Effects of Pregabalin on Postnatal Development of The Kidney in Albino Rat’s Offsprings

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Effects of Pregabalin on Postnatal Development of Kidney in Albino Rat’s Offsprings

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ABSTRACT

Background: Pregabalin is used for treatment of neuropathic pain, fibromyalgia, and migraine.

Objectives: This study was designed to assess the effect of pregabalin on the developing kidney of albino rat’s offspring.

Material and methods: This study was conducted between September 2019 and January 2020, at Faculty of Medicine for Girls, Al-Azhar University. Twenty pregnant rats were divided into two equal groups: group I (control) group and group II (treated) group which received pregabalin (150 mg/kg/day) from the first day of gestation up to 21 days after delivery. Offsprings were selected to be sacrificed at the 2nd postnatal day and at the 21st postnatal day, kidney specimens were prepared for light and electron microscopic examination.

Results: Maternal pregabalin administration disrupted the development of the kidney in offsprings with a significant increase in urea and creatinine level. Histological examination of the kidney offsprings at the 2nd postnatal day revealed irregular thickening of the glomerular basement membrane, fusion, and effacement of podocyte’s foot processes and tubulointerstitial injury. Examination of the kidney offsprings at the 21st postnatal day revealed thickened distorted glomerular basement membrane, broadening, and fusion of the foot processes and vacuolar degeneration of tubules.

Conclusion: Maternal pregabalin might disturbed the renal development in the offspring. So, it should be used with caution during pregnancy and lactation.

Keywords: Kidney development; Pregabalin; Rat.

INTRODUCTION

Anti-epileptic drugs (AEDs) have the possibility of affecting the fetal development throughout pregnancy1. Many AEDs have been shown to readily cross the placental barrier reaching the fetal circulation causing major and minor teratogenic effects 2. The new AEDs as pregabalin, felbamate, gabapentin, lamotrigine, levetiracetam, topiramate, etc., have been launched for clinical usage whereas, clinical data on their teratogenic adverse effects are still limited 3. Pregabalin is the latest compound that joins the list of approved new AEDs that shows efficacy in the treatment of uncontrolled partial seizures, neuropathic pain, generalized anxiety disorder, fibromyalgia, sleep disorders, and migraine 4. Pregabalin is a gamma-aminobutyric acid (GABA) analogue that acts by increasing neuronal GABA level 5. It is structurally related to gabapentin, but it has the benefit of more efficacy and absorption 6.

MATERIAL AND METHODS

Animals: The animals were housed in the animal facility at the Faculty of Medicine for Girls, Al-Azhar University. This study was conducted between September 2019 and January 2020, on twenty pregnant rats in accordance with ethical procedures and policies approved by Animal Care and Use Committee of Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

The pregnant rats were divided into two equal groups: Group I (Control group): composed of 10 pregnant female rats that received distilled water. Group II (treated group): composed of 10 pregnant female rats that were given pregabalin drug (150 mg/kg / day) from the first day of gestation and sustained up to 21 days after delivery. Offsprings included in the present study were sacrificed at the 2nd postnatal day and 21st postnatal day. The
offspring were divided into four groups (10 animals each):

- **Group (1):** composed of control male offspring at the 2nd postnatal day.
- **Group (2):** composed of treated male offspring at the 2nd postnatal day.
- **Group (3):** composed of control male offspring at the 21st postnatal day.
- **Group (4):** composed of treated male offspring at the 21st postnatal day.

**Chemicals:** Pregabalin capsules (Lyrica® 300 mg Pfizer-Egypt Company was purchased. The dose used was the minimal human therapeutic dose (150 mg/kg/day)\(^7\). The equivalent rat dose was calculated by using the formula of Paget and Baren's\(^8\).

**Methods:** Light microscopic examination, the right kidney was processed then stained with haematoxylin and eosin (H&E)\(^9\). Transmission electron microscope (TEM) examination, the left kidney was processed\(^10\) and examined by transmission electron microscope (JEOL1010, Japan) at the Regional Centre for Mycology and Biotechnology (RCMB)-Al-Azhar University, Cairo.

