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Effects of Paracetamol on Vascular Endothelial Growth Factor, Sclerostin and FETUIN-A in the Liver of Rat Fetuses

Sıçan Fetusların Karaciğerlerinde Parasetamolün Vascular Endothelial Growth Factor, Sclerostin ve FETUIN-A Üzerine Etkileri

Seher Yılmaz[1*], Ayşe Yeşim Göçmen[2], Adem Tokpınar[1], İlyas Uçar[3], Seda Avnioğlu[4], Mehtap Nisari[5]

1.Yozgat Bozok University, Faculty of Medicine, Department of Anatomy, Yozgat, Turkey

2.Yozgat Bozok University, Faculty of Medicine, Department of Biochemistry, Yozgat, Turkey

3.Ahi Evran University, Faculty of Medicine, Department of Physical Therapy and Rehabilitation, Kırsehir, Turkey

4.Alanya Alaaddin Keykubat University, Faculty of Medicine, Department of Anatomy, Antalya, Turkey

5.Erciyes University, Faculty of Medicine, Department of Anatomy, Kayseri, Turkey

ABSTRACT

Aim: Paracetamol is widely used by important societal groups such as pregnant women and the elderly. Paracetamol, taken in high doses especially during pregnancy, causes liver failure. The aim of our study is to investigate the effects of paracetamol, which is widely used during pregnancy, on the fetal liver.

Methods: In our study, five groups of randomly selected rats from 10 Wistar Albino rats (n=2) were formed as control group, 50 mg / kg paracetamol group, 125 mg / kg paracetamol group, 250 mg / kg paracetamol group and 500 mg / kg paracetamol group. Paracetamol by gavage was given to pregnant rats in specified doses. Fetuses were taken by cesarean on the 20th day of pregnancy (10 fetuses were taken from each group). The fetus livers were then taken for biochemical analysis. Biochemically, vascular endothelial growth factor A (VEGF-A), FETU-A (FETUIN-A) (α2-heremans-schmid glikoprotein) and Sclerostin (SOST) values were examined.

Results: In this study, changes in liver hepatocyte cells are seen as the dose of paracetamol increases. Regular increase is observed in VEGF-A and FETU-A. SOST increased at a dose of 250 mg / kg and decreased in the group of 500 mg / kg paracetamol. ($p<0.05$).

Conclusions: As a result, it is seen that the use of high doses of paracetamol in pregnancy causes changes in the liver and many biochemical values on the fetus. We think that an overdosing of paracetamol, which is sold without prescription and used as an innocent analgesic during pregnancy, should be examined and this study will be a reference for other studies to be conducted.

Keywords: VEGF-A, paracetamol, liver, FETU-A, fetus

ÖZ

Amaç: Parasetamol gebe, çocuk ve yaşlılar gibi önemli gruplar tarafından yaygın olarak kullanılmaktadır. Özellikle gebelikte fazla dozda alınan parasetamol karaciğer yetmezliklerine neden olmaktadır. Çalışmamızın amacı gebelik döneminde yaygın olarak olarak kullanılan parasetamolün fetüs karaciğeri üzerine etkilerini biyokimyasal olarak araştırmaktır.

Yöntemler: Çalışmamızda 10 adet (n=2) gebe sıçanlardan Wistar Albino cinsi rastgele seçilen kontrol grubu, 50 mg/kg parasetamol grubu, 125 mg/kg parasetamol grubu, 250 mg/kg parasetamol grubu ve 500 mg/kg parasetamol grubu olmak üzere beş gruba ayrıldı. Gavaj yoluyla parasetamol, belirlenen dozlarda gebe sıçanlara verildi. Gebeliğin 20. gününde sezeryan ile fetüsler alındı (her gruptan 10 adet fetus alındı). Sonrasında fetus karaciğerleri biyokimyasal analiz için alındı. Biyokimyasal olarak vascular endothelial growth factor A (VEGF-A), FETU-A (α2-heremans-schmid glikoprotein) ve Sclerostin (SOST) değerleri incelendi.

