dg α-mannosidosis brain containing engorged lysosomes (large arrow) and swollen mitochondria (arrowhead). (D, F) Electron micrographs of dark cerebrocortical neurones of the 51-dg unaffected brain containing swollen mitochondria (arrowhead) and small tissue processing artefacts (small arrow). E and F show higher magnification of area indicated in C and D, respectively. Scale bars: 10 μm (A, B), 5 μm (C, D), 1 μm (E, F).
Figure 5. Electron micrographs of vacuolated cells in the brain of α-mannosidosis guinea pig foetuses at 30, 36 and 38 dg. (A) At 30 dg, engorged lysosomes were seen in undifferentiated neuronal cells. At 36 or 38 dg, engorged lysosomes were seen (B) in differentiating neuronal cells, (C) in perivascular cells and (D) in macrophages/microglial cells. Scale bars: 2 μm (A, D), 10 μm (B), 5 μm (C).

Figure 6. Electron micrographs of vacuolated cells in the cerebrum of an α-mannosidosis guinea pig foetus. At 51 or 65 dg, engorged lysosomes of various sizes were seen (A) in cerebral neurones, (B) in pial cells (B) and (C, D) in perineuronal microglial cells and (D) in microglia located in the neuropil. Scale bars: 10 μm (A), 5 μm (B–D).
α-mannosidosis neurones (including Purkinje cells) occupied a relatively small amount of cytoplasmic space without appearing to disturb adjacent organelles (Figures 4C,E, 6A). In contrast, the large coalescent lysosomes present in macrophages/microglial cells occupied the majority of the cytoplasmic space, and displaced the nucleus to an eccentric position (Figures 6C,D and 7C).

In unaffected guinea pig foetuses at any gestational age, a very small number of lucent vacuoles (1–3 per cell; with or without limiting membrane) were occasionally seen in some developing neuronal cells (not shown). Furthermore, as expected, some vacuoles of various sizes containing material of different density were seen inside many cells of the monocyte/macrophage lineage due to normal phagocytic activity (especially common when rapid cellular turnover is occurring). These vacuoles could generally be differentiated from the distended lysosomes that characterize α-mannosidosis by their number, size and appearance of content.

Pathology of visceral tissues

Pathological signs indicative of α-mannosidosis were not detected on paraffin-embedded sections of visceral tissues. On 1-μm resin-embedded sections, abnormal vacuolation was only noticed in the kidneys at 51 and 65 dg as well as in the lungs at 65 dg (not shown). In the kidney cortex, the vacuoles appeared to be localized principally inside the parietal cells of the Bowman capsule and inside the epithelial cells of the proximal and distal convoluted tubules. In the pulmonary parenchyma, the vacuoles seemed scattered unevenly throughout the alveolar epithelium (not shown).

Using TEM and low magnification, distended lysosomes were predominantly seen in the parietal and visceral cells of the Bowman capsules of α-mannosidosis guinea pigs at 36, 38, 51 and 65 dg (Figure 8A,C,D). Overall lysosomal vacuolation within renal corpuscles increased as maturation occurred, reflecting an increase in severity with age (Figure 8A,C,D). At 65 dg, lysosomal vacuolation was
also detected in the epithelial cells of the proximal and distal convoluted tubules in α-mannosidosis kidneys (Figure 8A vs. 8B).

Within the pulmonary parenchyma of α-mannosidosis foetuses, no difference was found between unaffected and α-mannosidosis immature lungs at 36 and 38 dg. No lung samples were available for the 51-dg α-mannosidosis foetuses. At 65 dg, relatively small intracytoplasmic vacuoles were seen in pneumocytes and capillary endothelial cells while larger vacuoles were seen in macrophages (Figure 8E).

The spleen of the older α-mannosidosis foetuses only (that is, at 51–65 dg) exhibited some lysosomal vacuoles in a small number of parenchymal cells and in some large macrophages (Figure 8F).

Many droplets (without limiting membrane) were seen inside hepatocytes in both unaffected foetuses and α-mannosidosis foetuses at 36, 38, 51 and 65 dg (Figure 9A–D). Some Kupffer cells exhibited a small amount of lysosomal vacuolation as early as 36 dg (Figure 9A). At 51 and 65 dg, inside affected hepatocytes, a few intracytoplasmic vacuoles presumed to be lysosomes

Figure 8. Electron micrographs of kidney cortex, lung and spleen. Electron micrographs of kidney cortex of α-mannosidosis guinea pig foetuses (A) at 65 dg, (C) 51 dg and (D) 36 dg and (B) of an unaffected guinea pig at 65 dg. Engorged lysosomes were predominantly seen in the parietal and visceral cells (arrowhead) of the Bowman capsule (A) at 65 dg, (C) at 51 dg and (D) at 36 dg and inside the epithelial cells of the proximal (large arrow) and distal convoluted tubules (small arrow) (A) at 65 dg in α-mannosidosis foetuses. Electron micrographs of (E) pulmonary parenchyma and (F) spleen of one α-mannosidosis guinea pig foetus at 65 dg (E). In the lung, engorged lysosomes were predominantly seen in pneumocytes (large arrowhead), in capillary endothelial cells (small arrowhead) and in pulmonary macrophages (arrow). (F) In the spleen, engorged lysosomes were in cells of the parenchyma (arrowhead) and in macrophages (arrow). Scale bars: 20 μm (A, B, E), 10 μm (C, F), 5 μm (D).
were seen among the more predominant droplets (Figure 9C). Many circulating macrophages clearly contained a large number of distended lysosomes at 51 and 65 dg (Figure 9C vs. 9D). Visceral tissues were not examined at 30 dg.

