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EFFECTS OF TRANSIENT HYPOTENSION ON GLUTATHIONE LEVELS AND LIPID PEROXIDATION IN THE LIVER DURING PREGNANCY IN RATS

Bilge PEHLIVANOĞLU[1], Sibel BAYRAK[1], Dicle Zeynep BALKANCI[1], Yasemin AKSOY[2]

ABSTRACT

Objective: Pregnancy is associated with physiologically reversible but important changes in various body systems like blood pressure regulation or oxidant status in various organs. The effects of transient hypotension on the fetus and mother are not studied extensively although experienced frequently. During hypotension, blood flow of many tissues is altered. The liver is vulnerable to hypoxia and the affected oxidant balance may lead to changes in other tissues, including the blood. On the basis of these accumulated data, in the present study we tested the effects of transient hypotension on oxidant stress in the liver in pregnant rats.

Materials and Methods: Twenty-eight 3-month-old female Wistar-albino rats were divided into virgin control (VC), virgin hypotension (VH), pregnant control (PC), and pregnant hypotension (PH) groups. On day 15 of pregnancy in the PH and PC groups and a week after admission to the laboratory in the VH and VC groups the right femoral artery was catheterized and hypotension was created for 30 minutes by blood withdrawal. Forty-eight hours after the surgery all the animals were sacrificed and liver tissues were stored at -80 °C until biochemical analysis for malonyldialdehyde (MDA) and reduced glutathione (GSH) was performed.

Results: MDA levels were higher in the pregnant and hypotensive groups compared to the control animals. Amongst all the groups, the PH group had the lowest GSH and the highest MDA levels ($p<0,05$).

Conclusion: Pregnancy as well as increasing lipid peroxidation weakens the antioxidant defense system, which becomes more prominent especially in the liver tissue during the transient hypotensive periods in late pregnancy.

Key Words: Blood Pressure, Pregnancy, Liver, Hypotension, Rat.

SIÇANLARDA GEBELİK SIRASINDA GEÇİRİLEN GEÇİCİ HİPOTANSİYONUN KARACİĞER LİPİT PEROKSİDASYONU VE GLUTATYON DÜZEYİNE ETKİSİ

ÖZ

Amaç: Gebelik, kan basıncının düzenlenmesi veya çesitli organlardaki oksidan durum gibi çok sayıda farklı vücut sistemlerinde fizyolojik olarak önemli ve çoğu zaman geri dönüsümlü değisikliklere neden olur. Sıklıkla rastlanmasına karsın geçici hipotansiyonun anne ve fetus üzerindeki etkileri çok fazla çalısılmamıstır. Hipotansiyon sırasında pek çok dokunun kan akımında ve oksijenizasyonunda değisiklikler gözlenir. Karaciğer hipoksiye duyarlıdır ve oksidan dengedeki değisiklikler kan gibi diğer dokulardaki değisikliklerin öncülü olabilir. Bu çalısmada literatür bilgilerine dayanarak, gebelerde geçici hipotansiyonun karaciğerdeki oksidan strese etkisini arastırdık.

Gereç ve Yöntem: 28 adet üç aylık Wistar albino sıçan gebe olmayan kontrol (GOK), gebe olmayan hipotansiyon (GOH), gebe kontrol (GK) ve gebe hipotansiyon (GH) olmak üzere dört gruba ayrıldı. GK ve GH grupları gebeliğin 15. gününde GOK ve GOH grupları ise laboratuvara geldikten bir hafta sonra sağ femoral arter kateterizasyonu yapılarak, kan alınması yoluyla 30 dakikalık hipotansiyona maruz bırakıldı. Cerrahiden 48 saat sonra hayvanlar feda edildi ve karaciğer dokusu çıkarılarak malonyldialdehid (MDA) ve redükte glutatyon (GSH) düzeyleri için biyokimyasal analizler yapılana kadar -80° C'da saklandı.

Bulgular: MDA düzeyleri gebe ve hipotansiyon gruplarında kontrol hayvanlarına göre anlamlı olarak yüksekti. Tüm gruplar arasında GH grubunda en düsük GSH ve en yüksek MDA düzeyleri ölçüldü ($p<0,05$).