Blood samples were collected from the retro-orbital sinus of each offspring by a fine capillary glass tube then centrifuged. Sera were collected for assessment of serum urea and creatinine then were carried out\(^11\) and\(^13\) at the Biochemistry Department, Faculty of Medicine, Cairo University. Statistical analysis of urea and creatinine in all studied groups was carried out using the SPSS statistical package. The collected data were analysed (Expressed as means ± SD) and statistical significance was performed using one-way ANOVA followed by a Tuckey post-Hoc test for multiple comparisons. So, Values of \(P \leq 0.05\) were statistically significant\(^13\).

**RESULTS**

**Light microscopic examination:**

- **Group (1):** revealed that the kidney was formed of an outer cortex and inner medulla with medullary rays passing through the cortex and medulla dividing it into renal lobules. The renal cortex showed two cortical zones, the subcapsular nephrogenic zone and the juxtamedullary zone. In the subcapsular nephrogenic zone, immature forms of renal glomeruli, which include cell clusters, ureteric buds, hemispherical glomeruli, comma-shaped and S-shaped glomerular structures close to the capsule surrounded by primitive convoluted tubules. The juxtamedullary zone contained more mature renal corpuscles, convoluted tubules, and interstitial tissues (Fig.1a). In the juxtamedullary zone, the renal corpuscle was formed of a glomerulus (glomerular capillary tuft) surrounded by Bowman’s capsule. The Bowman’s capsule was formed of parietal layer lined by flat squamous epithelium and visceral layer closely applied to the glomerular capillaries with Bowman’s space in between. Proximal convoluted tubules (PCTs) were lined by cubical cells with oval basal nuclei and acidophilic cytoplasm with a narrow lumen. Distal convoluted tubules (DCTs) were lined by simple cuboidal epithelial cells with central rounded nuclei with wide lumina (Fig. 1b).

- **Group (2):** the kidney was divided into the outer cortex and inner medulla with faint medullary rays. The renal cortex showed crowded immature renal glomeruli with primitive tubules (Fig.1c), while the deeper cortex showed glomerular and tubulointerstitial injury. The glomeruli appeared distorted, congested with a widening of Bowman’s space. In addition, there was dilatation in the PCTs and DCTs lumina as well as degeneration and vacuolation associated with exfoliation of their epithelial lining. The interstitial tissues revealed haemorrhage with congested peritubular capillaries in between the renal tubules (Fig.1d).

- **Group (3):** the kidney acquired full maturation and differentiated into an outer cortex and the inner medulla. The renal cortex showed numerous uniform renal corpuscles, PCTs, DCTs surrounded by interstitial tissues with disappearance of most of the immature forms (Fig.2a). The renal corpuscles consisted of renal glomeruli surrounded by Bowman’s capsules which consisted of parietal and visceral layers. The parietal layer was formed of flat cells which were separated by the Bowman’s space from the visceral layer which was closely applied to the renal glomeruli. PCTs were lined by cuboidal cells with oval basal nuclei and acidophilic cytoplasm with a narrow lumen. DCTs were lined by cuboidal cells with large apical vesicular nuclei and acidophilic cytoplasm with wide lumina (Fig.2b).

- **Group (4):** the kidney was differentiated well into outer cortex and inner medulla with the disappearance of most of the immature forms. The renal cortex exhibited disturbed architecture with congested shrunken glomeruli, wide Bowman’s space with interstitial congestion and extravasation of blood (Fig.2c). Dilatation in the PCTs and DCTs lumen with degeneration and vacuolation in their epithelial lining with congested peritubular capillaries. (Fig.2d).

**Transmission electron microscopic examination:**

- **Group (1):** the renal corpuscles formed of glomerular blood capillaries which may contain red blood cells (RBCs). The glomerular blood capillary was formed by a thin glomerular basement membrane lined by endothelial cells and surrounded by podocytes and mesangial cells. Endothelial cells had euchromatic nuclei with cytoplasmic fenestrations. The podocytes had large euchromatic nucleus, abundant cytoplasm, with primary foot processes that split up into numerous secondary foot processes with filtration slits in-between (Fig. 3a & 4a).

