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Study of Therapeutic Effect of Minocycline in Treatment of Chronic Periventricular Leukomalacia in Rat Model

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ABSTRACT
Introduction: *This study investigates the therapeutic potential of minocycline in treating chronic periventricular leukomalacia (PVL) in albino Wistar rats. By assessing minocycline's neuroprotective and anti-inflammatory effects, the study aims to determine its efficacy in reducing neurological impairments associated with PVL, offering insights into potential treatments for human patients.*
Materials and methods: *Rats received intraperitoneal lipopolysaccharide (LPS) injections to induce periventricular leukomalacia (PVL). Following LPS exposure, the treatment group received minocycline, while controls received saline. Neurodevelopmental reflexes and behavioural tests assessed neurological function. Post-euthanasia, brain and organ samples were collected for immunohistochemistry, ELISA, and qPCR to evaluate molecular and cellular effects.*
Results: *Minocycline treatment significantly improved neurodevelopmental outcomes in rats with periventricular leukomalacia (PVL), reducing deficits in key metrics like forelimb and hindlimb grasping, and cliff avoidance. Histological analysis revealed that minocycline reduced ventricular dilatation, indicating its therapeutic potential in mitigating brain damage and preserving neural architecture in PVL-affected rats.*
Conclusion:*The study demonstrates that minocycline exhibits significant therapeutic potential in treating periventricular leukomalacia (PVL) in a rat model. Neurodevelopmental testing indicated that minocycline improved motor coordination, posture, and reflexes, reducing the neurological impairments typically associated with PVL*
Keywords: *Minocycline, Periventricular Leukomalacia, anti-inflammatory, antioxidant, Albino Wistar Rats, neurodevelopment, ventricular dilation*

INTRODUCTION

This study looks into the possibility of using minocycline as a medication to treat the Albino wistar rats with chronic PVL. PVL, a frequent brain injury seen in preterm children, is caused by damage to the white matter that envelops the ventricles of the brain. (1,2) Because of its anti-inflammatory and neuroprotective qualities, minocycline is thought to lessen damage caused by PVL. The purpose of this study is to determine whether Minocycline is effective in reducing neurological impairments caused by PVL using rat models. By better understanding its therapeutic impact, human patients with PVL may be able to receive treatment that lessens the condition's long-term effects.

Albino Wistar rats are pink skinned rats with white fur. (3) They are large in size which makes them suitable for anatomical studies and surgical procedures. They have a well proportioned body and their skeletal structure is similar to humans. They have a broad head and a short nose. They have large eyes which are pink or red due to albinism. They have large and prominent ears and continuously growing incisors. Albino Wistar rats have further been utilised to assess the wound healing properties of various herbal formulations like Pruthvisara taila showing significant wound contraction. (4) Albino Wistar rats are usually inbred and thus have a high degree of genetic variability. They have high reproductive rates and have a gestation period of 21 to 23 days making them ideal for both short term and long term studies. Albino Wistar rats were also used to study the cognitive effects of hypoxia therapy, showing improved mobility, reduced anxiety and enhanced memory retrieval. (4,5) Albino Wistar rats have also been used in research to develop animal models of Periventricular Leukomalacia (PVL), a condition associated with white matter injury in the ventricles of neonates. (2)

Periventricular leukomalacia is a major health concern found in premature infants. It is characterised by diffuse white matter injury caused near the ventricular region. (6) (7) PVL is linked to cerebral palsy, various neurodevelopmental issues including mental retardation and visual impairment. (8) PVL is linked to numerous factors such as hypoxia, ischemia, free radical damage, cytokine toxicity and excitotoxicity. (9) Following hypoxia/ischemia and systemic infection, periventricular white matter injury occurs in premature infants, resulting in hypomyelination and cognitive impairments in later life. When periventricular leukomalacia (PVL) occurs, the cerebral white matter exhibits inflammatory infiltrates.

