Cortical Oligodendrocytes in Kaolin-Induced Hydrocephalus in Wistar Rat: Impact of Degree and Duration of Ventriculomegaly

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Keywords

Oligodendrocyte · Hydrocephalus · Endoplasmic reticulum · Mitochondria · Ventriculomegaly

Abstract

Background: Oligodendrocytes are critical to the function of the brain. They generate the myelin sheath which ensures saltatory conduction, which is a more energy saving and efficient means of axonal impulse transmission. Ventriculomegaly results in neuronal degeneration and astrogliosis. **Purpose:** The effect of the degree of ventriculomegaly on oligodendrocyte in kaolin-induced hydrocephalus and the timeline have not been extensively documented, hence this study. **Methods:** A total of 81 rats that were 3 weeks old were divided into 4 groups each consisting of control and experimental subgroups. Kaolin suspension was intracisternally injected to induce hydrocephalus and the animal sacrificed post-induction at 1, 2, 3, and 4 weeks. Two 1-mm-thick coronal slices at optic chiasma level were fixed in 10% buffered formal-saline and Karnovsky's fixative for light and transmission electron microscopy (TEM), respectively. The former slices were processed and stained with hematoxylin and eosin for glial density, cortical thickness, and oligodendrocyte evaluations. Subcortical white matter region of the latter were processed by conventional techniques for TEM. **Results:** Compared with their corresponding control rats, thickness of the dorsolateral cortex was significantly reduced across the 2–4 week post-induction (WPI), glial density was signifi-

cantly increased in the mild and moderate ventriculomegaly subgroups 1 WPI but only in mild ventriculomegaly subset 2 WPI. In the 4 WPI group, there was significant increase in glial density across the 3 ventriculomegaly subsets. Early hydroptic changes of oligodendrocytes were noted in the inner pyramidal layer mostly in the 4 WPI experimental rats. Dilation of the endoplasmic reticulum precedes that of mitochondria, while mitochondrial crista disruption was noted in the 3 and 4 WPI rats. The nuclear membrane of the oligodendrocytes was progressively deformed from the 2nd to 4th WPI. **Conclusion:** This study reported degenerative changes of oligodendrocytes and its organelles in kaolin-induced hydrocephalus. Degeneration was worse with duration and in the deep cortical layers.

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Introduction

The complex proliferation, migration, and differentiation of oligodendrocytes is a critically timed process that ensures the insulation of the axons of the central nervous system (CNS) [1]. Moreover, their unique physiology makes them one of the most vulnerable cells of the CNS. In rodents, oligodendrocyte precursor cells (OPC) originate from the medial ganglionic eminence, anterior entopeduncular area of the ventral portion of the forebrain, the lateral, and or caudal ganglionic eminences and the postnatal cortex [2].

Contact mediated mechanism involving the astrocyte, axons, and the extracellular matrix contribute significantly to the migration of the OPCs. Cytokines such as platelet derived growth factor, fibroblast growth factor and hepatocyte growth factor, and chemotrophic factors such as netrins and the chemokine CXCL1 affect migration [3–7]. Notably, some OPCs persist while majority differentiate into myelin producing oligodendrocytes [8]. Oligodendrocytes generate approximately 100 times its cell body weight in membrane, hence have an extremely high metabolic rate [9–11].

A congenital hydrocephalus mice model has implicated an oligodendrocyte morphological defect in the degeneration of cortical axons [12]. Hence, oligodendrocyte function is not restricted to myelination, but includes a possible supportive role in the survival and development of cortical axon. In another study, depletion of the glial precursor cells was noted in a 14-day-old model of kaolin-induced hydrocephalus [13]. Also the optic nerve of a rat model of 21-day kaolin-induced hydrocephalus revealed a reduction in the density of oligodendrocytes [14]. Hydropic changes affecting the mitochondria and endoplasmic reticulum have also been reported in oligodendrocytes of infant hydrocephaly [15]. The morphology of oligodendrocytes is essential for successful myelination under the modulatory role of integrin [16]. It is plausible to assume that the altered morphology of oligodendrocytes may lead to myelin sheath dysfunction leading to distorted axon of the developing neuron thereby affecting cognitive and motor function that is associated with hydrocephaly.

