Pre and Postnatal study of the developing kidney and Testes of the albino Rat under the effect of antibiotic Maxipime (Cefepime hydrochlorid)

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Summary

Subject of the thesis:
Pre and postnatal study of the developing kidney and testes of the albino rat under the effect of antibiotic "Maxipime "(Cefepime hydrochlorid).

Introduction and review of Literatures:
All available literature that specific for causing most types of infection and specific antibiotics especially Cephalosporin group in regard to its action and resistance of bacteria to it, has been mentioned. The classification of cephalosporin into several generations and the effect of these antibiotics on embryos, fetouses at different time of pre and postnatal period, also its side effects on the growth and developed embryos and infants . Special references which is related to Cefepime drug as, its synthetic character, mechanism of action, absorption, distribution , extraction, and its side effects, has been mentioned .Also, the relation between Cephalosporin group and penicillin was mentioned . Some studies explained its effects on experimental animals such as mice, rats, cats, rabbits and dogs, also its effect on human selected organs for study in specific form. The literature of the normal development of kidney and testes of the embryos and fetuses of mammals and other animals had been reported .
Materials and Methods:

First: Materials used in this investigation.

In this study (83) Albino rats were used in which (55) of this number were mature females and (28) were mature male, their weighs were about (200-250) gm. The animals were given balanced foods; water and put under adjust proper temperature. After mating, the pregnant females were divided into three groups as follow:

First: control group.

This group was used for studying the normal development and structure of kidney and testes. It consisted of (20) pregnant females which were injected by (normal saline) for 7 repeated doses through the 2nd week of gestation (from 8-14 days) each dose was (0.7 mg/rat). Then it was divided into 2 subgroups:

A– The First sub group (20) gestational prenatal old days (pre20) This group consisted of (5) pregnant females rats which were examined in the day (20) the day of gestation. The mothers were anaesthetized and the embryos were extracted through an opening in the abdominal wall to examine the 2 uterine horns. The pregnant females gives (43) embryos, (22) of them were male, which were examined for any morphological deformities, the weights and the heights were measured. Then the kidney and testes were extracted for morphological and histological examination.

B– The Second sub group: postnatal group (post C). It was consisted of (15) pregnant females which divided into (5) category, each one contains (3) females. This group deliver (129) fetouses in which (56) were male fetouses and were examined at (7, 14, 21, 30, and 45) days of their ages. Each fetus was examined separately for any morphological deformities. The heights and weighs for each fetus was measured and essential statistics were done. The kidney and testes were extracted for histological examination. The number of fetuses at (7 – 14- 21- 30-45) postnatal days were (10-12-13-11-and 10) respectively.

Second: Treated group during gestation.

This group formed of (20) pregnant female which were injected through intraperitoneal membrane with repeated (7) doses of Maxipime,
each dose was equal to the human therapeutic dose (0.7mg/rat) and was modified to rat species according to (Paget and Parnnes, 1964) in the same injected days as the control group. This period was specific for organogenesis to know the effect of the drug through penetration of the placental barriers. This group was subdivided into (2) subgroups.

**C- The first group prenatal at 20 – gestational days old (preT20).** It was consisting of (5) pregnant females which produced (35) fetouses from them (19) male fetouses were examined at the same age as the control group.

**D- The second postnatal group (post T).** It was consisting of (15) pregnant female rats which were subdivided into (5) sub groups, each one contained (3) female rats. This sub group delever (112) fetouses, (55) fetouses males, their examined at the postnatal ages (7, 14, 21, 30, and 45) days. These( sub groups) were treated as the control group. The number of fetouses at (7 –14 – 21- 30 and 45) post natal days were (9-10-12-13- and 11) respectively.

**Third: The treated group during breast feeding (post F).** In this group (15) pregnant females were injected through intraperitoneal membrane with the same dose as the treated group but in the first postnatal week (starting from the first day to the 7 days). The dose was (0.07mg/rat) for 7- days, to study the effect of 7 - drug passing through the milk barrier. The pregnant mothers were subdivided into (5) subgroups, each one consisted of (3) female rats. The female rats produced (126) fetouses. They collected all ages of (7, 14, 21, 30, and 45) days the number of fetouses were (11-12-12-10-and finally 11) one respectively.

