

Semua laporan kemajuan dan laporan akhir yang dikemukakan kepada Bahagian Penyelidikan dan Pembangunan perlu terlebih dahulu disampaikan untuk penelitian dan perakuan Jawatankuasa Penyelidikan di pusat pengajian

**LAPORAN AKHIR PROJEK PENYELIDIKAN
R & D JANGKA PENDEK**

RUJUKAN

A. MAKLUMAT AM

Tajuk Projek: ..Effect of Experimental Hyperbilirubinemia
on visual system in Rat Brain.....

Tajuk Program:

Tarikh Mula: ...1 August, 1996.....

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B. PENCAPAIAN PROJEK:

(Sila tandakan / pada kotak yang bersesuaian dan terangkan secara ringkas di dalam ruang di bawah ini. Sekiranya perlu, sila gunakan kertas yang berasingan).

Penemuan asli/peningkatan pengetahuan

Neuronal damage was seen in lateral geniculate
nucleus in experimentaly hyperbilirubinemic neonatal rat
brain.

PERKHIDMATAN PERUNDINGAN BERBANGKIT DARIPADA PROJEK (Klien dan jenis perundingan)

- (1)
- (2)
- (3)
- (4)

F. PATEN/SIJIL INOVASI UTILITI
(Nyatakan nombor dan tarikh pendaftaran paten. Sekiranya paten/sijil inovasi utiliti telah dipohon tetapi masih belum didaftarkan, sila berikan nombor dan tarikh fail paten).

- (1)
- (2)
- (3)

G. PENERBITAN HASIL DARIPADA PROJEK

(i) LAPORAN/KERTAS PERSIDANGAN ATAU SEMINAR

- (1) Presentation of paper in third National Conference on Medical Sciences, PPSP, USM, 25 - 26 May, 1997.
- (2) Siddiqui, MS; Othman, M and Singh (1997).....
Neuronal damage in lateral geniculate nucleus without bilirubin,
② Staining in Hyperbilirubinemic neonatal rat brain.
Abstract OP - 8, Page 19.....
- (4)
- (5)

EFFECT OF EXPERIMENTAL HYPERBILIRUBINEMIA ON VISUAL SYSTEM IN RAT BRAIN

by

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OBSERVATIONS

The average serum bilirubin concentration in the control and the hyperbilirubinemic group was 0.19 mg% and 3.39mg% respectively.

Gross examination of the brains the control and the experimental animals did not show bilirubin staining (Fig.1 and Fig.2).

The coronal sections of brain passing through the caudal part of diencephalon showed the cavity of third ventricle lined by ependyma. Specialised elongated ependymal cells were seen in subcommissural organ (SCO)(Fig.3). Posterior commissure (PC) was seen as bundle of transverse fibers dorsal to subcommissural organ (Fig.4). The lateral geniculate nucleus with dorsal (D) and ventral (V) parts was identified (Fig.5).

Lateral Geniculate Nucleus (LGN)

In control animals

The coronal sections of the fore brain passing through the caudal part of diencephalon stained with haematoxylin and eosin, Nissl and myelin stains showed the dorsal (D) and ventral (V) parts of lateral geniculate nucleus (Fig.5). The nucleus was sharply outlined by the fibers of the external medullary lamina medially(EML) and those of the optic tract(OT) laterally (Fig.6).

In the LGN medium sized neurons, nerve fibres and glial cells were seen. The neurons showed large oval relatively pale nucleus with well-defined nucleolus. The cytoplasm was studded with Nissl granules. In some neurons the Nissl granules were so much abundant that the nucleus was not clearly visible (Fig.7 ,9). Neuroglial cells were seen and were distinguished by small sized nucleus .Neurons were much larger than the glial cells.