Bulgular: Bu çalışmada parasetamol dozu arttıkça karaciğer hepatosit hücrelerinde değişiklikler görülmektedir. VEGF-A ve FETU-A'da düzenli artış görülmektedir. SOST ise 250 mg/kg dozunda yükselirken, 500 mg/kg parasetamol grubunda azalmıştır. ($p<0.05$)

Sonuç: Sonuç olarak gebelikte yüksek dozda parasetamol kullanımının fetüs karaciğerinde ve biyokimyasal değerde değişiklik meydana getirdiği görülmektedir. Bu çalışmada gebelikte masum analjezik olarak reçetesiz satılan ve çok kullanılan parasetamol'un yüksek dozda kullanımı konusunda dikkat edilmesi gerektiğini ve bu çalışmanın yapılacak diğer çalışmalara referans olacağını düşünmekteyiz.

Anahtar Kelimeler: VEGF-A, parasetamol, karaciğer, FETU-A, fetüs

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*Corresponding author: Seher Yılmaz, Yozgat Bozok University, Faculty of Medicine, Department of Anatomy, Yozgat, Turkey. Phone: + 90 3542126201 E-mail: seher.yilmaz@yobu.edu.tr

ORCID: 0000-0003-4551-995X

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INTRODUCTION

Paracetamol has been widely used worldwide since its release in the mid-1950s due to its pain-relieving and antipyretic effects [1]. Nonsteroidal anti-inflammatory drugs are frequently used among pregnant women, preferred for its availability to be purchased without a prescription [2]. Paracetamol is a pain reliever that is widely used due to its reliability, cheapness and effectiveness [3]. However, unchecked use of paracetamol may affect fetus health negatively. Recent studies have reported that exposure to high doses of paracetamol before birth was linked to autism, attention deficit and hyperactivity [4]. Paracetamol taken in excessive doses passes through the placenta and causes both fetal and maternal hepatotoxicity [5]. As a weak acid, nonionized in the physiological pH range and weakly bound to plasma proteins, it crosses the placenta with the blood-brain barrier. The passage of substances dissolved in lipids from mother to baby is directly proportional to placental blood flow [6]. Although the use of standard doses of paracetamol in any trimester of pregnancy does not cause any side effects in fetal development, some studies on the use of paracetamol in pregnancy have attracted attention in recent years. Studies in humans have not shown a link between prenatal paracetamol exposure and complications such as miscarriage, low birth weight and preterm delivery. In addition, many studies argue that there is a positive link between paracetamol used during pregnancy and the risk of asthma in children, and there are publications both criticizing and supporting these views in the literature [7].

Along with increasing frequency of paracetamol use and overdose intake, an increase in liver toxicity and mortality rates are also observed. Paracetamol intoxication is responsible for about 500 deaths annually in the United States alone, causing hepatocellular necrosis. Although hepatotoxicity frequently develops in toxic doses of paracetamol intake, symptoms of renal failure, metabolic acidosis, coagulopathy, encephalopathy and recurrent gastrointestinal tract may also be seen. After taking paracetamol orally, it is converted into the toxic metabolite N-acetyl-p-benzoquinonimine by the cytochrome p450 enzyme system and detoxified with endogenous glutathione. In high doses, glutathione stores decrease, and liver toxicity occurs because toxic metabolites cannot be detoxified. N-Acetylcysteine is a glutathione precursor. It prevents the binding of toxic metabolites of paracetamol to hepatic macromolecules and refreshes reduced glutathione stores; it also reduces hepatic necrosis with antioxidant mechanisms. In acute paracetamol poisoning, it is reported that the N-Acetylcysteine applied within the first 8 hours following the intake largely prevents the development of toxicity [8].

Angiogenesis is a multifactorial and progressive process that leads to the formation of new blood vessels. It plays an important role in the development of the tumor. The most important and best-known factor affecting both physiological and pathological angiogenesis is VEGF-A [9].

It is a multifunctional growth factor family that has specific effects for endothelial cells [10]. While this growth factor plays a critical role in vascular formation, it has been found that it is necessary in many functions performed by endothelial cells [11].