Sonuç: Gebelik lipit peroksidasyonunu artırmanın yanı sıra antioksidan savunma sistemini zayıflatır. Bu etki geç dönem gebelikte maruz kalınan hipotansiyondan sonra özellikle karaciğerde belirgindir.

Anahtar Kelimeler: Kan Basıncı, Gebelik, Karaciğer, Hipotansiyon, Sıçan.

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[1] Hacettepe Üniversitesi Tıp Fakültesi, Fizyoloji, Ankara, Türkiye

[2] Hacettepe Üniversitesi Tıp Fakültesi, Biyokimya, Ankara, Türkiye

INTRODUCTION

Altered blood pressure (BP) and regulatory mechanisms during pregnancy are a matter of great concern in obstetrics.[1,2] Short-term regulation of blood pressure is accomplished via the autonomic nervous system, baroreceptors, and peripheral vessel resistance.[3] In pregnancy, due to various reasons, such as insufficient BP regulating mechanisms, a physiological increase in vascular bed capacity, blood retention in lower extremities, changes in the sympathetic nervous system, and hormonal shifts, BP is lowered.[4-6] Reflex increases in heart rate, vasopressin, adrenocorticotropic hormone, and cortisol concentration were found to be attenuated in pregnant dogs and blamed on altered baroreceptor function. Pregnant animals are considered to be unable to achieve normal cardiovascular homeostasis in response to blood loss.[7] During pregnancy increased blood volume and cardiac output dampen the baroreceptor reflex.[4] On the basis of the accumulated data, it is now clear that BP regulation during the natural course of pregnancy is disturbed, resulting in hyper- or hypotensive periods. Although pregnancy related hypertension has been studied extensively, [5,8] there is a limited number of studies regarding hypotension. [9, 10] Since its results on mother and fetus physiology are not observed acutely following hypotensive attack, transient hypotension experienced by many pregnant women is neglected. On the other hand, recent data suggest that hypotension experienced during pregnancy although transient has important impacts on fetal and maternal tissues.[10] The hypotensive period may act as a stressor, regulating adaptive cellular response to hypoxia resulting from hypotension.[11]

Pregnancy is a period during which maternal physiology changes dramatically. Of the changes observed during the natural course of pregnancy, liver functions and balance between oxidants and antioxidants are significant.[12] During pregnancy, increased energy and oxygen demands cause reactive oxygen molecules to accumulate in proportion. Under physiological conditions antioxidant defense mechanisms limit and/or prevent the damage caused by these molecules.[2, 13] In uncomplicated pregnancies, lipid peroxidation is increased in the sera of the mother; on the other hand, serum antioxidant activity also increases as the pregnancy proceeds, peaking at the time of labor. These effects are found to be more prominent in hepatic samples.[2, 14] The liver is a well recognized target of injury in low flow states like hypotension. Decreased cardiac output is accompanied by a greater decrease in hepatic blood flow, resulting in liver ischemia. Hypotension and reoxygenation in resuscitation result in hepatocellular injury, which may be caused by generation of oxygen radicals.[15, 16]

Because pregnancy and hypotensive periods change the oxidant-antioxidant balance in the organism and liver is one of the most affected organs in either condition, in the present study we aimed to investigate the effects of transient hypotension on liver glutathione levels and lipid peroxidation in late pregnancy.

METHODS

Experimental procedure:

Twenty-eight female 3-month-old Wistar albino rats weighing 250-350 g were used in the present study. The animals were divided into four groups: VC: Virgin control (n=5), VH: Virgin hypotensive (n=7), PC: Pregnant control (n=7), PH: Pregnant hypotensive (n=9). They were maintained on standard laboratory chow and water ad libitum. All the animals received humane care in compliance with the "Codes of Practice for the Care and Use of Animals for Scientific Purposes".[17] The experimental protocol was approved by the Hacettepe University Animal Ethics Committee with decision number 2003/42-1. Food was withheld 12 h prior to the experiments. While the pregnant rats were anesthetized with 90 mg/kg of ketamine and 10 mg/kg of xylazine intramuscularly on day 15 of pregnancy, the non-pregnant animals had the same anesthesia a week after their admission to the laboratory. Each animal was secured on an animal board and allowed to breathe spontaneously. The right femoral artery was dissected free of the associated vein and nerve and cannulated with preheparinized plastic tubing (2F). The catheter was attached to a three-way stopcock joined to the pressure transducer (Biopac SS13L), which was connected to a data acquisition system (Biopac MP 30) The animals were allowed to stabilize for 15 minutes after catheterization. Based on the literature knowledge, a 30-35% decrease in MABP was aimed [6, 9, 18] and blood was withdrawn (an average volume of 1 ml/100 g of body weight) to lower the mean arterial blood pressure (MABP) to around 50 mmHg (Table 1). It was maintained at that value for 30 minutes by withdrawal and reinfusion if necessary. Blood (stored in heparinized syringes at the body temperature of the animal) was reinfused after 30 minutes of hypotension. The same procedures except blood withdrawal and hypotension were performed in the control groups. All the animals were allowed to recover for 48 hours. Under the same anesthesia as during surgery, all the animals were sacrificed before delivery to avoid additional stress or complications due to the birth itself, [9,19] and the liver tissue was collected and kept at -80 °C until biochemical analysis was performed.

Biochemical Analysis:

Level of Lipid Peroxidation;

Thiobarbituric acid reactive substances (TBARS) were measured as an index of lipid peroxidation by the method described by Uchiyama and Mihara.[20] Tissue samples were homogenized in 1:10 (w:v) potassium phosphate buffer (50 mM, pH: 7.4) by the use of a dounce homogenizer. Then 0.5 ml of homogenate was mixed with 3 ml of 1% phosphoric acid and 1 ml of 0.67% TBA was added. Tubes were placed into boiling water for 45 min. After the tubes were cooled, TBARS were extracted into n-butanol and the absorbance was read at 532 nm (=1.56 (105 M-1cm-1)). TBARS were given as nanomoles per gram of wet tissue.

Level of Glutathione

Reduced glutathione (GSH) was measured through total sulfhydryl groups using Ellman Reagent (DTNB) (DTNB: 5, 5'-dithiobis-2-nitro benzoic acid). Tissue homogenate was deproteinized with perchloric acid (5%) and neutralized using 0.7M K_3PO_4. The resulting precipitates were removed by centrifugation and the supernatants were used for GSH determination, as previously described.[21]

Statistics

The data are presented as mean±SEM. The statistical analysis of the data was carried out using SPSS 11.0 for Windows software. The distribution of the parameters was tested by Kolmogorov-Smirnov test. Since they exhibited a normal distribution the difference between the four groups was evaluated by one-way analysis of variance (ANOVA), followed by a post-hoc Tukey's test. A dependent samples t-test was used to evaluate blood pressure change in hypotension groups and a value of $p<0.05$ was considered significant.

RESULTS

Pregnant rats (PC and PH) had higher body weight compared to the virgin groups (VC and VH). There was no statistically significant difference between body weights of corresponding hypotensive (VH and PH) and normotensive (PC and VC) groups (Table 1). Although slightly lower in the pregnant groups, the MABP values measured initially did not differ between the experimental groups. When compared to initial values, MABP values after 30 minutes of hypotension were significantly lower in the hypotensive groups ($p<0.01$) (Table 2).

GSH levels:

GSH level was 4.35±0.74 mM/g wet tissue on average and significantly higher in the VC group than in the other groups ($P<0.001$). Pregnancy alone caused a reduction in the GSH levels to 2.24±0.44 mM/g wet tissue although not as much as hypotension alone. Pregnant animals had lower GSH compared to corresponding virgin animals ($p<0.001$). The lowest value was 0.64±0.21 mM/g wet tissue, in the PH group, where pregnant rats experienced hypotension ($p<0.001$) (Figure 1).

Table 1: Mean body weight of animals in virgin control (VC), virgin hypotension (VH), pregnant control (PC), and pregnant hypotension (PH) groups on the day of the experiment. Data shown are mean±SEM.