Therefore, the aim of this study was to evaluate the morphology of oligodendrocytes of 1–4 weeks kaolin-induced hydrocephaly in Wistar rats with a varying degree of ventriculomegaly.

Methods

Study Population

A total of 81 rats that were 3 weeks old of both sexes weighing between 19 and 36 g were divided into 4 groups viz., A, B, C, and D, each having control and experimental subgroups as shown;

1. Group A, 22 rats (experimental = 17; control = 5)
2. Group B, 20 rats (experimental = 15; control = 5)
3. Group C, 20 rats (experimental = 16; control = 4)
4. Group D, 19 rats (experimental = 14; control = 5)

Rats were provided water and standard rat chow freely. Bedding was changed regularly, and rats were monitored closely; 200 mg/mL of aluminum silicate (kaolin) suspension was prepared under sterile condition with normal saline. Under anesthesia (90 mg/kg body weight of ketamine and 12.5 mg/kg body weight of diazepam), hydrocephalus was induced by percutaneous injection

Table 1. Showing the distribution of the rats according to the groups and degree of ventriculomegaly

Groups	Degree of ventriculomegaly		
	mild	moderate	severe
A	6	6	5
B	6	4	5
C	6	4	6
D	5	5	5

of a kaolin suspension (0.04 mL) into the cisterna magnum with a 25 G needle. Rats in each group were sacrificed after 1, 2, 3, and 4 weeks post-induction respectively by cervical dislocation. Two coronal brain slices were obtained at the level of the optic chiasma and immediately immersion fixed. Ethical approval for the study was obtained from the University of Ibadan animal care and use research Ethics Committee and the University's animal care protocol was adhered to. This article conforms to the ICJME recommendation.

Light Microscopy

Tissue was fixed in 10% formal saline and processed using conventional dehydration techniques prior to paraffin wax embedding. Five micron thick sections were cut and stained with hematoxylin and eosin (H&E). Sections were viewed on a light microscope and morphometric image analysis of the width of the lateral ventricle, glia cell count and density was performed. Ventricular width classification corresponded with mild, moderate, and severe ventriculomegaly. Therefore, the experimental subgroups of weeks 1–4 were further divided into mild, moderate, and severe ventriculomegaly for assessment of glial density and electron microscopy evaluation, that is,

- i. Mild ventriculomegaly – ventricular width $\leq$ double the mean width of the corresponding age-matched control rats
- ii. Moderate ventriculomegaly – $>$ double the mean width of the corresponding age-matched control rats and $\leq$ quadruple of the mean width of the corresponding age-matched control rats
- iii. Severe ventriculomegaly – $>$ quadruple of the mean width of the corresponding age-matched control rats

Ventricular width measurements was done at the CA1 level of the hippocampus for all micrographs.

The distribution of rats in the different ventriculomegaly subsets of the experimental subgroup is shown in Table 1.

Calculation of Cellular Density

Serial photomicrographs of the dorsolateral cortex were obtained for all the groups. Photomicrographs were labelled appropriately for each rat and were transferred to Scope Photo DCM 510 software template. An automated 4×4 pixel grid frame was superimposed on the photomicrographs. Cells within alternate grids were counted per micrograph and carefully noted. Cells bisected by the grid line with more than half of the cell within the grid were counted, while those less than half within the grid were ignored. Double counting of cells on the grid line was avoided. Dimension of the grid was converted from pixel to micrometer by the equation below;