(10) Periventricular leukomalacia (PVL) involves necrotic and/or gliotic lesions in the periventricular white matter. This affects premature infants, with neuropsychological and potential motor disabilities. (11) Periventricular leukomalacia (PVL) is often diagnosed using real-time ultrasound by observing the echodense areas in periventricular regions, correlating with autopsy findings showing necrosis and cystic degeneration in premature infants with abnormal neurological signs. CT scans showing ventricular asymmetry, reduced periventricular white matter, ventriculomegaly and irregular walls is also used in identification and diagnosis of PVL. (12)

Responses from microglial cells are essential to the pathophysiology of PVL. Inflammatory and hypoxic-ischaemic brain injury are caused by the activation of microglia in response to endotoxin injection and hypoxic-ischaemic cortical injury. Microglia express cytokines linked to hypoxic and inflammatory ischemic brain injury as well as release reactive oxygen and nitrogen species. (13) M1 type of microglial cells give a neuroinflammatory response whereas the M2 type of microglial cells have a neuroprotective role. The use of Minocycline in treatment increased the number of M2 microglial cells (neuroprotective cells) and reduced cerebral infarction as compared to rats without Minocycline treatment. (14) Treatment with Minocycline also suppressed P-p38 MAPK in microglial cells. (P-p38 MAPK) phosphorylated form of p38 Mitogen-Activated Protein Kinase is a protein that plays an important part in cellular responses to inflammation and stress. (14,15)

It has been demonstrated that the drug minocycline has anti-inflammatory and antioxidant qualities. (16) In a rodent model of PVL, the drug minocycline, which inhibits microglial activation, has demonstrated potential in reducing white matter injury. Treatment with minocycline after hypoxic-ischaemic injury decreases the number of microglial cells and shields against white matter damage, indicating that it may be useful as a therapeutic approach for PVL. (10,13) In different parts of the brain, minocycline reversed the rise in TBARS and protein carbonyl levels brought on by stress. (17)

Minocycline increased the activity of antioxidant enzymes and reduced the levels of pro-inflammatory cytokines TNF_α and IL-1β. (18) (19)A histological analysis revealed that minocycline reduced the sciatic nerve's surrounding perineural inflammation. Minocycline may function via blocking the blood-brain barrier's deterioration, oxidative stress pathways, and the degradation of myelin basic protein. In an immature rat model of hypoxic-ischemic brain damage (HIBD), which resembles PVL in preterm neonates, minocycline has shown protective effects. (1) The duration of minocycline treatment after the insult is essential to the medication's protective effectiveness. Minocycline treatment protected pre-oligodendrocytes and had long-term neuroprotective effects in terms of neuroethology. (20)

Because minocycline may penetrate brain tissue, it directly reduces inflammation. Studying minocycline's therapeutic effects in treating a rat model of Periventricular Leukomalacia is the aim of this project. (21)

MATERIALS AND METHODS

The approval for this project is given by Saveetha Dental College Approval no: BRULAC/SDCH/SIMATS/IAEC/04-2024/05.

Inducing pre-term injury

Normally born rats pups will receive intraperitoneal injections of lipopolysaccharide (LPS 15 mg/kg) or E. Coli culture media on days 2, 4, and 6 in order to induce preterm brain injury, also known as Periventricular Leukomalacia.

Treatment group

In the treatment group, the test drug will be administered after the final LPS injection on day 6. This aims to evaluate the drug's effectiveness in mitigating preterm brain damage, specifically targeting the prevention or reduction of Periventricular Leukomalacia (PVL).

Control group

The control pups will receive equal amounts of normal saline intraperitoneally. This ensures for allowing any effects observed in the treatment group to be attributed to the test drug rather than the injection process.

Neurodevelopmental reflex testing

Pups will be assessed using neurodevelopmental reflex testing and a series of behavioural tests, such as the Forelimb Test, Hindlimb Test, Righting Reflex Test, Cliff Avoidance Test, Gait Test to identify any abnormalities. These assessments are designed to evaluate motor coordination, spatial learning, and overall neurological function. By comparing the results between the treatment and control groups, researchers aim to determine the effectiveness of the test drug in mitigating the neurodevelopmental impacts of induced preterm brain damage, particularly Periventricular Leukomalacia (PVL).

Day(PND) of start test	Test
Day 3	Forelimb Grasping
Day 3	Hindlimb Grasping
Day 3	Righting
Day 4	Hindlimb Placing
Day 4	Cliff Avoidance
Day 6	Gait
Day 10	Auditory Startle
Day 12	Posture
Day 12	Eye opening

Figure 1 and 2: forelimb grasp **Figure 3: hindlimb grasp**
Figure 4 and 5: righting reflex **Figure 6: cliff avoidance**
Figure 7 and 8: gait **Figure 9: cliff avoidance**

Tests

At the age of 15 or 35 days, the pups will be euthanized using carbon monoxide gas inhalation in a CO chamber after sedation with a suitable anaesthetic agent. Brain, liver, kidneys, and other organs will be collected for analysis. These samples will undergo immunohistochemistry, ELISA, qPCR and other tests to evaluate the molecular and cellular impacts of the treatments on organ systems.

