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Original Research Article

Nephrotoxic Effects of Carboplatin, A New Potential Anticancer Agent, in Mice: A Teratological Study

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Conflict of interest: Nil

Abstract:

Cisplatin [cis-dichlorodiammineplatinum (II)], the first generation anticancer platinum complex, is one of the most effective anticancer agents currently available for the treatment of testicular, ovarian, and bladder carcinomas. But its clinical usefulness has frequently been limited by undesirable side effects such as nephrotoxicity, gastrointestinal toxicity, ototoxicity, and neurotoxicity. The present study was conducted in collaboration with Department of anatomy, Institute of medical sciences, Banaras Hindu University, Varanasi. Animals - Fourty female albino mice of an average weight of 20 +/- 3 gm and an average age of 80-100 days were used in the study. Animals were housed individually in plastic cages in animal house on light – dark cycle of 12 : 12 hours. Mice were feed on diet in the form of pellets (Hindustan Lever Bombay, India), tap water and libitum and treated with a good antiseptic care. Mating- Female albino mice were kept overnight in the same cage with male mice of same stock in the ratio of 3:1 (female: male = 3:1). At 8:00 A.M. in next morning vaginal smear was examined. If vaginal smear was positive, then smear was made on glass slide and examined under light microscope. If sperms were found, pregnancy was confirmed, and it was considered as day ' ZERO' of pregnancy. The pregnant mice was weighted on alternate days and kept individually in separate cages. Microscopic examination of kidneys of both control and treated mice embryos were done. Staining method employed was Hematoxylin and Eosin (H&E) stain. Prepared slides of treated groups were compared with those of control groups. On examination of kidney of mice embryos, in control group kidney no abnormalities were observed [Plate A] while in treated kidney periglomerular space was increased. Severe degeneration and loss of proximal convoluted tubules [PCT] and distal convoluted tubules [DCT] was also observed [Plate B]. In the present study, Carboplatin was found to be teratogenic in developing mice embryos when injected intraperitonealy at a dose of 6 mg/kg body weight on 7th day of gestation. The fetuses were collected on 19th day of gestation. The drug was founded to be teratogenic because of its induced nephrotoxicity. The kidney of treated embryos showed increased periglomerular space and severe loss of proximal convoluted tubules [PCT] and distal convoluted tubules [DCT], establishing the nephrotoxic potential of carboplatin.

Keywords: Cisplatin, Carboplatin, Nephrotoxic, Anticancer Agent, Teratological Study.

Introduction

Teratology is the study of abnormal development in embryos and the causes of congenital malformations or birth defects. Congenital malformations account for approximately 20% of deaths in the perinatal period. [1] Approximately 3% of newborn infants will have major malformations and another 3% will have malformations detected later in life. These anatomical or structural abnormalities are present at birth although they may not be diagnosed until later in life. They may be visible on the surface of the body or internal to the viscera. [2] Cisplatin [cis-dichlorodiammineplatinum (II)], the first generation anticancer platinum complex, is one of the most effective anticancer agents currently available for the treatment of testicular, ovarian, and bladder carcinomas. But its clinical usefulness has frequently been limited by undesirable side effects such as nephrotoxicity, gastrointestinal toxicity, ototoxicity, and neurotoxicity. [3] Therefore, extensive efforts have been made to develop new cisplatin analogs with higher or equivalent antitumor activity and lower toxicity. Among them, Carboplatin is one of the commercially available platinum derivatives for clinical use. [4] Carboplatin (cis-diammine-

1,1cyclo butanedicarboxylateplatinum II), is a second-generation platinum-containing anti-cancer agent. It is well known that anticancer drugs are teratogenic because of their nonspecific attack on the embryos or fetuses, which have a very high cell proliferation rate. [4] This study was performed to evaluate the potential of Carboplatin to induce nephrotoxicity at a dose level of 6 mg/kg body weight in mice fetuses which correspond to the teratogenic dose in the previously described Bristol-Myres's report for rat fetuses. [5]

Materials and Methods

The present study was conducted in collaboration with Department of anatomy, Institute of medical sciences, Banaras Hindu University, Varanasi.