$$\text{Number of pixel} \times 2.1167 = Y (\mu\text{m})$$

Table 2. Showing the glial density in the dorsolateral cortex

	Control ventriculomegaly, $\times 10^{-3}/\mu\text{m}^3$	Mild ventriculomegaly, $\times 10^{-3}/\mu\text{m}^3$	Moderate ventriculomegaly, $\times 10^{-3}/\mu\text{m}^3$	Severe ventriculomegaly, $\times 10^{-3}/\mu\text{m}^3$
1 week	7.042 $\pm$ 0.278 (n = 5)	16.450 $\pm$ 1.048* (n = 6)	20.820 $\pm$ 1.166*, α (n = 6)	11.950 $\pm$ 2.975 α (n = 5)
2 weeks	4.667 $\pm$ 0.678 (n = 5)	9.426 $\pm$ 1.651* (n = 6)	5.232 $\pm$ 0.417 (n = 4)	8.664 $\pm$ 1.147 (n = 5)
3 weeks	7.775 $\pm$ 1.953 (n = 4)	5.325 $\pm$ 0.948 (n = 6)	5.605 $\pm$ 0.711 (n = 4)	7.185 $\pm$ 1.018 (n = 6)
4 weeks	4.232 $\pm$ 1.121 (n = 5)	10.730 $\pm$ 0.919* (n = 5)	8.364 $\pm$ 0.838* (n = 5)	10.650 $\pm$ 2.534* (n = 4)

Values are expressed as mean $\pm$ SEM.

* Statistically significant differences compare with control; α statistically significant difference between indicated columns. $p < 0.05$.

Cell count for the dorsolateral cortex of each rat was divided by the combined area (μm^2) of the grids counted in the rat. Mean cellular density for rats in each group were calculated and tabulated. Cell type identification was based on Histology of the CNS [17].

Data obtained were analyzed with ANOVA followed by Newman–Keul’s test of multiple comparisons using Graph Pad Prism 5.

Transmission Electron Microscopy

The subcortical white matter region was minced into 1 mm³ and fixed in Karnovsky fixative (Karnovsky, 1960). Following 2 washes in 0.2 M sodium cacodylate buffer (pH 7.2; 4°C; 5 min each), tissue were post-fixed in osmium tetroxide (1 h), and further washed in 0.2 M cacodylate buffer. Tissue were then dehydrated through an ascending series of ethanol with final dehydration in an intermediate solvent, propylene oxide prior to infiltration with a low viscosity Spurr epoxy resin (Sigma, USA; Spurr, 1969). Tissue were embedded and polymerized for 48 h at 60°C.

Ultrathin (50–60 nm) sections were cut and collected on 200 mesh copper grids. Sections were contrast enhanced with a saturated ethanolic uranyl acetate solution followed by Reynold’s lead citrate (3 min each). All sections were viewed on the Jeol 1011 transmission electron microscope.

Results

General Observation

Food consumption and a reduction in physical movement was observed across all experimental groups compared with their corresponding control subgroup. Twenty-four hours post-induction, the experimental rats remained lethargic with poor food consumption. These however improved toward the end of the first week of induction. Domed-shaped enlargement of the head developed in the 2nd week of induction alongside protrusion of the eyeball. However, poor motor activity persisted in the experimental rats and got worse with increasing duration of induction.

Fig. 1. A line graph illustrating cortical thickness (mm) of experimental and control subsets of 1–4 weeks post-induction. * Statistically significant difference at $p < 0.05$.

Light Microscopy

The thickness of the dorsolateral cerebral cortex over the 4 week period was consistently reduced in the experimental rats compared with the corresponding control rats (Fig. 1). This difference was statistically significant in the 3rd to 4th post-induction weeks ($p < 0.001$ across the groups).

The density of the glial cells of the experimental and control group across the 4 weeks are outlined in Table 2. In the 1 week post-induction (WPI) group, an increase in the glial density was noted across the 3 experimental groups compared with the control. The differences were however significant in the mild and moderate ventriculo-

Fig. 2. Photomicrographs of the molecular layer of the cerebral cortex of Control (C) and Experimental (E) Wistar rats following 1, 2, 3, and 4 weeks of hydrocephalus (superscript 1, 2, 3, and 4, respectively) showing normal oligodendrocytes (red arrow), degenerating oligodendrocyte (black arrow). Stain H&E. Magnification $\times 100$.

megaly subgroups compared with the control ($p < 0.001$). While a statistically significant difference was noted between the mild ventriculomegaly and control subsets of the 2 weeks post-induction group ($p = 0.028$), the increase in the density noticed in the moderate and severe subsets compared with the control was not statistically significant. In the 3 weeks post-induction group, the reduced glial density across the experimental subsets was not significant when compared with the control. However, a significant increase in glial density was noted in the experimental subsets compared with the control in the 4 weeks post-induction group ($p = 0.013$).