**Second: Methods used in this investigation:**

In this study (87) fetuses were used in prenatal group. In which (43) embryos were used in the control group and (35) embryos were used is a treated group. In the postnatal group (167) fetouses were used in which (56) of the mentioned number were used as a control group, while (55) fetouses. The treated gestational group, and (56) fetouses were used as a breast feeding groups in the postnatal fetouses were examined at (7,14,21,30 and 45) days to study the morphogenesis which included any
malformation and changes of the weights and leight measurements in control and treated groups to make the comparison between them and performing essential statistics.

The histological structures of kidney and testis were performed in all groups. The organs were fixed in Bouin's or 10% Formalin solution for all embryos. The fetuses The following stains (Haematoxylin and Eosin stain to study the general structure of the tissue in which the nuclei appeared with blue colour and the cytoplasm appeared with red colour. The ferrous Hematoxylin stain was used to study the division of the nuclei as it appeared with bluish black, and also Masson’s trichromie stain to study the collagen fibers which appeared with green colour and blood cells appeared with red colour. The measurement of the glomeruli and tubules were performed by using Haematoxylin and Eosin slides stain to study the structure of kidney at age of (20- gestation days old) at the prenatal period and at (30-days) old of the postnatal groups to make the comparison between the control group and the 2 treated groups during gestational and breast feeding periods for performing the essential statistics.

**Results:**

In this investigation the normal development of kidney and testes were examined and the effects of Maxipime on morphogenesis and histogenesis for the selected organs were studied and also some staticall analysis were done.

**The morphological effects .**

**First Maleformation :**

The results of this study showing some malformations in some embryos at the prenatal group (pre T20) such as slight congestion and hemorahage under skin and increase of size of embryos due to swelling especially at the abdominal part. As age advanced in the postnatal groups, the sign of hemorahage and congestion at 7- postnatal days were present in both treated groups (post T7) and (post F7). The congestion was advanced at 14-days; the presence of swelling was noticed in the fetouses of both treated postnatal groups (post T14) and (post F14), but the swelling was more obvious in the postnatal gestational treated groups (post T14) old. At 21- postnatal days old it was noticed that no hair
developed in some head areas. There was swelling in the post treated and breast feeding (post F21) which was more than that seen in postnatal treated group (post T21). Other deformities were seen at 30–day’s postnatal group, as the presence of clear shortness of the height of the front limbs with abnormal development of them. It was noticed that the presence of abnormal hair in the treated breast feeding group (post F30). Also, there were obvious swelling of the abdominal part in the treated group of 30–days old (post T30). The effect of the drug was continued till the age of 45-days (post F45) in the breast feeding groups as the head appeared small in size and obvious swelling in both treated postnatal groups (post T45) and (post F45), but the swelling was more obvious in the treated group (post T45).

**Second: Effect on weight:**

In the present study there were changes in the pre and postnatal period groups: there were significant increase in the weight of the treated prenatal gestational group at (pre T20) but in postnatal groups, in the groups of age( 7, 21, 30 and 45 there were significant decrease in weight and the (post T30) was the most effected one. But in the (post T14) subgroup there were significant increase of weight as compared with the control group. In the post treated breast feeding groups, there were significant decrease of the groups (post F7,30,45) and the most affected groups were (post F 7 and 45), while in the (post F14 and 21) there were significant increase of weight.

**Third: Effect on height.**

In this study the high of both control and treated prenatal group at (20 gestional days old) was nearly equal. In the postnatal groups there were obvious decrease in the height in all treated postnatal groups (post T7), (post T14), (post T21), (post T30) and (post T45). In the treated breast feeding groups there were more decrease in the height in the groups (post F21), (post F30) and (post F45) but the height was normal at the groups (Post F7) and (post F14) as they nearly equal to the control one.
Second: Histological Studies:

Normal development of rat embryos and fetuses:

1- Normal development of testis:

The results of this study showed the presence of testis of the prenatal (20- gestational days) old was lateral to the urinary bladder, posterior to muscles of anterior abdominal wall. It was oval in shape and contained many semineferous follicles which more dense at the preferal part than the central part. The testis was surrounded by a normal connective tissues capsule which sent fine septa to the testis to divide it into many lobules. The testicular capsule was formed of two layers, an external one the tunica albuginea and inner one the vascular layer. The inner layer consisted of loose connective tissues enriched with blood vessels. There was direct proportion in the increase of thickness of the 2 layers till the forth (4th) week of postnatal period. The testis was consisting of many semineferous follicles which differ in shape and size, each one follicle was surrounded by a basement membrane. It consisted fine spindle shape cells which have flat dark nuclei. The seminiferous follicles of 20- gestational days old have 2 types of cells, the supporting cells and spermatogonial cells. The supporting cells were the commenst cells one and ther were cubical in shape and had small dark rounded nuclei and pale cytoplasm. They were small in size, and lie vertically on the basement membrane. The spermatogonial cells were large in size and less in number and were found in the central part of the follicle, most of them had pale big round nuclei and clear cytoplasm.

At postnatal periode age ,the 7- days old the spermatogonial cells were arranged in more than one raw. They were about 2-3 rows inside the semineferous follicle. In this age the semineferous follicle had four different types of cells which were , the supporting cells, spermatogonia cells were very few and were divided into 2 types of spermatogonia (A and B). The spermatogonia (A) were round in shape and had large round or oval nucleus containing one or two nucleolus and were found on the basement membrane and inbetween the supporting cells and they were few in number and found in most seminiferous follicles at this age. The spermatogonia (B) were rounded in shape and small in size and lie on the supporting cells or
near to it and were few in number at this age. It was characterized by round nucloeus and contained small masses of chromatin on the nuclear envelop.

As the age advance at 14- postnatal days old the spermatogonia cells were arranged into 3-4 rows of cells which are the supporting cells, spermatogonia (A) and (B) and the initially appeared the primary spermatogonial cells which appeared for the first time and were characterized by their presence around the center of seminiferous follicles and they were scattered in few number. They were small in size and containing dark nucloeus with chromatin inside it which was distributed in disordered manner.

At the postnatal age 21- days old, there was increase in cellular density and rows of cells inside the seminiferous tubules. The spermatogonial cells were present in 4-5 rows inside the seminiferous tubules and they were like the cells which were present at age 14-days, but in this age the a cavity started to be appear in the middle of seminiferous tubules, for that it was termed semineferous tubules. The Sertoli cells which were pyramid in shape and lying vertically on the basement membrane. had been firstly appeared at this age.

As age advanced 30- days postnatal old, the testis would be found descent inside the scrotum which was consisting of connective tissues sac outside the abdominal cavity at the abdominal side of the anal opening. The cellular density lining the seminiferous tubules were increased and arranged in 5-6 raws but the number of supporting cells were decreased and there were presence of spermatogonia (A), and (B) and the primary spermatocytes which were found around the cavity it was formed of in 2-3 rows, this type of cells characterized by its large size with large nucloeus which containing dark masses of chromatin.

At postnatal 45-days old fetous, the testis was large in size and the spermatogonia were arranged into (6-7) rows and containing the supporting cells which were very few in number. The Sertoli cells, which were pyramid in shape and were lying vertically on the basement membrane. Spermatogonia (A) and (B) were few in number and the primary spermatocytes were large in size. The primary spermatocytes were arranged in one or two rows near the germinal
cells and secondary spermatocytes was a small in size and had a pale small nuclei and appeared around the lumen.

In between the semineferous tubules in all ages from 20- gestational days old till 45-days old there were interstitial tissue which appeared between seminiferous tubules, and containing loose connective tissue, small blood vessels and contained spindle shape cells which were present in single or in small groups in between the seminiferous tubules which increased in number as the age advanced, in all ages. They were arranged in groups or as single cells or in chain shape of cells. They were the Leydig cells which are oval cells with rounded nucleous and characterized by their acidophilic cytoplasm.