Animals: Fourty female albino mice of an average weight of 20 +/- 3 gm and an average age of 80-100 days were used in the study. Animals were housed individually in plastic cages in animal house on light – dark cycle of 12 : 12 hours. Mice were feed on diet in the form of pellets (Hindustan Lever Bombay, India), tap water and libitum and treated with a good antiseptic care.

Mating: Female albino mice were kept overnight in the same cage with male mice of same stock in the ratio of 3:1 (female: male = 3:1). At 8:00 A.M. in next morning vaginal smear was examined. If vaginal smear was positive, then smear was made on glass slide and examined under light microscope. If sperms were found, pregnancy was confirmed, and it was considered as day ' ZERO' of pregnancy. The pregnant mice was weighted on alternate days and kept individually in separate cages.

Drug Used: Trade name - CARBOWEL Contains - Carboplatin BP 10 mg Dose - 150 mg / 15 ml

Calculation of Dose: Average weight of mice = 20 gm Dose of drug = 6 mg/kg body weight Or (6 x 20) / 1000 = 0.12 mg / mice 1 vial = 15 ml contain 150 mg of drug 150 mg = 15 ml 0.12 mg = (15 x 0.12) / 150 = 0.012 ml Tuberculin syringe have 100 divisions. Therefore, 1 ml tuberculin syringe = 100 divisions Then, 0.012 ml tuberculin syringe = 1.2 divisions

Route of Drug Administration: Drug was administered intraperitoneally.

Procedure of Drug Administration: The drug was administered in a dose of 6 mg/kg body weight of mice (after calculation of dose = 0.12 mg/mice) on 7th day of gestation. At day 7th of gestation, pregnant mice were taken for drug administration. Calculated dose of the drug was drawn into sterile syringe. The drug was administered intraperitonealy by introducing the sterile injecting needle into the flank of the animal with the needle directed cranially and medially under rectus abdominus muscle. The injected mice were marked to indicate day of injection and dose. A group of pregnant control mice were nourished to term. These were given an identical volume of distilled water through same route (intraperitoneally) and on the same day (7th day of gestation).

Collection of Fetuses: The pregnant mice were anesthetized with an overdose of ether on day 19th of gestation (full term pregnancy). The uterine horns were exteriorized after opening the abdomen by midline caesarean incision. The fetuses were removed from the uterus. Fetuses were fixed in 10% formalin solution.

Collection of Organ from Fetuses: After examination and fixation, anterior abdominal wall of fetuses was opened and both Kidneys were dissected out. All dissected out kidneys were kept in fresh 10% formalin solution for

- Histological techniques and examinations
- Tissue processing and embedding (for H&E stain)

After fixing for 72 hours, 8-10 micron thick sections were cut for each kidney and then stained in H&E solution.

Microphotography: The microphotograph of the histological slides was taken with the help of digital microscope (MOTIC 2000, 1:3). Photographs were processed in the digital lab and pictures were developed.

Observations

Histological Observations: Microscopic examination of kidneys of both control and treated mice embryos were done. Staining method employed was Hematoxylin and Eosin (H&E) stain. Prepared slides of treated groups were compared with those of control groups. On examination of kidney of mice embryos, in control group kidney no abnormalities were observed [Plate A] while in treated kidney periglomerular space was increased. Severe degeneration and loss of proximal convoluted tubules [PCT] and distal convoluted tubules [DCT] was also observed [Plate B].