The molecular layer of the cerebral cortex showed few cells across the groups. However, in the control subsets of the different groups, normal oligodendrocytes with the surrounding halo were noted either in twos and threes. They appeared to be more in the control subsets than the corresponding experimental subsets which showed degenerating oligodendrocytes in different stages of degeneration. While some oligodendrocytes were completely degenerated with the remnant of the cell noted others appeared to be in the early stages of degeneration with features of hydropic changes (Fig. 2).

The outer granular and pyramidal layers of the cerebral cortex revealed an abundance of apparently normal oligodendrocytes (Fig. 3) in the control subset across the 4 groups. However, fewer albeit smaller in size oligodendrocytes were noted in the experimental subsets. The 4 week group showed increased number of apparently normal oligodendrocytes

in the experimental subset compared with the other experimental subsets. Several degenerating oligodendrocytes with hydropic features were noted in the experimental subsets of the different groups.

The inner pyramidal layer of the cortex showed few apparently normal oligodendrocytes in the experimental groups mostly in the 4 week experimental subset, the control groups showed abundance of apparently normal oligodendrocytes. The experimental subsets revealed abundant degenerating oligodendrocyte at different stages of degeneration across the 1–4 WPI group (Fig. 4). Vacuolation of the white matter bundle in the subcortical white matter region of the cortex was conspicuous in the experimental subset associated with an apparent disruption of the string-like arrangement of oligodendrocytes. It appeared to be worse with increasing duration of ventriculomegaly. The white matter bundle was compact in the control subset and the oligodendrocytes were arranged in a string-like fashion when they are not obstructed by the compact white matter (Fig. 5).

Electron Microscopy (Fig. 6)

1 WPI group; Oligodendrocytes of the control group appeared round with a largely euchromatic nuclei. A similar pattern of chromatin distribution was observed in the mild ventriculomegaly subset rats. Oligodendrocyte cytoplasm was rich in mitochondria and endoplasmic reticulum in both the control and mild ventriculomegaly subset rats. Endoplasmic reticula of the severe ventriculomegaly

Fig. 3. Photomicrographs of the outer granular/pyramidal layer of the cerebral cortex of Control (C) and Experimental (E) Wistar rats following 1, 2, 3, and 4 weeks of hydrocephalus (superscript 1, 2, 3, and 4, respectively) showing normal oligodendrocyte (red arrow), degenerating oligodendrocyte (black arrow). Stain H&E. Magnification $\times 100$.

Fig. 4. Photomicrographs of the Inner pyramidal layer of the cerebral cortex of Control (C) and Experimental (E) Wistar rats following 1, 2, 3, and 4 weeks of hydrocephalus (superscript 1, 2, 3, and 4, respectively) showing normal oligodendrocyte (red arrow), degenerating oligodendrocyte (black arrow). Stain H&E. Magnification $\times 100$.

subset appear slightly dilated whilst the mitochondria appear normal.

2 WPI group; The shape of oligodendrocytes of the control group varied, often appearing elliptoid with a rim of heterochromatin around a largely euchromatic nucleus. In the mild ventriculomegaly subset, the rim of heterochromatin was prominent and thicker than the control group. Nuclei crenation was observed. However, the endoplasmic reticulum and the mitochondria were mor-

phologically normal. In the severe ventriculomegaly subset, nuclei crenation was common with electron dense chromatin patches.