2- Normal development of kidney:

The results of this study showed that the kidney was similar in shape to the bean and had an outer convex border and inner concave border, it was starting to develop in the pelvis then it ascend to the abdomen, it was surrounded by a fibrous capsule. The kidney was divided into 2 parts, the outer part (the cortex) and inner part (the medulla). At prenatal 20- gestational days old, the kidney contained 2 types of renal corpuscles. The first type was the immature renal corpuscles which were large in number, small in size and were arranged under the renal capsule. The second type was mature as it had a large size and less in number, and were scattered in the medullary part. The renal corpuscles were elliptical in shape. As the age advanced at age 7-21 postnatal days old, the renal corpuscles would be found in about 2-3 rows under the renal capsule. They differed in size and shape, the most mature and large ones found in the medullary part.

As age advanced at 30-45 postnatal days, the cortex contained many mature renal corpuscles which were the largest in size and less in density. The renal corpuscles appeared in all age consisted of small blood capillary tuft which formed the glomerulus and was surrounded by Bowman’s capsule. The Bowman's capsule formed of two layers, the outer parietal layer and the inner vascular layer in between the Bowmen’s space. There were many types of renal tubules which were differentiated into proximal tubule which had small brush lumen and formed of cubical cells. The distal tubule had wide lumen and less brush border and the two
lobe of Henle. Which were small in size and near to each other and lined by small cubical cells.

**Effects of Maxipime on histological structure of (kidney and testis) of rats embryos and fetuses.**

The aim of this work was to study the effect of Maxipime drug on renal and testicular development. The pregnant rats were injected intraperitoneal by 7- repeated doses of the drug. Each dose was (.07 mg/rat) which was equal to human therapeutic doses in the second week of gestation (from day 8th to day 14th) because this period was the time of (organogenesis) and to know the effect of drug on the penetration through the placental barriers. In the breast feeding treated groups the same dose of drug was given in the first week after delivery to know the effect of the drug through the milk barrier. The drug caused many maleformations, delay in the development, morphological and histological changes in the tissues of both selected organs (testis and kidney).

**First: Retardation of growth:**

The retardation of the growth had been appeared in both testis and kidney in this work as in testis of all ages. In the prenatal group at 20-gestation days old, there were small size of the testis and the seminiferous follicles had a less cell density inside it and lost of its normal shape especially the supporting cells, also there was deficiency in the leydig cells. As the age advanced from 7-21-days, this delay was still and there was a few spermatogonial cells has been appeared, the spermatogonial cells were few in number. Also, the presence of interstitial edema between the follicles. As the age advance from 30-45 days, the effect of the drug was more obvious as the number of primary spermatocytes was more decreased and secondary spermatocytes had not be appeared. There were appearance of new giant cells at the middle of the semineferous tubules and most of semineferous follicle not differentiated into semineferous tubule. In comparison of the two treated groups the treated breast feeding groups were more affected than the gestional treated antenatal group in all examined ages (7, 14, 21, 30, and 45 days).
In the kidney: There were delayed of growth had been appeared in all ages examined at through this study. At 20- gestational days old the the thickness of the renal capsule was decreased. There were deficiency in the number and density of renal corpuscles with appearance of shrunken or atrophied ones. Also some Bowman's capsule has a thin parietal layer or detected one. As the age advanced from (7-21) postnatal days, the normal shape of the outer layer of Bowman's capsule was changed. Some renal tubules appeared collapsed and had degenerated cells with pyknotic nuclei. From (30-45) postnatal old days, the effect of the drug was more obvious in both the gestional treated group and the treated breast feeding group where there was increase in the interstitial spaces with decrease in both the glomerulus and the renal tubules. The treated breast feeding groups were more affected than the treated gestional groups, as mentioned before in the testes.

Second: The degenerated and histological changes.

In all the treated groups in the prenatal and the postnatal there were degenerative changes like necrotic cells, nucleic changes as pyknosis, karyohexses and karyolyses has been appeared in the cells of both testis and kidney. Also, most of the cells had many vacuolated cytoplasm. The cell division appeared less in most of the cells of the two organs. May interstitial edema had been appeared in between the tissue. The Leydig cells and the Sertoli cells also changed their shape. The renal corpuscle lost its normal shape in most of the treated groups.

Third: morphometric changes.