- **Group (2):** the PCT cells had large euchromatic nucleus, numerous mitochondria, and few lysosomes (Fig.5a).

- **DCT cuboidal cells** had large oval euchromatic nuclei, numerous mitochondria and short apical microvilli and wide lumina (Fig.6a).

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**Group (2):** the renal corpuscle had thickened irregular glomerular basement membrane. The endothelial cells had a large, intended nucleus with peripheral chromatin condensation. The podocyte had distorted primary foot processes with fusion and effacement of its secondary foot processes with narrow obliterated filtration slits (Fig. 3b & 4b). The PCT cells had irregular nuclei, cytoplasmic vacuolations, and few mitochondria with many lysosomes (Fig. 5b). The DCT cells had shrunken nuclei, cytoplasmic vacuolations and few mitochondria (Fig. 6b).

**Group (3):** the glomerular capillary formed by thin glomerular basement membrane which was lined with fenestrated cytoplasm of endothelial cells. The podocyte had a large nucleus with primary and secondary foot processes (Fig. 3c) and (Fig. 4c). The PCT columnar cells resting on a basement membrane with oval euchromatic nuclei with dense apical microvilli that filled most of the lumen. The cytoplasm contained many pinocytic vesicles, numerous mitochondria, and few lysosomes (Fig.5c). The DCT cuboidal cells had rounded nuclei, cytoplasmic vacuoles, few mitochondria, and many lysosomes (Fig.5d). The DCTs cells had irregular nuclei, cytoplasmic vacuolations and few mitochondria (Fig.6d).

**Biochemical study:**
The data illustrated in table (1), (2) and figures (7), (8) revealed a significant increase in the levels of serum urea and creatinine in group (2) and (4) if compared with group (1) and group (3) respectively.

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Fig. 1: Light photomicrographs of:

a: Group (1) showing subcapsular nephrogenic zone (SC), juxtamedullary zone (J), medulla (M) medullary rays (MR), hemispherical glomeruli(G), comma-shaped bodies (C), S-shaped bodies (S), primitive tubules (arrow) (H&E×200).

b: Group (1) showing glomeruli (G), proximal (P), distal (D) convoluted tubules (H&E×400).

c: Group (2) showing subcapsular nephrogenic zone (SC), juxtamedullary zone (J), medulla (M), faint medullary rays (MR), distorted glomeruli (G), comma-shaped (C), S-shaped bodies (S), primitive convoluted tubules (arrow) (H&E×200).

d: Group (2) showing distorted glomeruli (G), haemorrhage (H), exfoliated proximal (P) and distal (D) tubules (H&E×400).
Fig. 2: Light photomicrographs of:

a: Group (3) showing outer cortex (C), inner medulla (M) (H&E ×200).

b: Group (3) showing glomeruli (G) Bowman’s space (S), proximal tubules (P), distal tubules (D) (H&E ×400).

c: Group (4) showing cortex (C), medulla (M), interstitial extravasation of blood (arrow) (H&E ×200).

d: Group (4) showing congested glomeruli (G), dilated Bowman’s space (S), hemorrhage (H), dilated proximal (P), distal (D) tubules (H&E×400).

Fig. 3: Electron photomicrographs of:
a: Group (1) showing glomerular blood capillary (CL), fenestrated cytoplasm (E), podocyte (Po) primary foot processes (1F).
b: Group (2) showing glomerular blood capillary (CL) red blood cell (RBC), intended nucleus of endothelial cell (E), distorted primary foot processes (1F), podocyte (Po).
c: Group (3) showing glomerular blood capillary (CL), fenestrated cytoplasm (E), podocyte (Po), primary foot processes (1F).
d: Group (4) showing glomerular blood capillary (CL), red blood cell (RBC), intended nucleus of endothelial cell (E), distorted primary foot processes (1F) podocyte (Po) (TEM x12000)