FETU-A is a glycoprotein and is synthesized from the liver. Factors affecting it is secretion in humans have been reported to be in conditions such as severe liver injury, cirrhosis, acute viral, hepatitis and cancer [12].

SOST is a new biomarker associated with the bone-vascular axis. Zou et al. performed a study in which vascular effects of serum sclerostin level were examined [13].

In this study, we aimed to investigate the effects of high dose paracetamol use on the fetus during pregnancy, especially on the enzymes of the fetus liver.

MATERIALS AND METHODS

The approval for the study was received by the University Experimental Animals Ethics Committee on 16.11.2016 with the protocol number 16/145 Wistar albino rats aged at least 8 weeks old and with an average weight of 185-200 g, were obtained from Erciyes University Experimental Animals Clinical Research Center use in this study. Female rats were placed in the same cage with male rats at 17:00 in the evening

to launch the experiment. After the vaginal smear samples of the female rats were examined under a microscope at 7:00 am the next day, specimens with sperm in the vaginal smear were considered to be 0.5 days pregnant and kept in separate cages apart from male rats. Paracetamol doses were given to the pregnant rats in accordance with the literature and at the end of the experiment, livers of rat fetuses were analyzed. A total of 10 rat fetuses from each group were included in the study.

Experimental groups

In accordance with the literature [14] powdered paracetamol was dissolved with 1 ml SF (saline solution) and administered to the rats orally at 3.00 pm by gavage.

Control group: SF was administered to the rats by gavage 1-20 days of their pregnancy.

50 mg/kg paracetamol group: Paracetamol at a dose of 50 mg/kg was administered to the pregnant rats at 3.00 pm on 1-20 days of their pregnancy by gavage.

125 mg/kg paracetamol group: Paracetamol at a dose of 125 mg/kg was administered to the pregnant rats at 3.00 pm on 1-20 days of their pregnancy by gavage.

250 mg/kg paracetamol group: Paracetamol at a dose of 250 mg/kg was administered to the pregnant rats at 3.00 pm on 1-20 days of their pregnancy by gavage.

500 mg/kg paracetamol group: Paracetamol at a dose of 500 mg/kg was administered to the pregnant rats at 3.00 pm on 1-20 days of their pregnancy by gavage (Figure 1).

Homogenization and total protein concentration

Rat liver samples were weighed and homogenized in phosphate-buffered saline solution (PBS). Then homogenates were centrifuged at 10 000 RPM for 15 minutes at 4°C, and supernatants were collected and analyzed immediately. The total protein concentration was determined in each sample using direct colorimetric measurements of total protein with Bio-Rad reagents (Bio-Rad laboratories GmbH, Munchen, Germany) and bovine serum albumin as the standard.

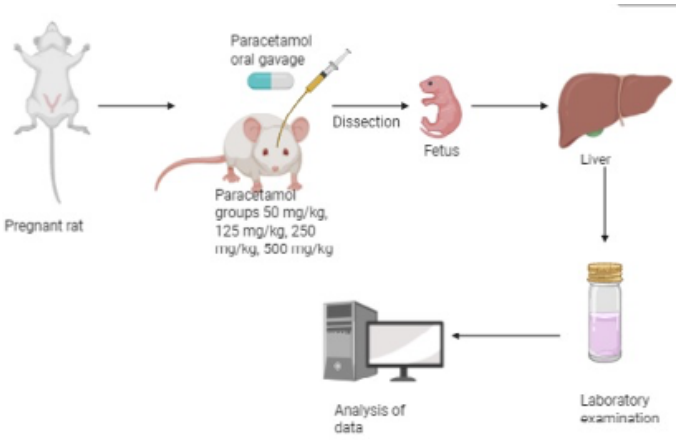

Figure 1. Summary diagram of the whole study. Biochemical analyzes of liver tissues were performed in five different groups