The lysosomal storage vacuoles found in visceral tissues were very similar in size and appearance to the vacuoles observed in nervous tissues of α-mannosidosis foetuses.

**Discussion**

This study is the first to attempt to identify and describe the early pathological signs encountered in the tissues of α-mannosidosis guinea pigs at different gestational ages. The presence of intracytoplasmic lysosomal vacuoles, the histopathological hallmark of the disease, was noticed around mid-gestation (30–38 dg) in a minority of cells in the CNS (differentiating neuronal cells, macrophages and endothelial cells), in the kidney cortex (podocytes) and in the liver (Kupffer cells). However, at the start of the last quarter of gestation (at 51 dg) a considerably increased number of cells containing distended lysosomes were detected throughout the brain, and to a lesser extent in the kidneys, lung, liver and spleen, indicating that a gestational interval of 2 weeks was sufficient for a substantial accumulation of storage material within a variety of cells. In α-mannosidosis, as in many other LSD, the cells of the macrophage/microglial lineage appear to accumulate storage material more avidly than other cell types, most likely due to their specific phagocytic function and high molecular turnover [26]. At 51 and 65 dg, few cerebral and brainstem neurones contained vacuoles while most Purkinje cells were vacuolated. As axonal spheroids indicate disruption of axonal transportation [16], the presence of axonal spheroids in the brainstem at 65 dg suggests that the pathology within the CNS has already progressed to a more advanced level not seen at 51 dg. The incidence of ubiquitin positive spheroids observed in various brain regions in α-mannosidosis guinea pigs has recently been shown to increase steadily from birth to end-stage disease [15]. Foetal axonal spheroids resembling those observed in guinea pigs have been observed in mucopolysaccharidosis IIIID and β-mannosidosis goats [18,20]. Axonal spheroids have also been identified at

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**Figure 9.** Electron micrographs of liver of α-mannosidosis guinea pig foetuses (A) at 36 dg and (C) at 65 dg; and of unaffected guinea pig (B) at 36 dg and (D) at 51 dg. In the α-mannosidosis foetus, engorged lysosomes were predominantly seen (A) in Kupffer cells (small arrowhead), (C) in circulating macrophages (large arrowhead), and possibly (C) in hepatocytes (large arrow). In all electron micrographs, droplets without delimiting membrane (small arrow) are seen within the hepatocytes of unaffected and α-mannosidosis guinea pig foetuses. Scale bars: 5 μm (A, C, D), 10 μm (B).
various postnatal ages in mice affected with α-mannosidosis [27].

Gross macroscopical and microscopical examinations of brains and visceral organs did not suggest noticeable differences in degree of maturation between age-matched unaffected and affected guinea pig foetuses. We found no evidence that a deficiency in α-D-mannosidase could induce premature interruption of development, given that the only two resorbing foetuses recovered in utero were carriers (+/−). Irrespective of whether some storage material was engorging the lysosomes, most cells around mid-gestation still appeared to be in the process of proliferation and/or differentiation, while most cells examined during the last quarter of gestation were relatively differentiated. As some myelinated axons and non-vacuolated oligodendrocytes were seen in the brainstem of 51-dg α-mannosidosis guinea pigs, it appears that the onset of myelination was not delayed and that it took place near the expected time for this species [28,29]. In fact, although demyelination has been described in α-mannosidosis cats [30], this abnormality was not detected in two mature guinea pigs [13] and in some human patients [31] affected with the disease. Jones et al. found no morphological evidence that the metabolic perturbations of N-acetylglucosamine 6-sulfatase deficiency in mucopolysaccharidosis IIID goats interfered with CNS foetal development despite the presence of increased storage material and occasional misalignment of Purkinje cells [20].

