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Effect of nebivolol treatment during pregnancy on the genital circulation, fetal growth and postnatal development in the Wistar rat

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ABSTRACT

The aim of study was to evaluate the effects of nebivolol, a cardioselective beta-1 adrenergic receptor blocker of the third generation with vasodilatory properties, *vs.* bisoprolol on the genital circulation, uterine vasculature, fetal growth and postnatal development in pregnant Wistar rats. Non invasive measurements of systolic and diastolic blood pressure (SBP and DBP) and heart rate (HR), and invasive measurement of genital blood flow (GBF) were taken in pregnant rats, by tail cuff and transonic probe methods respectively, after an oral treatment by gastric gavage with nebivolol (8 mg/kg/day) or bisoprolol (10 mg/kg/day) from day 11 to day 18 of pregnancy. Other morphometrical and histological measurements were performed on the ovarian and uterine arteries to evaluate the effect of nebivolol on the uterine vasculature. Furthermore, postnatal mortality and pup growth were recorded. The data demonstrated that nebivolol (compared with bisoprolol) induced a significant decrease in SBP, HR and GBF while DBP remained unchanged. Moreover, nebivolol increased the diameter and the length of ovarian and uterine arteries and the number of uterine artery segmental branches. The results also showed that the body weight gain of newborns in the nebivolol group was significantly lower *vs.* bisoprolol and *vs.* control with a higher mortality rate. The nebivolol action is not only limited to its favorable hemodynamic effects represented by a decrease in blood pressure, but it also produces adverse effects on fetal growth and postnatal development that may limit its therapeutic use in females during pregnancy.

1. Introduction

There are increasing interests in the use of beta adrenergic receptor blocker drugs as first-line treatment of all types of arterial hypertension including gestational hypertension (Magee et al., 2000; Mancia et al., 2013). In general, the most common beta adrenergic receptor blockers used during pregnancy are those of the first or second generation such as labetalol and bisoprolol, respectively. Despite the beneficial antihypertensive effect of these drugs which is mainly represented by the reduction in high blood pressure to less than 160/100 mmHg (Thuillez, 2010) by blocking the effect of the sympathetic nervous system on the heart (Magee et al., 1999), their adverse effects on the uterine blood flow and their tendency to a high incidence of fetal hypotrophy (Von Dadelszen et al., 2000) limit their clinical use and their prescription requires a great caution and carefulness. Recent data from experimental studies and clinical trials meets the need to assess the effectiveness of a new beta adrenergic receptor blocker drug for use during pregnancy, not only in terms of its ability to lower the blood pressure level but also for its ability to protect the function of the vital organs and particularly the fetal growth.

Nebivolol, which is a cardioselective beta-1 adrenergic receptor blocker of the third generation with vasodilatory properties, may be the most effective drug for treatment of gestational hypertension. Indeed, this drug is currently used in the treatment of arterial hypertension (McNeely and Goa, 1999) because it is characterized by its ability to lower the blood pressure attributed to its vasodilatory properties mediated by the L-arginine/NO pathway (Ritter, 2001; Ignarro et al., 2002; De Nigris et al., 2008a). All the recent studies have shown that nebivolol possesses endothelio-, nephro- and cardio-protective effects (Sorrentino et al., 2011; De Nigris et al., 2008b; Georgescu et al., 2005; Tzemos et al., 2001). Taken together, these studies lead to the hypothesis that nebivolol may also have beneficial effects on the genital system and fetal growth during pregnancy. As the ability of nebivolol to improve the uterine perfusion and protect fetal growth in Wistar rats were

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not evaluated to date, we investigated the effects of nebivolol treatment on the genital circulation, uterine vasculature, fetal growth at late gestational life and postnatal development in normotensive Wistar rats. Furthermore, the second objective of this study was to compare the effects of nebivolol *vs*. bisoprolol, which is a cardioselective beta-1 adrenergic receptor blocker of second generation but without vasodilatory properties.

2. Materials and methods

2.1. Animals

Experiments were performed in 10–12 week-old pregnant or non-pregnant normotensive Wistar rats weighing 230 ± 12 g, purchased from Janvier labs (Le Genest St Isle, France). The mating for the rats (for pregnant groups) took place at the breeding center. After arrival to the laboratory animal housing, all animals were raised in a temperature-controlled environment (20–24 °C) with free access to food and water ad libitum. The experimental study was performed according to the standard ethical guidelines for laboratory animals and was accredited by the local ethical committee of Pays de la Loire (Accreditation number study: CEEA 2012.128).

A total of 200 normotensive Wistar rats were divided into six groups (Fig. 1). The 1st group (G_1) composed of pregnant rats, served as pregnant control group, received no treatment and was given only distilled water (n=47); the 2nd group (G_2) constituted of pregnant rats (n = 48) was orally treated with nebivolol (8 mg/kg/day) by gastric gavage for 8 days, from day 11 to day 18 of pregnancy; the 3rd group (G_3) composed of pregnant rats (n = 30) was orally treated with bisoprolol (10 mg/kg/day) by gastric gavage for 8 days, from day 11 to day 18 of pregnancy; the 4th group (G_4) constituted of non-pregnant rats served as non-pregnant control group and received only distilled water (n = 25); the 5th group (G_5) made up of non-pregnant rats (n = 25) was orally treated with nebivolol (at exactly the same dose used in pregnant rats, for the same period and by the same way of administration); the 6th group (G_6) was composed of non-pregnant rats (n = 25) orally treated with bisoprolol (identically to the treatment in pregnant rats).