VEGF-A, SOST and Fetu-A Levels

The concentrations of VEGF-A, SOST and Fetu-A were assayed in rat liver homogenates by ELISA method (8-point standard worked) according to the protocol booklet of the manufacturers of the ELISA kits for VEGF-A (Elabscience Rat VEGF-A ELISA kit catalog No: E-EL-R2603, Elabscience (Wuhan, China)), SOST (Elabscience Rat SOST ELISA kit catalog No: E-EL-R2427, Elabscience (Wuhan, China)) and FETU-A (Elabscience Rat FETU-A ELISA kit catalog No: E-EL-R2451, Elabscience (Wuhan, China)) assay kit instructions in duplicates. The absorbances were measured using Multiskan GO plate reader (Thermo Scientific, U.S.A) and calibrated according to standard curves. The results obtained were calculated for liver homogenate total protein contents in mg.

Statistical Analysis

All analyses were performed with an SPSS version 23.0 (IBM Co., NY, USA) and data was given as mean (standard deviation). In the data obtained, 5 parameters were evaluated (kurtosis), skewness, mean-standard deviation ratio, Gauss curve, Shapiro-Wilko test) and normal distribution analysis was performed. When 3.5 + points out of 5 parameterswere obtained, we accepted that our data was distributed normally and parametric tests were applied a one-way Anova test for the same day in multi-group comparisons Tukey test, since variances are equal in post hoc evaluations.

RESULTS

VEGF-A, FETU-A and SOST levels were measured in accordance with the protocol, fetus livers samples by ELISA method. When the levels of VEGF-A were examined, it was found that the paracetamol 500 mg / kg group was statistically higher than the other groups (Figure 2). In the FETU-A group, the paracetamol was also higher in the 500 mg / kg group (Figure 3). In the SOST level, paracetamol was more common in the 250 mg / kg group than in the other groups (Figure 4). In groups with liver damage, sclerostin level increased.

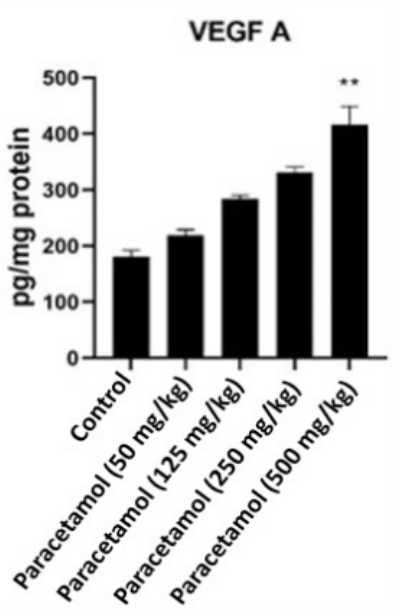

Figure 2. Levels of VEGF-A by experiment groups. One-way ANOVA, post hoc Tukey test (**p<0.01)

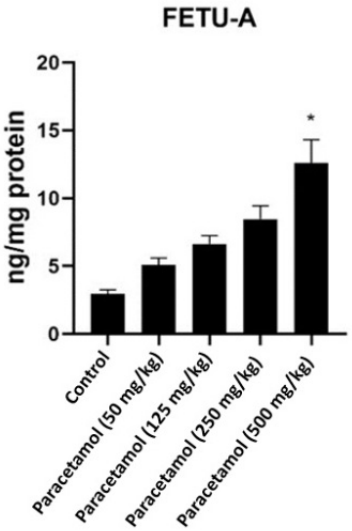

Figure 3. Levels of FETU-A by experiment groups. One-way ANOVA, post hoc Tukey test (* p<0.05)

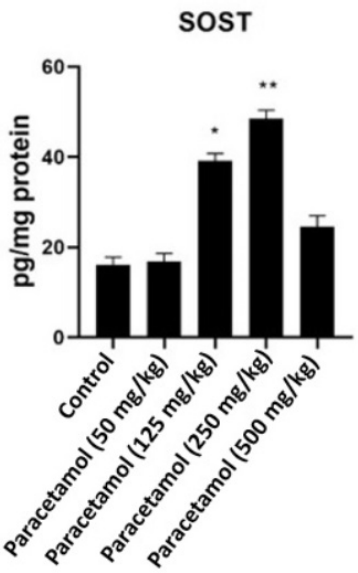

Figure 4. Levels of SOST (Sclerostin) by experiment groups. One-way ANOVA, post hoc Tukey test (*p<0.05, **p<0.01)