In the α-mannosidosis guinea pig brain, during the last quarter of foetal development, the most substantial alteration in tissue appearance was the presence of distended lysosomes in a large variety of cells, including some neurons, and the presence of axonal spheroids in the brain of 65-dg guinea pigs. Another striking CNS histopathological abnormality observed in α-mannosidosis guinea pigs foetuses was the presence of darkly stained vacuolated cerebrocortical neurones at 51 and 65 dg. These dark neurones could reflect higher susceptibilities to fixation artefacts (ex vivo phenomenon), as well as early signs of neurodegeneration or apoptosis (in vivo phenomenon). As a small number of darkly stained neurones were also noticed in the brain of some unaffected foetuses and as the method of tissue fixation used in this study was not optimized for foetal tissue, we assume that a small number of fixation artefacts were also present in the α-mannosidosis brain. Furthermore, as specific detection methods for signs of neurodegeneration and/or apoptosis were not used in the present study, we cannot demonstrate that these events occurred to a larger extent in the α-mannosidosis brain. The fact that many brain cells are selected to undergo apoptosis during normal foetal development is well known [22,32]. In guinea pigs, apoptotic cells have been detected in normal-developing cerebellum up to 40 dg [33]. However, the existence of a correlation between the level of apoptotic and/or degenerated cells and the extent of intracytoplasmic storage material within the brain still remains to be demonstrated [20]. Moreover, in order to systematically quantify and compare the changes happening during the last quarter of gestation in both α-mannosidosis and unaffected guinea pigs, the use of morphometric analysis would be required.

At present, we do not know whether the cellular changes observed in the brain of α-mannosidosis guinea pigs at 51 and 65 dg were severe enough to produce malfunction of the CNS. The behaviour and growth pattern of affected guinea pigs in the first postnatal weeks do not suggest functional abnormalities. Likewise, kidney or liver appears to function normally in mature α-mannosidosis guinea pigs [13]. In human, the most severe form of α-mannosidosis (type I, infantile) is usually not associated with clinically detectable signs of kidney or liver dysfunction, although abnormal patterns of urinary oligosaccharide excretion and hepatomegaly are present at an early age [1].

Wide individual phenotypic variations during foetal life have already been reported for various animal models of LSD [20]. In the present study, one of the two 51-dg α-mannosidosis foetuses seemed to have accumulated more storage material in Purkinje cells than the 65-dg foetus, again confirming that phenotypic variation appears early on during development. Interestingly, Jones et al. reported that the smaller and rounder Purkinje cells observed in younger mucopolysaccharidosis IIID goat foetuses appear to contain more storage material than the larger and non-spherical Purkinje cells seen in older foetuses [20].

Morphopathological features of α-mannosidosis in human foetuses or neonates have not yet been reported in the literature. Whether the pathology in α-mannosidosis guinea pig brain follows a similar developmental pattern to that of an affected human brain remains to be demonstrated. However, due to obvious limitations in obtaining direct information from human patients, a rough comparison of developmental stages between both species can be useful. In this study, the youngest guinea pig foetuses...
(20 dg) were recovered from the uterus at an age corresponding to human embryonic days 49–56 [34]. The observations made around mid-gestation (30–38 dg) preceded the time of the brain growth spurt occurring between 45 dg and 55 dg in guinea pigs [35]. Therefore, guinea pig mid-gestation is likely to correspond to the period preceding the beginning of the last trimester of gestation in human [36]. The brain of a 51-dg guinea pig most likely correspond to the human brain near term or during early postnatal development, while the 65-dg guinea pig brain is probably equivalent to the brain of a 1- to 2-year-old child [36,37]. Indeed, the fact that newborn guinea pigs (approximately 68 dg) are able to stand and walk within minutes after birth and can start ingesting dry food within 3 days of life supports the view that, regarding motor functions and coordination, the brain of a guinea pig at term has reached a more advanced level of maturation compared with its human counterpart.

In summary, the observations made in α-mannosidosis guinea pig tissues suggest that the relatively small amount of storage material seen within a few differentiating cells around mid-gestation had very little impact on the early stages of ontogenesis. However, the considerable amount of lysosomal vacuolation seen in a variety of differentiating cells during the last quarter of gestation already changed the overall appearance of the tissues and, in the CNS, was associated with the presence of axonal spheroids. It is our hope that the amount of information on early ontogenic progression of α-mannosidosis will continue to expand and to enrich our knowledge on metabolically inherited LSD.

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References


Crawley AC, Walkley SU. Developmental analysis of CNS pathology in α-mannosidosis guinea pigs. 9th International Symposium on Mucopolysaccharide and Related Diseases, Venice, Italy. 2006: Abstract P044.

Walkley SU. Cellular pathology of lysosomal storage disorders. Brain Pathol 1998; 8: 175–93


Vite CH, McGowan JC, Braund KG, Drobatz KJ, Glickson JD, Wolfe JH, Haskins ME. Histopathology, electrodiagnostic testing, and magnetic resonance imaging show significant peripheral and central nervous system myelin abnormalities in the cat model of alpha-mannosidosis. J Neuropathol Exp Neurol 2001; 60: 817–28


Herschkowitz N. Brain development in the fetus, neonate and infant. Biol Neonate 1988; 54: 1–19


Byrnes ML, Reynolds JN, Brien JF. Effect of prenatal ethanol exposure during the brain growth spurt of the guinea pig. Neurotoxicol Teratol 2001; 23: 355–64


Levitt P. Structural and functional maturation of the developing primate brain. J Pediatr 2003; 143: S35–45

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