3 WPI group; A thin rim of heterochromatin around a largely euchromatic nucleus predominated in oligodendrocytes of the control subset. Their cytoplasmic organelles (mitochondria and endoplasmic reticulum) were morphologically normal. However, in the mild ventriculomegaly subset nuclei were largely euchromatic with highly

Fig. 5. Photomicrographs of the subcortical white matter layer of the cerebral cortex of Control (C) and Experimental (E) Wistar rats following 1, 2, 3, and 4 weeks of hydrocephalus (superscript 1, 2, 3, and 4, respectively) showing normal oligodendrocyte (red arrow), degenerating oligodendrocyte (black arrow). Stain H&E. Magnification $\times 100$.

dilated endoplasmic reticulum cisternae. Mitochondrial dilation with disruption of the cristae and intramitochondrial vacuolation were noted. In the severe ventriculomegaly subset, the nuclear membrane were often crenated, endoplasmic reticulum and mitochondria were sparse.

4 WPI group; The control subset revealed an elliptoid oligodendrocyte with dense heterochromatin within nuclei and abundant mitochondria and endoplasmic reticula. In the mild ventriculomegaly subset, oligodendrocyte nuclei were small with nucleolemmal disruption. Cytoplasm was degenerative and limited by a “halo”. Organelles were sparse. Microglia cells with cellular debris were noted in the neuropil. In the severe ventriculomegaly subset, the oligodendrocyte nuclei were euchromatic with nucleolemmal disruption. Microglia occurred in close proximity to oligodendrocytes. Cytoplasm were very thin with degenerative endoplasmic reticulum and mitochondria.

Discussion

Oligodendrocytes are critical to the functioning of the nervous system. The production of myelin sheath and its role in axonal conduction ensures the conservation of energy use as well as reduce the time required for axonal transmission. Since oligodendrocytes generate myelin sheath membrane, their deregulation probably leads to cognitive and motor dysfunction in hydrocephalus.

In our study, the oligodendrocytes in the control subsets of the different groups appeared healthy with a sur-

rounding halo, some of them are arranged in pairs and triplets. A reduction in the number of oligodendrocytes across the experimental subsets of the 4 groups was noted (Fig. 2–4). However, the glial density assessment revealed an increase in the experimental subsets in 1, 2, and 4 weeks post-induction groups respectively while a reduced density was noted in the 3 weeks post-induction group. Members of the glial family include astrocytes, microglial, and oligodendrocytes. Our observations are indicative of an early degeneration of oligodendrocytes when compared to other glial cells in kaolin-induced hydrocephalus. While oligodendrocytes are primarily concerned with the production of myelin sheath, thereby enhancing axonal conduction, the other glial cells particularly astrocytes are associated with functions such as mobilization of Ca^{2+} , clearance of glutamate, and regulation of blood flow [18]. This shows that the process of myelin formation will be adversely affected in kaolin-induced hydrocephalus while the above-stated functions of astrocyte are spared. Since microglial are largely invisible at light microscopy level, the glial density are a reflection of the oligodendrocyte and astrocyte in the cortex. The increased density of glial cells may therefore be a reflection of increased mobilization of astrocytes as a compensatory mechanism in kaolin-induced hydrocephalus. The ability of astrocytes to cause cerebral vasodilation and its antioxidant properties make it a candidate to compensate for the increased intra-cerebral pressure and generation of reactive oxygen species which are synonymous with kaolin-induced hydrocephalus. Reactive gliosis is a known manifestation of hydro-

Fig. 6. Electron micrographs of the subcortical white matter region of the cerebrum of 1–4 week post-induction rats (1, 2, 3, and 4), control rats (C), mild ventriculomegaly (M) and severe ventriculomegaly (S) showing oligodendrocyte (OL) with morphologically

intact mitochondria (short thick white arrow), endoplasmic reticulum (long thin white arrow), deformed mitochondria (short thick black arrow), dilated endoplasmic reticulum (long thin black arrow). Mg, microglia.

cephalus which is amenable to shunting thereby associating it with the ventriculomegaly of hydrocephalus [19, 20]. Of note, in our study, the increase in the density of glial cell was significant across the experimental subsets at 4 weeks of induction. This points to a possible gradual mobilization of astrocytes which peaks at about the 4th week of kaolin-induced hydrocephalus.

Hydropic changes in oligodendrocytes are features that are suggestive of early degeneration of the myelin-producing cell of the CNS [21]. The hydropic changes of oligodendrocyte in this study worsen with the duration of hydrocephalus and this is associated with a disruption of the chain-like arrangement of oligodendrocyte particularly in the subcortical white matter region of the cortex.

