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Review

Supplementation of maternal omega-3 fatty acids to pregnancy induced hypertension Wistar rats improves IL10 and VEGF levels

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ABSTRACT

Objectives: Our recent study demonstrates the beneficial effect of a combined supplementation of vitamin B_{12}, folic acid, and docosahexaenoic acid in reducing the severity of pregnancy induced hypertension (PIH). It is also known to be associated with angiogenic imbalance and inflammation. The current study examines whether the individual/combined supplementation of folic acid, vitamin B_{12} and omega-3 fatty acid during pregnancy can ameliorate the inflammatory markers and restore the angiogenic balance in a rat model of PIH.

Materials and methods: There were total of six groups, control and five treatment groups: PIH Induced; PIH+vitamin B_{12}; PIH+folic acid; PIH+Omega-3 fatty acids and PIH+combined micronutrient supplementation (vitamin B_{12}+folic acid+omega-3 fatty acids). Hypertension during pregnancy was induced using L- Nitroarginine methylester (L-NAME; 50 mg/kg body weight/day). Dams were dissected at d20 of gestation and placental tissues were collected for further analysis.

Results: Animals from the PIH induced group demonstrated lower ($p < 0.01$ for both) IL-10 and VEGF levels as compared to control. However, PIH induction did not alter the protein levels of eNOS, IL-6, Flt and mRNA levels of VEGF and VEGFR-1/ Flt-1. Individual micronutrient supplementation of vitamin B_{12} and folate did not offer benefit. In contrast individual omega-3 fatty acid as well as combined micronutrient supplementation showed IL-10 and VEGF levels comparable to that of control.

Conclusion: Omega 3 fatty acid supplementation plays a key role in reducing inflammation in pregnancy induced hypertension.

1. Introduction

Pre-eclampsia (PE) is a hypertensive multisystem disorder resulting in maternal and fetal mortality and morbidity [1]. The pathophysiology of PE is not clearly understood, although it is believed to be of placental origin [2–4]. It is known that the placental function is dependent on a proper vascular network [5]. Over the last one decade several reviews have highlighted the association between altered placental vascular development and PE [6–9]. Vascular endothelial growth factor (VEGF) plays a key role in vasculogenesis and angiogenesis both of which are important in the development of the placenta [10,11].

Several studies by us [12–14] and others [15,16] have reported altered levels of VEGF and its receptors in PE which may disrupt angiogenesis, leading to placental insufficiency and endothelial dysfunction. Studies also suggest that PE may be associated with inflammation, oxidative stress and angiogenic imbalance [17–21]. It has been reviewed that both oxidative stress and inflammatory processes are interlinked and both may be a cause and consequence of the cellular pathology [22].

Studies indicate that the imbalance between pro- and anti-inflammatory cytokines play a role in the pathogenesis of PE [23–28]. Further it is also reported that the increased levels of pro-inflammatory cytokines may trigger the maternal endothelial cell dysfunction seen in PE [29]. Omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to have anti-inflammatory effects [30].

A series of our studies both in women with preeclampsia and in preterm pregnancies have shown that altered folate, vitamin B_{12} levels and reduced DHA levels leads to increased homocysteine and oxidative stress [18–21,31,32]. In addition we also report

Abbreviations: DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; eNOS, Endothelial nitric oxide synthase; IL-10, Interleukin-10; IL-6, Interleukin-6; L-NAME, L- Nitroarginine methylester; PE, Pre-eclampsia; PIH, Pregnancy Induced Hypertension; VEGF, Vascular endothelial growth factor

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altered maternal proportions of long chain polyunsaturated fatty acids and their transport leading to disturbed fetal stores in women with preeclampsia [33]. Further, our animal studies have shown that vitamin B_{12} and omega-3 fatty acids together regulate lipid metabolism [34,35] and affect placental [36] and liver fatty acid desaturases and transport proteins [37]. Recently we have reported increased oxidative stress and lower levels of placental DHA in a rat model of pregnancy induced hypertension [38].

Studies examining the effect of individual supplementation of omega-3 fatty acids, folic acid and vitamin B_{12} in reducing the risk of PE are few and these findings are inconsistent. Further most of these studies have examined the effect of these supplements on birth outcome or associated them with the risk of PE [39–42]. A review by Jones et al., suggests that maternal dietary ω-3 PUFA supplementation limits placental inflammation and oxidative stress [43].

Although earlier studies have reported that micronutrient deficiencies or supplementation can modulate immune and inflammatory responses [44–46] there are no reports examining such effects in PE. Similarly no information is available regarding the effect of such supplementation on angiogenic markers inspite of the fact that inflammation and angiogenesis have been extensively studied in relation to PE.

We have extensively demonstrated that micronutrients (vitamin B_{12}, folic acid) and omega-3 fatty acids such as DHA are interlinked in the one carbon cycle [47–49]. We hypothesize that combined supplementation of micronutrients (folate and vitamin B_{12}) and omega-3 fatty acids may be have a beneficial effect on the levels of inflammatory cytokines (Interleukin-IL-6 and IL-10), angiogenic markers (VEGF, VEGFR-1/Flt-1) and endothelial nitric oxide synthase (eNOS).