Hyperbilirubinemic Animals:

Damaged neurons were seen in the dorsal and the ventral parts of lateral geniculate nucleus. Damaged neurons were identified by clear cytoplasm due to chromatolysis, hyperchromatic and distorted nuclei and insignificant nucleolus(Fig.8, 10).Some damaged neurons showed shrunken perikarya. Ghost like cells containing granular debris of chromatin showing the disintegration of neurons were not seen. There were no significant changes in the glial cells.The capillaries did not show the congestion and ecchymosis. The surrounding ground substance was loosely textured.Nerve fibres did show any significant change.

Visual Cortex

In Control Animals

The coronal section of the brain passing between posterior commissure and occipital poles of cerebrum showed visual cortex and underlying hippocampus(Fig.11). Visual cortex consisted of six layers .The neurons were distinct in arrangement of their perikarya. The neurons showed large spherical nucleus and prominent nucleolus and cytoplasm showed the Nissl granules(Fig.12,14). Pyramidal neurons were seen in all layers of visual cortex except in plexiform layer and were predominant in layer five.Non pyramidal neuron were present in all layers. Glial cells were seen between neurons.

In Hyperbilirubinemic Animals

In the hyperbilirubinemic rats sparseness of neurons in the visual cortex (reduction in the number of neurons) in different layers especially layer I and II was not detectable. Neurons were evenly stained and nuclei were not hyperchromatic. Outlines of the nuclei were regular (Fig.13,15).

Cytoplasm of neurons was not vacuolated and Nissl substance was present and "ghost" cells were not seen. There was no proliferation of glial cell in comparison to control animals. There was not much change in nerve fibres in comparison to control animals. The ground substance was not loosely textured.

Superior Colliculus

The coronal sections of midbrain passing through superior colliculus stained with cresyl violet and luxol fast blue showed neuronal layers (Fig.16).

Control animals

Neurons in different layers of superior colliculus were of medium size. The neurons in the control animals showed a large oval nucleus with a well defined nucleolus. The cytoplasm was studded with Nissl granules. Neuroglial cells with small condensed nuclei and clear cytoplasm were seen (Fig,17,19).

In hyperbilirubinemic animals

In the experimental animals neurons did not show significant changes. Neurons in different neuronal layers were normal looking in hematoxylin-eosin and Nissl stained sections. Neurons were having large nucleus with prominent nucleolus. Cytoplasm was studded with Nissl granules and there was no vacuolation in the cytoplasm (Fig.18,20). Glial cells and nerve fibers did not show significant changes.

MATERIAL AND METHODS

The animals used in the present study were 10 days old neonatal weaning Sprague Dawley rats weighing about 15 g. For this purpose ten days old neonatal rats along with their mothers were obtained from the animal house of PPSP, USM. These neonatal rats along with their mothers were kept in the dark room during the experimental period to avoid the conversion of bilirubin into photo-bilirubin in the presence of light. The lactating females were fed on standard pellet diet and tap water at libitum.

Bilirubin powder (Sigma) was dissolved in a small volume of 0.1 NaOH. The volume of the solution was made up by normal saline so as to obtain bilirubin concentration of 100 μ g/0.1 ml. The solution was prepared in a tube covered with black paper and was kept in dark room. Insulin syringe was used to inject the Bilirubin solution intraperitoneally. Fresh solution was prepared every time.

Fifty neonatal rats comprising of 25 control and 25 for treatment with bilirubin were used for reproducing the experimental model on the line as described by Kaul (1981)

Experimental animals : All the 25 animals from the experimental group were injected intraperitoneally with bilirubin solution prepared as previously described in the doses of 100 μ g/g body weight, hourly six injections per day for three days.

Control animals : All the 25 animals of control group were given intra-peritoneal injections of the same volume of normal saline at hourly intervals to a total of six injections/day for three days.

At the end of the experiment under ether anaesthesia blood samples were collected from the heart of control and experimental animals. The serum bilirubin was estimated by the method of Malloy and Evelyn(1937) using diazo A and diazo B.

For histological study, fixation was done by transcardiac perfusion of 10% buffered formaline under ether anaesthesia in control and experimental animals.