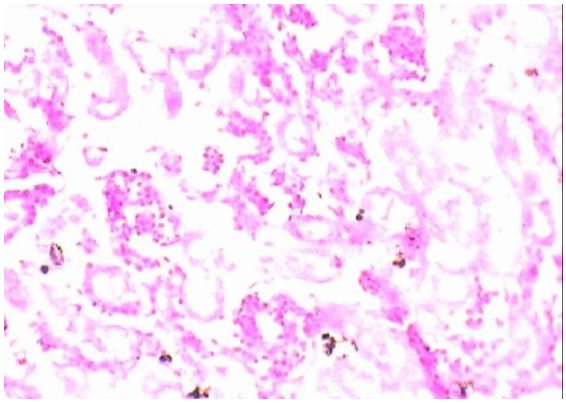

Figure 1: PLATE(A)

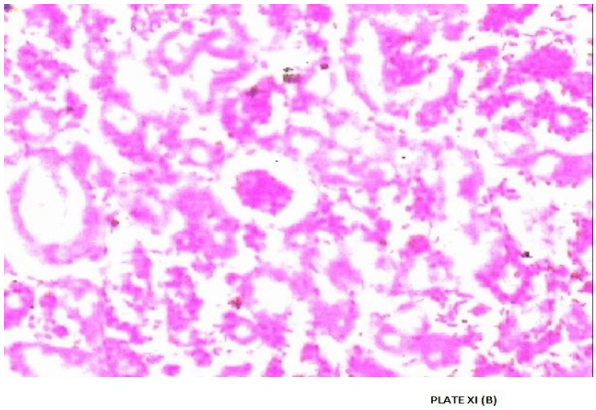

Figure 2: PLATE(B)

Discussion

The present study has demonstrated that Carboplatin (a platinum based chemotherapeutic agent) is teratogenic to albino mice. Present study establishes dosing regimens that will produce histological abnormalities in the form of nephrotoxicity. Teratogenecity in the present study occurred without significant maternal toxicity.

Since cisplatin, one of platinum coordinated complexes, is a bifunctional agent which causes interstrand/intrastrand DNA crosslinking in mammalian cells (Mansy et al., 1973 ; Munchausen, 1974; Pascoe and Roberts, 1974 ; Munchausen and Rahn, 1975 ; Van Den Berg and Roberts, 1976), [6-10] its major mechanism has been proposed to be the inhibition of DNA replication due to these crosslinking effects (Roberts and Pascoe, 1972 ; Roos, 1977 ; Zwelling et al., 1979; Zwelling and Kohn, 1980). [11-14] the mechanism of action of carboplatin seems to be similar to that of cisplatin because of resemblance in the chemical structure between these two compounds. In present study we observed decrease in size of kidney in carboplatin treated fetuses.

Microscopically, we revealed increased periglomerular space and severe loss of proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). It is currently thought that carboplatin principally kills malignant cells by forming DNA crosslink and to a lesser extent by the generation of toxic metabolites. Free radicals and reactive oxygen species may also contribute to chemotherapeutic agent induced side effects, such as cisplatin induced neurotoxicity, adriamycin induced cardiotoxicity, bleomycin induced pulmonary fibrosis (Conklin et al., 2000). [15] Carboplatin is secreted into breast milk. Carboplatin should not be used during pregnancy or breastfeeding. Mice are the foremost mammal studied by developmental biologists, providing greater availability of molecular probes, antibodies and transferable knowledge with transgenic studies. The teratologic effects of Carboplatin in mice have provided a reliable, easily reproducible method of studying the embryology and molecular biology for a range of complex congenital anomalies.

Conclusion

In the present study, Carboplatin was found to be teratogenic in developing mice embryos when injected intraperitonealy at a dose of 6 mg/kg body weight on 7th day of gestation. The fetuses were collected on 19th day of gestation. The drug was founded to be teratogenic because of its induced nephrotoxicity. The kidney of treated embryos showed increased periglomerular space and severe loss of proximal convoluted tubules [PCT] and distal convoluted tubules [DCT], establishing the nephrotoxic potential of carboplatin.

Thus, the present study establishes Carboplatin as a teratogenic drug in mice as it produces significant degenerative changes in kidney, establishing the nephrotoxic potential of the tested agent i.e. Carboplatin.

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