These changes were noted very early in kaolin-induced hydrocephalus confirming the fact that they represent early features of oligodendrocyte degeneration. Similar early cytopathological changes had been reported in Schwann cells of rat's sural and tibial nerves [22] following injection of lysophosphatidyl choline (LPC) type IV. However, to ascertain a possible role of LPC in the hydropic changes of oligodendrocyte and whether or not there is an increase in endogenous LPC in kaolin-induced hydrocephalus will require further research efforts. The hydropic changes in oligodendrocyte and disruption of its chain-like arrangement appeared to tally with the disruption of its organelles.

The subcortical white matter region is occupied by a conspicuous white matter bundle. However, this study revealed a progressive vacuolation of the white matter bundle in direct relation to the duration and severity of ventriculomegaly in kaolin-induced hydrocephalus (Fig. 5). As the duration and severity of the anomaly increases, the white matter was transformed from a compact bundle into a loosened bundle. Decompaction of white matter had been attributed to several factors such as intraventricular pressure-driven movement of the cerebrospinal fluid (CSF) from the ventricle into the cortical tissue across a denuded ependymal layer [23]. Other factor that has been found to be causal to decompaction of white matter is a downregulation of the production of myelin-related proteins. The correlation between the severity of ventriculomegaly and decompaction of white matter in the subcortical white matter region in this study points to a possible role of intraventricular pressure-driven CSF edema.

A correlation between early oligodendrocyte degeneration and organelle disruption was reported in infant hydrocephalus and associated pathologies in humans [15]. A similar finding was noted in this study. However, while dilation of endoplasmic reticulum was noted in the oligodendrocyte after 1 week of hydrocephalus, mitochondrial dilation was noted after 2 weeks of ventriculomegaly. This showed that disruption of energy generation is not an early contributor to or cause of oligodendrocyte pathology in kaolin-induced hydrocephalus. However, in addition to the dilation of mitochondria, crista disruption and intramitochondrial vacuolation were noted as the duration of hydrocephalus increases. This shows that despite the relatively late commencement of mitochondrial deformity, increasing duration of hydrocephalus results in more devastating morphological distortion of the organelle.

Deformity of the nuclear membrane was noted from the 2nd week of ventriculomegaly and progressed in direct relation to the duration of hydrocephalus. It also ap-

peared to be in direct relation to the severity of the ventriculomegaly. A disruption of the nuclear membrane will result in the release of the contents of the nucleoplasm into the cytoplasm. This might explain the congealed cytoplasm that was noted after 3 weeks of hydrocephalus. It is critical to note that the congealing of the cytoplasm was preceded by a disruption of the nucleoplasm which was noted after 2 weeks of hydrocephalus. Organelle disruption will result in the release of the content of the various organelles into the cytoplasm and might explain the destruction of the cytoplasm. However, the possibility of such cytotoxic content of the organelle to effect a gradual degeneration of the nuclear membrane and eventually gain access into the nucleus will be the focus of subsequent research effort.

The aforementioned intracellular events in the oligodendrocyte of the subcortical white matter region following kaolin-induced hydrocephalus might explain the shrinking of oligodendrocyte and presence of microglial cells noted in the 4 weeks post-induction group. Degrading oligodendrocytes are not only shown as shrunken cell, others are seen with a thin rim of cytoplasm. This shows that the degeneration of oligodendrocyte in kaolin-induced hydrocephalus occur gradually and in stages.

Conclusion

The findings of this study demonstrated that duration and severity of ventriculomegaly are critically responsible for extensive morphological alteration of oligodendrocytes in kaolin-induced hydrocephalus in Wistar rats.

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Author's Contribution

A.O.A. has done conception of the research work, animal care, induction of hydrocephalus, tissue harvesting, processing and histology, light and electron microscopy, data analysis, and Manuscript writing. N.T. has done tissue processing for electron microscopy, electron microscopy, data analysis, and manuscript writing.

Disclosure Statement

This article conforms to the ICJME recommendation.

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