The current study examines the effect of folic acid, vitamin B_{12} and omega-3 fatty acids given individually or in combination on cytokines, angiogenic markers and eNOS levels in the placenta using a rat model of PIH.

2. Materials and methods

This study was carried out at the animal house facility of our institute and was in accordance with the CPCSEA guidelines (Committee for the purpose of control and supervision of experimental animals) Govt of India. The study was initiated with prior approval from the animal ethical committee of Bharati Vidyapeeth.

The current study used the L-NAME induced rat model of pregnancy induced hypertension which has been recently reported by us [38].

2.1. Animals, Breeding and Induction of L-NAME

Pregnant rats were randomly assigned to either the control or the five dietary groups. These six dietary groups ($n=8$ per group) as follows: Control; PIH (Pregnancy Induced Hypertension); PIH+Vitamin B_{12} supplemented group (PIH+B_{12}); PIH+Folate supplemented group (PIH +F); PIH+Omega-3fatty acid supplemented group (PIH+O) and PIH+Vitamin B_{12}+Folate+Omega-3 fatty acid supplemented group (PIH+B_{12}+F+O) and have been shown in study design (Fig. 1). This protocol has also been recently reported by us [38].

L-NAME administration of a dose of 50 mg/kg body weight/day was used to induce hypertension in the pregnant rat. This administration of L-NAME was done by oral gavage from d14 to d19 of gestation. It induced maternal hypertension and has been reported earlier [38].The dams were dissected by C section on d20 of gestation and placentas were collected.

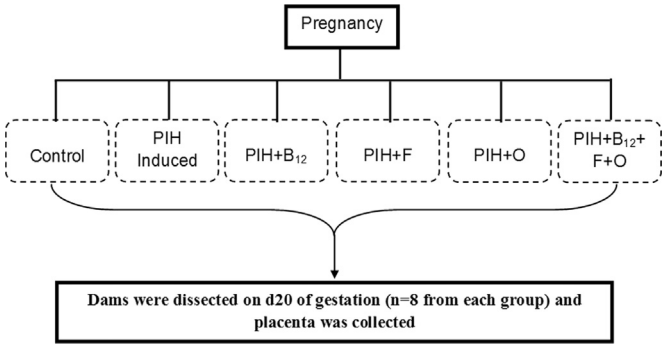

Fig. 1. Study Design Dietary Groups: Control; PIH: PIH Induced; PIH+vitamin B_{12}: PIH Induced+vitamin B_{12} supplementation; PIH+F: PIH Induced+folate supplementation; PIH+O: PIH Induced+omega 3 fatty acid supplementation; PIH+B_{12}+F+O: PIH Induced+vitamin B_{12}+folate+omega 3 fatty acid supplementation.

2.2. Diet preparation

The protocol for preparation of control and treatment diets (Table 1) in accordance with the AIN-93 guidelines [50] for purified diets for laboratory rodents The treatment diets were prepared using vitamin-free casein. Table 1 gives the composition of diets in each group and has been recently reported by us [38]. Fish oil capsules (MaxEPA, Merck Darmstadt, Germany) which contained both DHA (120 mg) and EPA (180 mg) were crushed and added to the AIN 93 dietary ingredients. The folic acid content in PIH+F and PIH+B_{12}+F+O groups was 8 mg per Kg diet; while 50 μg vitamin B_{12} per kg diet was added in the groups PIH+B_{12} and PIH+B_{12}+F+O (Table 1).

2.3. Preparation of tissue lysates and estimation of total protein

Whole placental tissue was taken and homogenized to prepare tissue lysate by a method reported by us previously [51]. Lowry method was used to estimate the total protein content [52].

2.4. Placental endothelial nitric oxide syntahse (eNOS), interleukin-6 (IL-6), interleukin-10 (IL-10), vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1) levels

Placental eNOS (USCN Life Science Inc. SEA868Ra), IL-6 (CUSABIO CSB-E04640r), IL-10 (CUSABIO CSB-E04595r), VEGF (Abcam ab100787) and VEGFR-1/Flt-1 (CUSABIO CSB-E07350r) levels were analyzed using the commercially available rat ELISA kits. The absorbance was measured spectrophotometrically at a wavelength of 450 nm. The protein levels were expressed as pg/mg protein.

2.5. RNA isolation and cDNA synthesis

Total RNA was isolated from placenta tissue using Trizol reagent (Invitrogen) and was quantified using the Biophotometer (Eppendorf, Germany). The high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) was used for reverse transcription (RT) of total RNA to single-stranded cDNA.

2.6. Analysis of mRNA levels of VEGF and VEGFR-1/Flt-1

Real-time quantitative PCR for the VEGF and VEGFR-1/Flt-1 genes was performed using the Applied Biosystems 7500 Standard system. cDNA equivalent to 100 ng total RNA was used for Real-time PCR using the TaqMan Universal PCR Master Mix procured from Applied Biosystems, USA and has been previously reported