By examination of slides kidney which stained by H&E of the age 20-gestional days prenatal and 30-days old postnatal to perform the morphometric measurements through application of resent and advanced computer program (Image analyzer computer image - proplus (v5.1) system – England), it was detected that the Maxipime drug induce significant decrease in all treated groups at 20- gestional days prenatal (preT20) and this decrease was significant in compare with control group of the some age in the cortical and medullary regions of the kidney. Also, significant decreases in the proximal and distal tubules were recorded in this group. There was significant decrease in postnatal group at 30- days old in the cortical and medullary glomerulus in both treated ante natal group (post T30) and the treated group during breast feeding period.
The decrease was more significant in the treated group during suckling period. Also, there was significant decrease in both treated groups.

**Discussion:**

This study included the discussion of subject results in comparison with previous subjects which were performed in relation to the development and normal growth of the kidney and testis of the rat embryos and fetuses. The effects of Maxipime and others similar drugs on tissues and different organs of different animals with explanation and illustration of the results and confirmation of that by the scientists researches in the same field. All points of the study were discussed starting from morphological studies as maleformations (weight and height), the histological studies as (delay of the growth, degenerative and necrotic changes and changes in morphometric measurements) for the selected organs (kidney and testis) as, they were very important organs.

**Summarization.**

It was found from the previous studies and different researches which dealt with the effect of Cefepime on the embryo and fetuses during the antenatal and postnatal period that it led to:

- An increase in the congenital abnormalities in the treated groups and the most affected one was (post F30) group and this depended on the time of dose given.
- There was hair falling from some parts of the head in (post F21) group and appearance of abnormal, falling ill hair with shortness of height in the anterior for-limb in (post F30) group.
- Decrease of the weight in the treated group especially (postF7), (post T30), (post F45) where the (post F45) group was more affected with decreased in weight.
- Changes of cellular structure (degeneration), nuclear changes, atrophy, apoptosis, edema of the interstitial tissue in all treated groups.
- Decrease in morphometric measurements in the cortical and medullary glomerulei and in the renal proximal and distal tubules at
20- gestational days old prenatal and 30-days old postnatal in all groups.

This variation and changes could be explained by scientific explanation to detect how it occurred:

1) The effect might be resulted by the drug interference on the process of ribosome's association with (ribo-nucleic) acid (RNA) as regarding to its main control effect on the cell activity and then, deficiency of protein synthesis process which is essential for formation of protein receptors which are specific for fats, and also in the average of cell divisions and its regeneration.

2) It might be caused by the presence of methyl tetrazole (MTT) group which led to undesirable effects.

3) The toxic effect of the drug might lead to increase preens of apoptosis cells.

4) Or might be more than one rezones from the previous ones or all of them together.

5) The placental barrier may could not prevent the passage of drug or its toxic effect on the fetuses.

6) The treated group during the breast feeding period was the most affected group as, the milk barriers could not prevent the passage of the drug or its toxins.

**Conclusions and recommendations.**

This study showed that, the Cefepime had toxic effect on the investigated organs and tissues and this toxic effects depended upon the time of dose given and the dose quantity as, the drug effected the tissue organs as a result of (toxic effect on nucleic acid which affect cellular division or by presence of methyl tetrazole group (MTT) which led to undesirable effects, the toxic effect of the drug might lead to increase the apoptosis cells).

**Recommendations:**

1) The pregnant female should avoid the intake of drugs during gestation period to prevent its harmful effect on the embryos and fetouses development.
2) Avoidance of drugs intake especially the antibiotic during the first 3 months, second 3 month of pregnancy (organogenesis) to avoid growth retardation and maleformation.

3) In case of drug intake, the pregnant female should be under medical supervision and follow up to protect her secure and the fetus secure.

4) Avoid antibiotic intake by big amount and lessen the intake doses.

5) The pregnant mother should be control and the drug should be given with serious caution during lactating period where it was proved that the drug passed through the milk barrier and affected both kidney and testis formation and its effects had a wide rang extended from the 2nd week of gestation to 45-days after delivery and from 1st week postnatal till 45-days postnatal.

**English summary:**

Contain the summary for this investigation by English language.

**Scientific references:**

Contain all references used in subject.