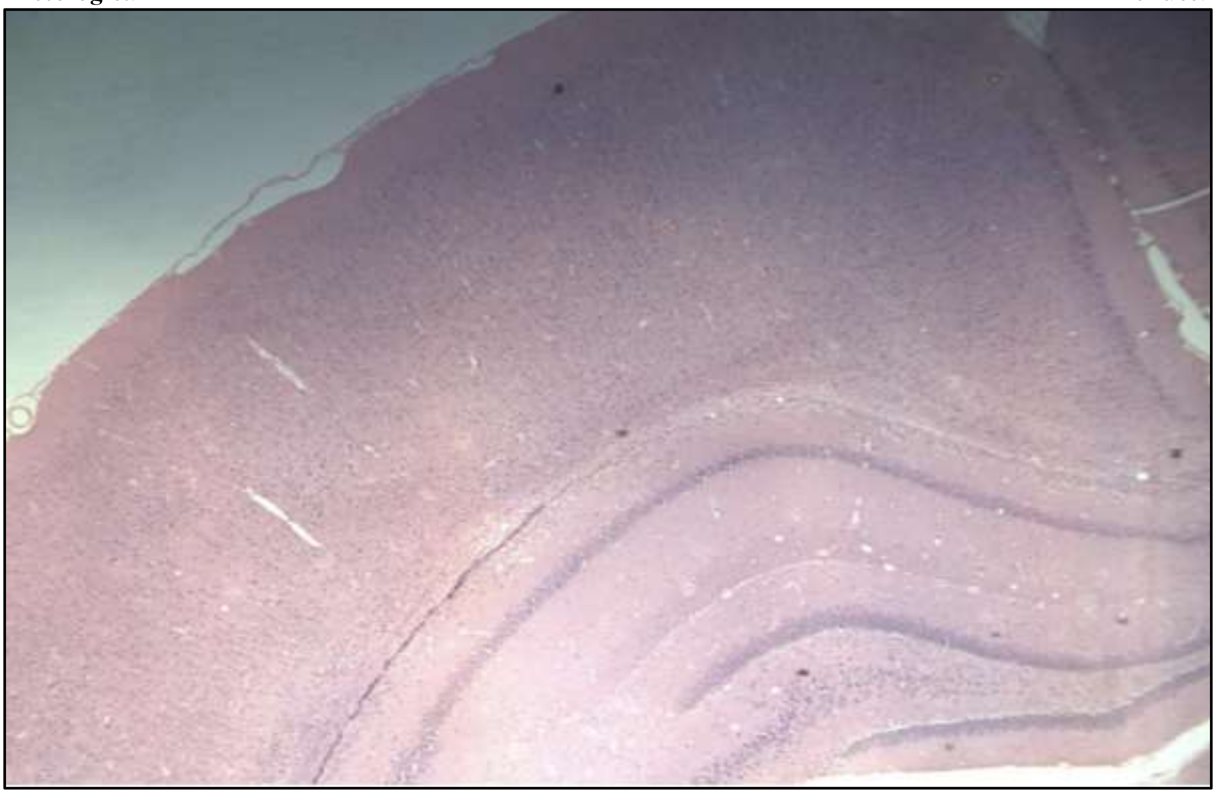
RESULTS

Tabular Data:

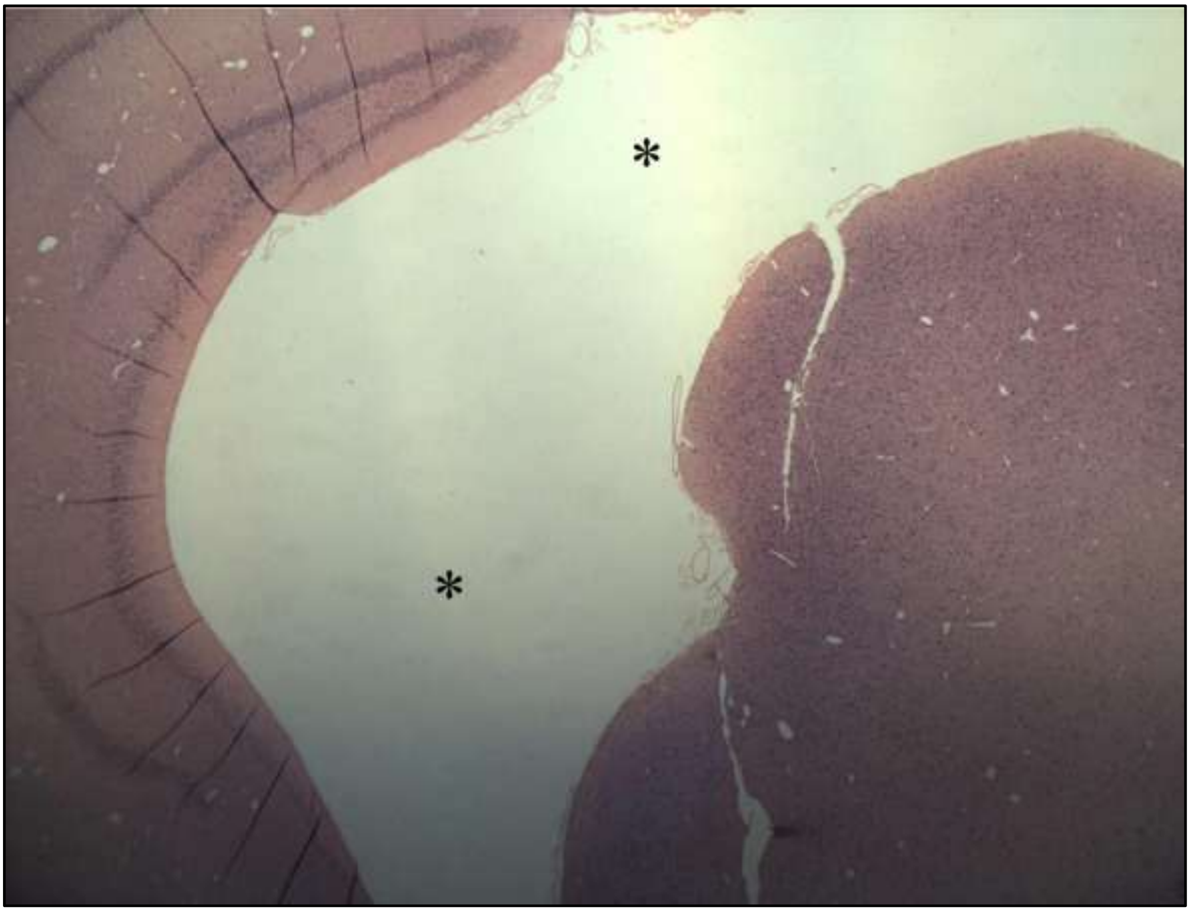
Groups	Forelimb Grasping	Hindlimb Grasping	Righting	Hand limb placing	Cliff avoidance	Gait	Auditory startle	Posture	Eye opening
Negative control (Dam 1)	5	6	6	6	6	9	10	12	15
Negative control(Dam 2)	5	6	5	6	5	9	11	13	16
Disease control	8	10	4	10	5	7	13	17	15
Treatment control	4	6	5	5	6	10	11	13	16
Test group (Dam 1)	3	5	6	9	6	10	10	14	16
Test group (Dam 2)	5	7	5	4	6	10	12	15	16

Table 1: The maximal developmental activity reached by the pups throughout their postnatal days, based on the grading of the neurodevelopmental tests for the test groups, disease control group, treatment control group, and negative control group.

Histological slides:



G1 (CONTROL)-Photomicrograph of Brain section (H&E,X100)showing normal histoarchitecture of hippocampus and cerebral cortex.



G2 (Induced) -Photomicrograph of Brain section (H&E,X100)showing marked Ventricular dilation.