Body weight (g)	VC	VH	PC	PH
	(n=5)	(n=7)	(n=7)	(n=9)
	284±8.59	270±6.6	283.6±11.07	292±10.6

Table 1: Mean arterial blood pressure (MABP) values at the beginning (time 0) and at the 30th minute of the experiment in the virgin control (VC), virgin hypotension (VH), pregnant control (PC), and pregnant hypotension (PH) animals. Data shown are mean±SEM

MABP (mmHg)	VC	VH	PC	PH
	(n=5)	(n=7)	(n=7)	(n=9)
Time zero	80.3±2.9	89.7±5.3	74.9±4.5	73.2±2.8
30th minute	82.1±2.3	48.6±3.5*	83.4±2.9	48.4±1.7*

* Significantly different compared to initial MABP ($P<0.01$)

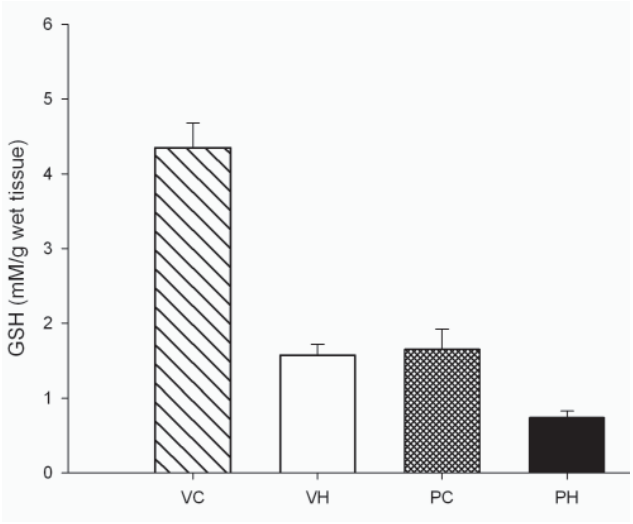

Figure 1: Reduced glutathione (GSH) levels in the virgin control (VC), virgin hypotension (VH), pregnant control (PC), and pregnant hypotension (PH) animals. Data shown are mean±SEM.

Significantly different compared to, * virgin control group, ** pregnant control group, *** virgin hypotension group ($p<0.001$)

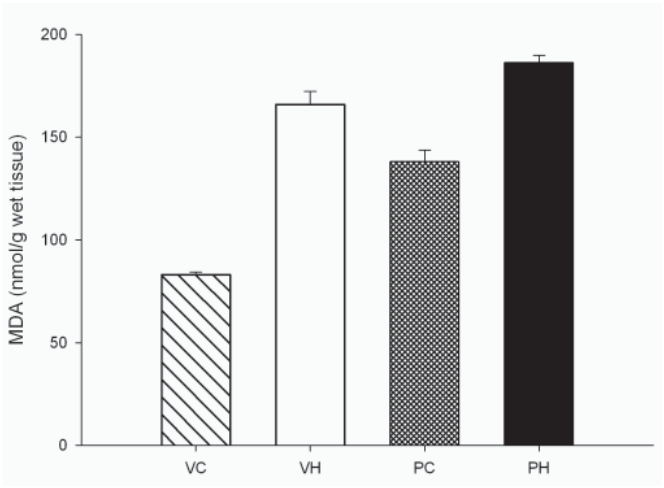

Figure 2: Malonyldialdehyde (MDA) levels in the virgin control (VC), virgin hypotension (VH), pregnant control (PC), and pregnant hypotension (PH) animals. Data shown are mean±SEM.

Significantly different compared to: * virgin control group ($p<0.001$), **pregnant control group ($p<0.001$), *** virgin hypotension group ($p<0.01$)

MDA levels:

MDA level was found to be lowest in the VC group, with a mean of 83.14 nmol/g wet tissue ($p<0.001$), and highest in the PH group, with a mean of 186.14 nmol/g wet tissue (Figure 2). Lipid peroxidation exhibited changes in all groups. The impact of hypotension or pregnancy alone was to increase MDA levels in a similar manner, although hypotension increased lipid peroxidation more. On the other hand, the MDA levels were higher in the PH group than in the VH or PC groups, and these differences were statistically significant ($p<0.01$).

