The stereological ratio of stromal/parenchymal components in postnatal prostate tissue of rats prenatally treated with diclofenac sodium

Mikail Kara**, Cennet Rağbetli*, Necat Koyun*, Neşe Çölçimen*, Murat Çetin Rağbetli*

*Department of Histology & Embryology, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey
**Department of Microbiology, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey

ARTICLE INFO

ABSTRACT

Since diclofenac sodium extends the pregnancy and affects the fetus, our study aimed to stereologically investigate the components of the rat prostate with prenatally treated with diclofenac sodium. The rats were left overnight to mate. Pregnant animals were kept in a room temperature with normal light and dark cycles and fed with normal diet. After injections to sham and treated groups for 15 days, 18 male offsprings were housed in three groups (6 placebo; 6 control; 6 treated). The 20 weeks-old animals were perfused and the prostates removed and embedded in paraffin. Tissue sections at 5µm thickness were taken at regular intervals and stained with Hematoxylin-eosin and Masson trichrome. Cavalieri principle was used for volume fractions analysis. Stereological examination revealed 55% acinar epiteli and 45% connective tissue in both control and placebo groups, as well as 60% acinar epiteli and 40% connective tissue in the treated group. Prenatally applied diclofenac sodium did not cause a statistically significant change in the prostate stroma-parenchymal ratio in 20-week old rats compared to the control animals (p>0.05).


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**Correspondence to:**
Mikail Kara
Department of Histology,
Faculty of Medicine,
Yuzuncu Yil University, Van, Turkey
e-mail: dr.karamikail@gmail.com

Keywords:
Diclofenac sodium
Prenatal term
Rat
Prostate
Stereology

1. Introduction

Diclofenac sodium (DS) is one of the most useful a nonsteroi- dal anti-inflammatory drugs (NSAIDs) and widely used because of its strong analgesic, antipyretic and anti-inflammato- ry effects (Skoutakis et al., 1988; Mitchell and Warner, 1999). Numerous studies have been carried out in order to achieve a desirable release rate of DS in the treatment of rheumatoid arthritis, degenerative joint diseases, ankylosing spondylitis and osteoarthritis (Todd and Sorkin, 1988). They are also used in painful conditions, renal colic, trauma, dysmenor- rhoea, dental medicine, and in the treatment of pain resulting from minor surgery. Previous studies reported its effect on osteoclast formation, platelet function, liver and kidney mor- phology (Nobukawa et al., 2001; Power et al., 2007; Rağbetli et al., 2009; Besen et al., 2009). To stereologically analyse the histological components of the zone in both normal human prostate and benign prostatic hyperplasia, and to determine the volumetric density of the total stroma, connective tissue and acini. Their results of that in benign prostate hyperplasia there is an increase in the stromal components (Chagas et al., 2002). The effect of DS on the urinary tract tissue was found to be more relaxant on ureteral smooth muscle (Sivrikaya et al., 2004). At the present time, DS is also used widely in the women of child-bearing age for the treatment of common gynecological problems. Diclofenac inhibited preterm delivery but had no effect on mifepristone-induced cervical maturation. The tocolytic effect of diclofenac was reported in a study using mifepristone (RU 486) to induce preterm labor in rats (Cabrol et al., 1991). Prostaglandin synthetase inhibitors diclofenac and indomethacin reduce smooth muscle activity in the renal pelvis (Lundstam et al., 1985). DS may lead to vascular changes, a decrease of the elastic fiber in rats that are prenatally subjected to increased volume of maternal blood resulting from as injection (Zengin et al., 2013). A review on toxicity of non-steroidal anti-inflammatory drugs has been recently published (Aygün et al., 2012). The prostate is basi-
cally composed of acinar and stromal components. We could not find a study investigating the effects of prenatal (prostate development) DS administration on prostate development in rats. Since DS can cross the placenta barrier and affect the fetus, our study dealt with the effects of prenatally applied DS on the morphometry of adult prostate by stereological methods. The aim of the present study was to investigate the effects of DS administrations during pregnancy on the components of rats prostate 20 weeks after the birth.

2. Materials and methods
This study was approved by the Animal Use Ethics Commission (04-2009) of Yuzuncu Yil University.

Animals and experimental procedures:
In the present study, three-month old male and female Wistar rats (150-200 g) were used. Totally 12 animals were housed as control, sham and DS treatment groups. Each group (3 female, 1 male) was separately left to mate on the same day. After observing the vaginal plug produced by male vesicular and coagulating gland secretions on the next day, the animals were accepted as pregnant. Pregnant animals were kept in a standard plastic cage on sawdust bedding in an air-conditioned room (20°C), under a 12:12-h light/dark cycle and fed ad libitum (Odaci et al., 2010). After mating, DS (Voltaren, NOVARTIS, Istanbul, Turkey) in a dose of 1 mg/kg was injected daily intraperitoneally (i.p.) to pregnant rats for the gestational period, 5-19 days. The rats in the sham group received physiological saline (1ml) alone during the same period and the control group did not receive any injection. After delivering, the pups male were separated into control (n=6), sham (n=6) and DS (n=6) groups. At postnatal week 20, the pups from each group were deeply anesthetized with i.p injection of urethane (1.25 g/kg) and perfused through the left ventricle of the heart with 0.9% saline, which was followed by 10% neutral buffered formalin. Prostate tissue samples of the groups were obtained from a total of 18 male offspring. They were fixed in 10% buffered formalin, and paraffin-embedded, sectioned and stained for histological examination. The preparations were examined under a light microscope (Zeiss axioskop 40, Göttingen, Germany). Tissue sections of about 5µm thick were taken at regular intervals. The first section was chosen at random and every 195th section pairs were taken. From each prostate, approximately eight sections were stained with Hematoxylin-eosin and Masson trichrome.