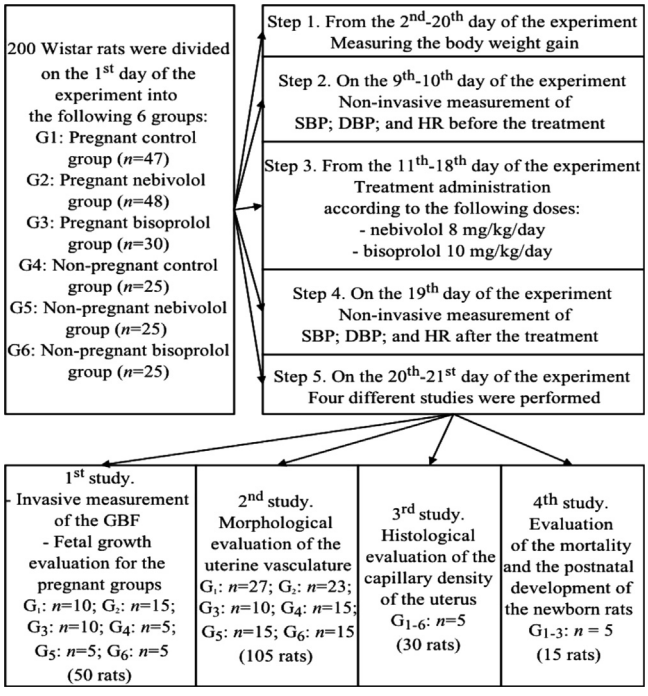

Fig. 1. Experimental flowchart of the key steps describing the main parts of the protocol. SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; HR=Heart Rate; GBF=Genital Blood Flow. n = number of rats in each tested group.

2.2. Drugs used

Many drugs were used in the study such as: pentobarbital sodium (pentobarbital sodique, Ceva, France), heparin (héparine Choay, France), white latex (Latex-Néoprène, type 601, Société Safic-Alcan, France), nebivolol (nébivolol sandoz 5 mg, Salutas Pharma GmbH, Germany) and fumarate of bisoprolol (bisoprolol sandoz 2.5 mg, Salutas Pharma GmbH, Germany).

2.3. Experimental procedure

The experimental procedure was carried out for a period equal to the period of pregnancy i.e. for 21 days, according to the experimental flow chart in Fig. 1. Four different evaluations were performed in this study: hemodynamic, macroscopic and histological evaluation in addition to assessing the mortality and the newborn postnatal development. Before any evaluation, animals were treated by gastric gavage with water, nebivolol (8 mg/kg/day) or with bisoprolol (10 mg/kg/day) for 8 days from day 11 to day 18 of pregnancy. The body weight gain of all experimental rats was recorded daily to adapt the doses of administered drugs and to evaluate the impact of nebivolol *vs*. bisoprolol treatment on the body weight gain during the pregnancy

2.3.1. Hemodynamic evaluation

The hemodynamic effects of nebivolol or bisoprolol treatment were evaluated on the one hand by measuring the systolic and diastolic blood pressure (SBP and DBP) and heart rate (HR) of pregnant (or non-pregnant) treated (or non-treated) rats, and on the other hand by measuring the genital blood flow (GBF).

2.3.1.1. Measurement of SBP, DBP and HR. SBP, DBP and HR were measured non-invasively in conscious rats by Coda Mouse & Rat Tail-Cuff Blood Pressure System (Kent Scientific CO, USA). The measurements were performed twice; the first time was on the 9th day of pregnancy i.e. before nebivolol or bisoprolol treatment, while the second time was on the 19th day of pregnancy i.e. after administration of treatment. Rats were placed into restraint tubes over a warming plate, to keep the body temperature at around 37 °C during the measurement, and the blood pressure tail cuff was attached. After a period of acclimation, a total of 30 cycles per animal was decided on to optimize the number of measurements, according to literature (Johns et al., 1996; Hosoda et al., 2007). The first 10 cycles were not used in the final analysis. The remaining 20 cycles were evaluated according to the manufacturer's recommendations. Five-second intervals separated each of the 30 measurement cycles, with a cumulative measurement time of approximately 15 min for each rat.

2.3.1.2. Measurement of Genital Blood Flow (GBF). Genital blood flow was evaluated, in pregnant (or non-pregnant) treated (or non-treated) groups (Fig. 1), on the 20th day of the experiment in internal iliac and uterine arteries. GBF was measured invasively using a transonic flow probe (Transonic system, ADInstruments®). Determinations of GBF were performed under sodium pentobarbital (60 mg/kg i.p.) anesthesia and a single dose of 5000 IU of intra-peritoneal heparin. The rat was placed horizontally in dorsal position over a warming plate to maintain the body temperature. The abdominal cavity was opened with a midline 3 cm-long laparotomy. After the intestines were slightly moved, the left internal iliac and uterine arteries were carefully dissected (on a length of about 2 cm). A 2 mm ultrasonic transit flow probe was