DISCUSSION

Excessive drug consumption may result in health problems in the liver and kidneys and cause acute insufficiency. Analgesic and non-steroidal drugs are used very frequently today [15] and over-the-counter medications are widely used in mild and moderate pain. Paracetamol in particular is a pain medication in use at large in the population by the elderly, for children and in pregnancyt [16]. Despite this, its effects on the fetus, especially in pregnancy, are not fully known. In experimental studies, paracetamol given for the purpose of treatment caused changes in the cognitive abilities and behaviors of animals [17]. It is a reliable drug when used in prescription dosage, however, when taken in doses above 4000 mg daily, it causes liver damage [18].

Schilling et al. determined that high doses of paracetamol would damage the liver and that some patients may experience liver damage at doses slightly above the recommended daily limit of 4 grams [19].

In another study, Holm et al. applied 50 mg / kg and 150 mg / kg paracetamol to the rats by gavage until the 7th day after mating. When the ovaries of the female offspring were examined 7 weeks after birth, they found that the number of primordial follicles decreased by approximately 50% in groups exposed to paracetamol, and that there was a significant decrease in the number

of primary and secondary follicles. Based on these data, the author points out that exposure to prenatal paracetamol subsequently leads to decreased fertility [20].

In a study by Momma et al., analysis was performed of the potential effect of paracetamol given to Wistar rats on the cardiovascular system on the 21st day of their pregnancy, which reported that paracetamol, ductus arteriosus caused narrowing in the inner diameter, increase in the volume of the ventricles and heart failure. In another study, it was reported that glutathione concentration decreased in the fetal mouse liver without any malformation due to the paracetamol application to the mice between the 6th and 13th days of their pregnancy [21]. Oxidative stress stimulates the formation of mitochondrial peroxynitritis, which triggers mitochondrial DNA damage, and hepatic macrophages advance liver damage from paracetamol [2].

There are studies indicating that when paracetamol is taken in overdose, it causes a decrease in the activity of antioxidant enzymes. At the same time, pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α can cause apoptosis, necrosis of hepatocytes, and liver damage by stimulating oxidative stress [22].

Chronic paracetamol poisoning occurs at doses of more than 60 mg/kg daily, intakes more than a week. In particular, drug use takes place mostly in the first three months of pregnancy, and some expectant mothers use drugs prior to being aware of their pregnancy. In the late period of pregnancy, fetal kidneys start to work quite effectively and quickly eliminate hydrophilic drugs. Since the urine of the fetus passes into the amniotic fluid, hydrophilic drugs are less likely to pass to the mother, even though the maternal plasma concentration is reduced. Therefore, amniotic fluid can be considered as a reservoir for hydrophilic drugs [23]. The risk of toxicity is increased because the blood-brain barrier permeability is higher in the fetus than the adult and the detoxification function of the liver is less effective. In individuals with chronic liver disease, SOST level increases [24]. This result is similar to our study where we that VEGF-A and FETU-A ratios increased in 500mg/kg paracetamol group and SOST amount increased in 250mg/kg paracetamol group. Considering the literature, it was seen that high doses of paracetamol in the liver increased VEGF-A, FETU-A and SOST levels compared to the control group.

Paracetamol crosses the blood-brain wall in the fetus during pregnancy. In our study, growth factors in fetus livers were investigated in pregnant rats given paracetamol. Depending on the dose of paracetamol, VEGF-A, FETU-A and SOST values increased and the VEGF-A and FETU-A were found to be similar to other studies [25]. More clinical and experimental studies are needed to determine the extent to which paracetamol affects the organs and development of the fetus in pregnant women.

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Conflict of interest: The authors declare that there are no conflicts of interest.

Limitations: Lack of cell viability test in the fetal liver and failure to look at different growth parameters constitute the limitations of the study.

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