G3(Treatment)-Photomicrograph of Brain section (H&E,X100)showing moderate Ventricular dilation

DISCUSSION

The study aimed to evaluate the therapeutic effects of Minocycline in treatment of Periventricular Leukomalacia in Rats. Based on neurodevelopmental tests, the table shows the maximum developmental activity of rat pups across different groups: illness control, treatment control, test group (Dam 1 and Dam

2), and negative control (Dam 1 and Dam 2). The disease control group, which represents PVL without treatment, appears to have significantly higher day count in forelimb and hindlimb grasping as well as cliff avoidance but lower day count in other metrics like righting, gait, and auditory startle when compared to the treatment and test groups in the context of researching the therapeutic effect of minocycline in treating chronic periventricular leukomalacia (PVL) in rats. Numerous changes, particularly in posture and eye opening, are seen in the treatment control and test groups receiving minocycline, indicating a possible therapeutic advantage. all-encompassing therapeutic advantages. These findings demonstrate that minocycline is effective in reducing several neurodevelopmental deficiencies linked to PVL, however the degree of improvement varies depending on the developmental task.

The provided histological images contribute to a better understanding of the therapeutic effects offered by minocycline in treating the chronic periventricular leukomalacia (PVL) in rats. The control group (G1) shows normal hippocampal and cerebral cortex architecture, serving as a baseline for healthy brain tissue. The induced group (G2) shows marked and extreme ventricular dilatation which is a characteristic of PVL, indicating severe pathological changes and potential brain tissue damage due to the condition. The treatment group (G3), which received minocycline, exhibited moderate ventricular dilatation compared to the induced group which suggested that minocycline mitigates the severity of PVL. This reduction in ventricular size implies that minocycline may help preserve brain structure and reduce the extent of damage in chronic Periventricular Leukomalacia. Hence these images suggest that minocycline has a therapeutic effect in reducing pathological changes associated with PVL in this rat model and highlights its potential as a treatment option.

The research "Animal Models of Periventricular Leukomalacia" on Periventricular Leukomalacia (PVL) in neonates identifies hypoxia-ischemia and infections as common causes and explores new animal models using hypoperfusion, bacterial products, microbes, and excitotoxins. Combined hypoxia-ischemia-lipopolysaccharide models are effective for inducing white matter injury and evaluating treatments. (2) Recent studies emphasise the role of intrauterine or perinatal infection in brain injury, with bacterial endotoxin sensitising the brain to hypoxic-ischemic injury. Minocycline, a microglial inhibitor, has shown promise in reducing white matter damage in a rat model of chronic cerebral hypoperfusion by decreasing microglial activation and MMP-2 immunoreactivity. It also demonstrated protective effects on axonal integrity, highlighting its potential in treating conditions like vascular dementia and PVL as shown. (22) Another study on minocycline's effects following hypoxic/ischemic injury in a rodent model of PVL found that timely treatment significantly protected against white matter injury and reduced microglial cell numbers. (10) These findings suggest that minocycline and similar anti-inflammatory treatments could be effective for PVL in infants. This research was supported by the NIH and the United Cerebral Palsy Foundation.

Minocycline has been found to have protective effects on pre-oligodendrocytes in a rat model of hypoxic-ischemic brain damage (HIBD), leading to milder pathological changes in periventricular white matter and reduced loss of O4 cells as shown in the research "Role of minocycline in an immature rat model of hypoxic-ischemic brain damage." (20) Another study showed Minocycline also reduced neuropathic pain in a rat model of chronic constriction injury by attenuating oxidative stress and inflammatory response (decreased pro-inflammatory cytokines). (13) Early treatment of minocycline alleviated white matter and cognitive impairments after chronic cerebral hypoperfusion, promoting oligodendrocyte progenitor cell (OPC) proliferation and preventing mature oligodendrocyte loss as shown in the study. (23) According to the research "Minocycline protects against oxidative damage and alters energy metabolism parameters in the brain of rats subjected to chronic mild stress", Minocycline also showed neuroprotective effects by reducing oxidative damage and regulating energy metabolism in the brain, suggesting it as a potential treatment for depression, targeting oxidative stress and energy metabolism pathways. (17)

CONCLUSION

The study demonstrates that minocycline exhibits significant therapeutic potential in treating periventricular leukomalacia (PVL) in a rat model. Neurodevelopmental testing indicated that minocycline improved motor coordination, posture, and reflexes, reducing the neurological impairments typically associated with PVL. Histological analysis further supported these findings, showing that minocycline treatment led to a marked reduction in ventricular dilatation, suggesting preservation of brain structure and mitigation of white matter damage. These results align with previous studies highlighting minocycline's anti-inflammatory and neuroprotective effects, particularly its ability to inhibit microglial activation and reduce oxidative stress. Overall, this research suggests that minocycline could be a promising therapeutic option for managing PVL, potentially translating to better clinical outcomes in human patients with this condition.

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