Fig. 4: Electron photomicrographs of:
a: Group (1) showing glomerular blood capillary (CL), fenestrated endothelial cytoplasm (E), glomerular basement membrane (star), filtration slits (arrows), podocyte (Po).
b: Group (2) showing glomerular blood capillary (CL), red blood cell (RBC), thickened irregular basement membrane (star), intended nucleus of endothelial cell (E), obliterated slits (arrows), podocyte (Po).
c: Group (3) showing glomerular blood capillary (CL), glomerular basement membrane (star), filtration slits (arrows) (Po).
d: Group (4) showing glomerular blood capillary (CL) thickened irregular glomerular basement membrane (star), obliterated filtration slits (arrows), podocyte (Po) (TEM x20000)
Fig. 5: Electron photomicrographs of:

a: Group (1) showing PCT cells nuclei (N), microvilli (Mv), mitochondria (stars).
b: Group (2) showing PCT, the lining cells had irregular nuclei (N), cytoplasmic vacuolations (v), microvilli (Mv), mitochondria (stars), lysosomes (L).
c: Group (3) showing PCT cells had oval nuclei (N), microvilli (Mv), mitochondria (stars), few lysosomes (L).
d: Group (4) showing PCT cells had irregular nuclei (N), vacuolations (v), microvilli (Mv), mitochondria (stars), lysosomes (L). (TEM x8000)

Fig. 6: Electron photomicrographs of:
a: Group (1) showing DCT cells nuclei (N), mitochondria, (stars).
b: Group (2) showing DCT cells nuclei (N), vacuolations (v), mitochondria (star).
c: Group (3) showing DCT cells nuclei (N), mitochondria (star).
d: Group (4) showing DCT cells nuclei (N), vacuolations (v), mitochondria (star). (TEM x8000)

Fig. (7): Level of urea (mg/dl) in all experimental groups

Fig. (8): Level of creatinine (mg/dl) in all experimental groups
Hala Mossalam – Maternal pregabalin renal effect.

**DISCUSSION**

Nephron development (nephrogenesis) continues postnatally in the rat until postnatal day 15 while the renal cortex is considered histologically mature by postnatal day 21.1

In this work, two postnatal ages were carefully chosen to detect the effect of maternal pregabalin drug during gestation (the 2nd postnatal day) and lactation (the 21st postnatal day) on the developing renal cortex.

In the present study, light microscopic examination of the renal cortex of group (1) demonstrated that the renal cortex was formed of two zones: the subcapsular nephrogenic zone and the juxtamedullary zone. The subcapsular nephrogenic zone contained immature forms of renal glomeruli while the juxtamedullary zone contained more mature renal...
compared with the control groups, this might be
creatinine were observed in groups (2) and (4) if
In this study, significant elevation in serum urea and
and DCTs cells revealed nuclear degeneration with
Furthermore, the renal cortex of group (3) revealed
renal cortex revealed glomerular and tubulointerstitial
In the current work, the effect of pregabalin
In the current work, the renal histopathological
CONCLUSION
Pregabalin should be used with caution during
Pregabalin use during pregnancy.

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15. 16. The electron microscopic examination of group (1)
17. However, the renal cortex of group (3) revealed
18. These zones were present during the early neonatal period until
19. In the current work, the effect of pregabalin
20. who found in their study that gabapentin, an ADE similar
21. The PCT cells showed oval basal nuclei, dense
22. suggested that foot processes effacement leads to
23. mentioned that podocytes injury could affect
24. In this study, significant elevation in serum urea and
25. On the contrary, the renal cortex of group (3) revealed
26. explained as the elevation of serum urea and
27. Zaken et al.25 stated that in the early stages of
28. Susceptible to high levels of oxidative stress because
29. Although placental enzymes could play a part in fetal protection against oxidative
30. In the current work, the renal histopathological changes with pregabalin use during gestation (at the
31. The author has a substantial
32. No conflicts of interest.
33. The author has a substantial contribution to the article.
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