Formalin fixed brains were dissected out from the cranial cavity and examined for bilirubin staining. Coronal sections of brain passing through caudal part of diencephalon upto occipital pole were cut with the help of Craige's charts (1953). Horizontal sections of cerebrum were also taken. These sections were having lateral geniculate nucleus, visual cortex and superior colliculus.

The tissue pieces were fixed in formalin, processed (dehydration, clearing and paraffin embedding) and paraffin blocks were sectioned at 4 micron and 10 micron with rotatory microtome. Thin sections were stained with haematoxylin and eosin stain. Thick sections were stained by Nissl stain using Cresyl violet. Myelin staining was done by Luxol fast blue stain.

Sections were examined under Olympus microscope with photographic attachment. Photography of important observations was done using computerised exposuremeter.

Aim of the study

The aim of the present study is to investigate the histological changes in visual system (Lateral geniculate bodies and visual cortex) in experimentally hyperbilirubinemic neonatal rats.

DISCUSSION

The results of the present study represent the histological study of visual system in experimental hyperbilirubinemia. The results demonstrate that bilirubin causes neuronal damage in the visual system. The damage is selective. Although bilirubin entry has been shown in visual cortex, lateral geniculate bodies and superior colliculus by autoradiographic studies using [3H] bilirubin (Roger,1996), neuronal damage is seen only in lateral geniculate nucleus. This may be due to more entry of bilirubin in the lateral geniculate nucleus than in visual cortex. Lowest entry of bilirubin has been shown in the superior colliculus (Roger,1996). We have not observed neuronal damage in the visual cortex as reported in a single case of neonatal jaundice (Crome,1955).

The reason for the differences in neuronal damage in different parts visual system is not known. However, several mechanisms appear to be possible. These may involve the uptake, redistribution, local metabolism or excretion and may be mediated by the regional differences in brain blood flow, permeability of blood brain barrier, concentration of bilirubin-binding molecules and bilirubin oxidizing enzymes.

However, bilirubin may not be equally toxic to all class of neurons (Notter,1986; Aoki 1985). Purkinje cells show the earliest and most severe manifestation of bilirubin toxicity. The binding of bilirubin to Purkinje cells may be a precondition for this increased sensitivity ((Danbolt,1993).

Rates of bilirubin clearance from brain regions may be different (Hansen ,1995). In several studies clearance of bilirubin from rat brain has been examined. (Broderson,1969; Hansen,1995,1996). These studies have shown a rapid clearance of bilirubin from brain than serum. There are significant differences between rat brain regions in the ability to oxidize bilirubin. Highest clearance activities were found in the cerebellum and midbrain. (Hansen,1996). Brain regions may differ with respect to metabolic activity and number and size distribution of mitochondria in the cells and activity of oxidizing enzymes. But these differences could not explain the kernicterus staining phenomenon, because the highest activities were found in brain regions which are more heavily stained in kernicterus. Bilirubin increases the affinity of the N-methyl-D-aspartate (NMDA) receptor and modifies the function of the NMDA receptor/ion channel complex in the brain of newborn piglet. Modification of the receptor by bilirubin may adversely affect the neuronal function. (Hoffman,1996).

Our observations support the hypothesis that neuronal susceptibility is an important factor in bilirubin neurotoxicity. Tissue culture studies also confirm that mitotically active neuronal cells are more sensitive to bilirubin treatment than mature neurons but mitotically active rat glial cells were resistant to bilirubin toxicity (Notter and Kendig 1986) Astrocytes are less sensitive to bilirubin cytotoxic effects, impairment of mitochondrial activity, than fibroblast (Chuniaud,1996).

This selective neuronal susceptibility of lateral geniculate nucleus neurons may be related to difference in bilirubin oxidising ability of neurons as there are significant differences between rat brain regions in the ability to oxidise bilirubin by mitochondrial membranes (Hansen and Allen 1996). Differential susceptibility may be due to differences in bilirubin binding to neuronal cell bodies and intracellular binding to mitochondrial membranes.