Microscopy, imaging, and stereological analyses:
The preparations were examined with a light microscope (Zeiss axioskop 40, Göttingen, Germany). With a 40x objective, the whole section images were captured on a computer as TIFF files by an attached video camera system (Sony-Japan). The areas of aciner epithelium and connective tissue of prostate were analyzed by using Shiteroem 1.5 software running on a personal computer. Calibration was set with an objective micrometer slide. Then, volumetric measurements were performed using the following formula modified from Cavalieri Principle (Gundersen, Kaplan et al., 1988; Howard and Reed, 1998). For stereological analysis (Gundersen and Jensen, 1987; Chagas et al., 2002, Kaplan et al., 2005) the volume fraction of the histological components (acinar or connective tissues) was calculated as Vp/Pc, Vp=Volume fraction (component, ref), the number of points hitting the components, Pc=the number of points hitting the references. As the ratio of the fine to coarse points is 4:1 each coarse point counts as four fine points, so the equation is Vp/Pc, then the total volume of a defined component calculated as (comp)=(ref), (comp) is an estimate of the total volume of components; (ref) is an estimate of the volume of the references (prostate) and an estimate of the volume of the fraction of component per unit volume of prostate tissue is 16. The coefficient of error (CE 5%) and the coefficient of variation (CV 10%) are also valuable data to see whether the fraction of volume in each animal and the number of subjects in each group. These were calculated with the formula by Gundersen and Jensen (1987). The mean coefficient of variation for each group and the mean coefficient of error for stereological estimation of tissue volumes were observed in an acceptable level. Stroma-parenchyma volume fraction was measured with combined field scale (Fig. 1).

Statistics
Descriptive statistics are expressed as mean, standard deviation, minimum and maximum values. The data from the present study was compared in relation to experimental groups and gender by using SPSS (Version 13) statistical package program. Kruskal-Wallis test was used for comparisons of connective and acinar tissues, by converting the findings to numeric counterparts. P value of 0.05 and less was considered statistically significant for analyses and comparisons.

3. Results
Stereological examination revealed 55% acinar tissue and 45% connective tissue in the control and sham groups as well as 60% acinar tissue and 40% connective tissue in the DS group (Figure 1-5). Prenatally applied DS did not cause any statistically significant change in the 20-week old rat prostate stroma-parenchyma ratio compared to the control animals (p>0.05). We excluded smooth muscle tissue from statistical evaluation because there are not enough smooth muscles in prostate.
4. Discussion

NSAIDs are inhibitors of cyclooxygenase and have the potential to interfere adversely with pregnancy. NSAIDs given during pregnancy have the potential effects for maternal and fetal period. The reported adverse effects of NSAID treatment are: prolongation of pregnancy and labour, and increased maternal blood loss associated with delivery in the mother. The major pharmacological action of NSAIDs on the fetus is mediated through inhibition of prostaglandin synthesis. Many of the serious adverse effects reported in newborns after intrauterine exposure to NSAIDs have occurred in infants delivered preterm and exposed to the drug close to delivery, such as fetal anuria, oligohydramnions and other prostanoid related side-effects (Ostensen, 1996; Ostensen et al., 1998). NSAIDs (indomethacin) have been reported to have a direct inhibitory action upon smooth muscle obtained from human upper urinary tract (Angelo-Khattar et al., 1985; Besen et al., 2009). Since muscle tissue of the rat from the present study was observed to be extremely small, it was not included in our study.

It was reported that chronic usage of NSAIDs causes negative urinary tract effects such as tubular necrosis (Kim et al., 1999) tocolytic effects on the cattle uterine strips (Das et al., 2013) and drug induced renal toxicity (Roth et al., 1985).

Our study did not find any significant morphomet-

ric change by prenatally applied DS in the 20-week old rat prostate stroma and parenchyma. Diclofenac-induced teratogenicity on rat embryos was reported (Chan et al., 2001). In the present study, there were also no congenital anomalies in postnatal 20 week-old animals. Dosage used in our study might not be high enough to cause a congenital anomaly.

In conclusion, the present study found originally no pathological effects of prenatal diclofenac administration on prostate in rats. However, the present study should be confirmed by the further studies.

Declaration of interest

This work was approved by the Animal Use Ethics Commission from Yüzüncü Yil University and all procedures were performed according to the Animal Experimentation Ethics Committee. The authors thank the Yuzuncu Yil University Scientific Research Projects (2009-TFU-U075). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgement

The researchers would like to thank Prof. Dr. Atif Aydınlioglu who passed away an expertedly in a traffic accident, for his opinion on the stereological subject.
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