DISCUSSION

In the present study we compared the effects of pregnancy and/or hypotension on liver lipid peroxidation and GSH levels in rats. The general tendency of blood pressure during normal pregnancy is to decrease until midterm and to increase steadily until the labor.[22, 23] In addition to this information, altered baroreceptor activity during pregnancy may result in hypo- or hypertension. [4, 5] It is not studied extensively, although the incidence of arterial hypotension in pregnant women, especially in primigravids, is rather high. As indicated by Eliseev, Bergman et al. stated the incidence of hypotension to be as high as 10.9%.[6, 18] It usually develops in the 13th-14th week of gestation and is revealed mostly in the 17th- 24th weeks, while near term it is established in about 8% of women.[18] It is usually overlooked because its symptoms are less severe than those of hypertension and is sometimes ignored by the patient. However, recent publications indicate that hypotensive attacks, even for short periods, may have fetal and maternal outcomes.[10, 19] A temporary reduction or paucity of blood flow to the liver and low perfusion state related to tissue hypoxia cause ischemia/reperfusion injury in the liver. This situation is closely related to the oxidant status of the liver and there is considerable interest in its prevention. [24] Hepatic sinusoidal cells are vulnerable to low perfusion states, activating a cascade of events starting with neutrophil attraction that ends up in increased reactive oxygen species (ROS) production. [25, 26] Although endogenous intracellular defense mechanisms of hepatocytes such as superoxide dismutase, catalase, and GSH try to unarm the ROS, their effects are quickly overcome when the amount of ROS produced increases. [27] Any hormonal imbalance results in altered liver oxidant status. Pregnancy itself associated with or without hypoxia causes increased production of ROS and decreased liver GSH, [28, 29] despite high levels of estrogen, which acts as an antioxidant, antiapoptotic, and free radical scavenger in hepatocytes.[30, 31] Our results on the antioxidant status of the liver also support the findings in the literature. GSH was significantly lower in pregnant animals and lowered even further in pregnant animals experiencing transient hypotension. The data showing effects of hypotension on liver morphology indicate that the increased apoptosis in these animals might be initiated by the disturbed oxidant balance of the liver.[32] Glutathione peroxide uses GSH as its cofactor to convert lipid peroxides to less harmless hydroxylated fatty acids, water, and glutathione disulfide. This means that, if the GSH level is low, lipid peroxides increase in the tissues.[33] MDA makes up about 70% of the lipid peroxidation end products, which are more than 200 in number. [34] In accordance with this fact and other data, MDA levels were found to be significantly increased in animals experiencing pregnancy and/or hypotension. The impact of hypotension on lipid peroxidation seems to be greater than that of pregnancy alone, since hypotensive groups (VH and PH) exhibit significantly higher MDA levels compared to control groups (PC and VC). The PH group exhibited the highest level of lipid peroxidation among all the groups, indicating that hypotension during pregnancy increases lipid peroxidation, which cannot be covered by the na-

tural and pregnancy related antioxidants. We are not aware of any other reports on the effects of pregnancy and hypotensive periods on liver oxidant balance that allow a direct comparison, but it is likely that oxidative stress observed in this study is a part of multi-factorial and interdependent factors underlying dysfunction in liver tissue and complicate pregnancy or affect fetal and maternal outcome.

CONCLUSION

Pregnant females have increased oxidative stress in the liver, as demonstrated by increased lipid peroxidation and decreased GSH concentration, which is exacerbated by hypotensive attacks. These results will be more significant if they are supported by morphological data and late follow-up of liver functions. This study may also have implications for the designing of antioxidant base therapy to prevent liver damage due to hypotension during pregnancy.

Last but not least, for the sake of the future child, it should be kept in mind that changes in maternal hemodynamics have effects not only on maternal tissues but also on fetal tissues directly or indirectly.

Correspondence Addres
Bilge PEHLİVANOĞLU
Hacettepe Üniversitesi Tıp Fakültesi Fizyoloji ABD
Ankara, Türkiye
Tel: 0312 305 15 59
pbilge@hacettepe.edu.tr

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