Autoradiographic studies using [3H] bilirubin in adult rat brain sections in vitro have shown significantly higher rate of binding to neuronal cell bodies relative to other tissue elements. The binding sites were mainly intracellular. Bilirubin localizes preferentially to mitochondria in neuroblastoma cells (Schiff, 1991). Bilirubin is not toxic equally to all class of neurons (Notter, 1986) and there are regional differences in the concentration of molecules capable of binding bilirubin (Asaoka, 1986; Gurba, 1974; Kler, 1985; Nagaoka, 1978; Weil, 1975).

In our studies glial cells did not show appreciable change. This may be due to the resistance of neuroglial cells to bilirubin toxicity as shown by earlier tissue culture (Notter 1986) and autoradiographic studies (Roger, 1996). Our observations confirm the previous studies. There was no proliferation of glial cells in lateral geniculate nucleus, visual cortex and superior colliculus although reported in earlier studies in other regions of brain like basal ganglia and cerebellum following that neuronal damage (Schutta, 1967; Rodilsky, 1961; Crome 1955; Jew 1979).

Myelin staining did not show changes in experimental and control rat visual system. This is not in favour of previous observations of demyelination seen in optic nerve in neonatal jaundice (Zimmerman, 1935). In the rat, light microscopic evidence of myelin is not present in the cerebrum prior to the 10th postnatal day (Jacobson, 1963). Myelin sheath is first discernible in the pyramidal tract, posterior commissure, and internal capsule at the 10th postnatal day, and the corpus callosum at the 12th day (Schonbach, 1968). Demyelination as seen in human may not be detectable due to the early stage of degeneration in brain (Hansen, 1988).

In the present study bilirubin staining of brain and visual system was not seen in sections of brain. This confirms the earlier studies that functional brain toxicity and even death from hyperbilirubinemia may occur in the absence of both visible yellow staining and light microscopic cellular damage (Gartner, 1993). It may be possible that bilirubin may have been removed during fixation in formalin by cardiac perfusion, processing and embedding in paraffin as reported earlier (Dublin, 1951). However staining is not important because bilirubin staining of brain may occur without evidence of neuronal injury (Turkel, 1982). In the present study it is not possible to evaluate accurately, the extent of structural brain changes in experimental hyperbilirubinemia. Electron microscopy is judged to be the best way to evaluate morphological changes in neurons (Jew, 1979).

Therefore absence of neuronal damage in visual cortex and superior colliculus at the light microscopic level does not exclude possibility of neuronal damage at the ultrastructural and molecular level. Visual evoked potential abnormalities in neonatal jaundice (Chen,1995) and in Gunn rats (Silver, 1991,1995) may be due to neuronal damage in lateral geniculate bodies. But further ultrastructural and histochemical studies are needed for confirmation of results before application of these results.

Conclusions

In experimental hyperbilirubinemia in ten days old rats, histological changes have been observed in some areas of the visual system of brain under light microscopy.

1. Bilirubin staining of the brain including visual system was not a feature in this study.
2. Neuronal damage was seen in the lateral geniculate nucleus.
3. Features of neuronal damage were not seen in the visual cortex.
4. Neuronal damage was not identified in the superior colliculus.
5. Glial proliferation was not seen in the lateral geniculate nucleus, visual cortex and superior colliculus.
6. Myelin changes were not noticed in the lateral geniculate nucleus, visual cortex and superior colliculus.

Recommendations

Visual evoked potential abnormalities (reduced amplitude or delayed response) can be caused by disturbance of function of either optic nerve or the cerebral cortex. The procedure itself does not identify the anatomic site or the mechanism of the disturbance (Prosen and Stebbins 1980). Electroretinogram (ERG) changes (significant prolongation of wave b) which becoming apparent in hyperbilirubinemia after six hours indicates that primary damage in visual pathway is beyond the retina (Silver 1995).

The present study is limited to central visual pathways, did not show neuronal damage in the visual cortex under light microscopy. It is important to study the central visual system at the ultrastructural, histochemical and biochemical level for mitochondrial damage and synaptogenesis for confirmation of the results before application of these results.

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