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THE ROLE OF CALCIUM ANTAGONIST ISRADIPINE ON THE PREGNANCY AND OFFSPRING OF RATS

(Influência do antagonista do cálcio isradipina na gestação e nos filhotes de ratos)

VILELA-GOULART, M.G.[1]; BASTOS-RAMOS, W.P.[2]; MANCINI, M.N.G.[2]

[1]Departamento de Farmacologia, Anestesiologia e Terapêutica Medicamentosa, Faculdade de Odontologia, UNICAMP, Piracicaba, SP.;
[2]Departamento de Biociências e Diagnóstico Bucal, Faculdade de Odontologia, UNESP, S. José dos Campos, SP, Brasil.

ABSTRACT – Calcium blockers are used in cardiovascular diseases, usually in long term treatments and sometimes in pregnant women. Isradipine is an important antihypertensive drug, considered to be safe in pregnancy. In this study, the effects of isradipine were evaluated regarding to the uterine implantation, fetal reabsorption, plasmatic levels of calcium, phosphate and total protein of mother and offspring. Thirty-two female and 12 male quality-controlled Wistar rats were used. The drug was administered in drinking water for 56 days: 35 days before mating and 21 days along the pregnancy. In one group, caesarean surgery was performed on the 20th//day and in the other, isradipine treatment continued for the naturally born rats, which were observed along 30 days. During the caesarean, blood samples of mothers and newborns were taken and plasmatic / levels of calcium, phosphate and total proteins determined. To observe the drug influence on the bones, femur mineralization of mothers was evaluated. Results showed that isradipine stimulated uterine implantation; however, it increased the fetal reabsorption. No teratogenic effect was observed but newborns displayed a lower body weight. Plasmatic levels of calcium, phosphate and total proteins were not influenced by the drug. Dental eruption was not disturbed in the offspring born from the treated mothers.

Key words: isradipine, calcium channel blockers, calcium antagonists and pregnancy.

RESUMO – Os bloqueadores de cálcio são utilizados em doenças cardiovasculares, usualmente em tratamentos de longa duração e ocasionalmente em mulheres grávidas. A isradipina é uma importante droga antihipertensiva, considerada segura na gravidez e sem efeitos teratogênicos. Neste estudo foram estudados, em ratos Wistar, os efeitos da isradipina sobre a implantação uterina, reabsorção fetal e sobre os níveis plasmáticos de cálcio, fosfato e proteínas totais. Foram utilizados 32 fêmeas e 12 machos com qualidade controlada. A droga foi administrada na água de beber durante 56 dias, sendo 35 antes do acasalamento e 21 durante a prenhêz. Em um grupo, foi realizada operação cesareana ao 20º / dia de gestação e em outro, os ratos nasceram de parto natural e foram observados durante 30 dias, com manutenção da administração de isradipina. Por ocasião da cesareana foram obtidas amostras de sangue de mães e filhotes, para determinação de cálcio, fosfato e proteínas totais plasmáticas. Para observar a influência da droga no / tratamento prolongado sobre os ossos, a mineralização do fêmur das mães foi avaliada. Os resultados mostraram que a isradipina estimulou a implantação uterina porém aumentou a reabsorção fetal. Nenhum efeito teratogênico foi observado, entretanto ocorreu diminuição significativa do peso dos filhotes. Os níveis plasmãticos de cálcio, fosfato e proteínas totais não foram alterados pela droga. O período da erupção dental não foi alterado nos filhotes nascidos de mães tratadas.

Palavras chaves: isradipine, bloqueadores, antagonistas Ca, gestação.

Correspondence should be addressed to: Wilma P.Bastos-Ramos. Rua Albuquerque Lins, 1203/102, S. Paulo, SP, Brasil. CEP 01230-001. Tel. (011) 3822-2786. Fax (011) 3822-2787. E-mail: wilmabastos@uol.com.br.

Introduction

Calcium channel blockers are important therapeutic agents, used as vasodilator and antiarrhythmic drugs, usually in long term treatments. They act by inhibiting the slow voltage-dependent of L-type (NOWICKY *et al.,* 1985) in vascular smooth muscle, in a lower concentration than those required to interfere with the release of intracellular Ca^{2+} or to block receptor-operated channels, then, preventing ionized calcium from entering the cells and thus influencing calcium-dependent processes (FLECKESTEIN *et al.,* 1967, 1969; KERINS *et al.*, 2003). All Ca^{2+} channel blockers relax the arterial smooth muscle but have little effect on most venous beds. They act on cardiac cells by disturbing excitation-contraction coupling by a different mechanism, since in the heart, in the portion of the two inward currents, it is carried by Na^{+} through the fast channel in addition to that carried by Ca^{2+} through the slow channel. Although all blocking channel drugs share similar pharmacological properties, some are somewhat more specific as antihypertensive and others as antiarrhythmic. Verapamil was the first channel blocker clinically used and it is important nowadays as antiarrhythmic. Isradipine is indicated in hypertension, a condition in which it is considered by some authors as a first choice (HAMILTON, 1987; SAUTER and RUDIN, 1989, 1990; SOLAND, 1990; LUSCHER and WAEBER, 1991; SCHACHTER, 1991). An extensive revision on the pharmacology and clinical research of calcium antagonists was published by VANHOUTTE *et al.*, (1988). These drugs, indicated in chronic diseases, are often used in pregnant women, in which they are considered to be safe. Isradipine pharmacokinetics allows a continuous therapeutic activity for 24 hours in a single administration, with excellent tolerability and low side-effects (HANSON and DAHLOF, 1987; SOLAND, 1990). No teratogenic effect has been described for the use of this drugs in rats a significant reduction in body weight and in the number of offspring born from verapamil treated mothers (VILELA-GOULART *et al.,* 1999a). An interest arises whether calcium antagonists influence the calcium balance in bone or not. There is a great medical interest referring to the possibility of such drugs to be a risk factor for the development or aggravation of osteoporosis. However, a few studies refer to its influence on bone tissue. Receptors of the L type channels in osteoblasts were proposed by GRYGORCZYK *et al.*, (1989) and calcium receptors and/or calcium channels were suggested to occur in the osteoclasts (DATTA *et al.,*1990; RITCHIE *et al.,* 1994). Other authors referred to the subject (HERMANN-ERLEE *et al.*, 1977; LERNER and GUSTAFSON, 1982; SIMEKOVA *et al.,* 1987; CHAGNAC *et al.*, 1989; ZAIDI *et al.,* 1989, 1990; DURRIEZ *et al.,* 1990; RITCHIE *et al.,* 1994). It was reported by SAMNEGARD and SJÖDEN (1992) that verapamil increases bone volume and osteopenia in female rats but has the opposite effect in male rats. It was described by VILELA-GOULART *et al.,* (1999 b) that verapamil increases mothers´ femoral bone mineralization in female rats and induced a significant delay in their newborns´ dental eruption. In the present paper, it was intended to study the effects on pregnancy and offspring of a calcium blocker isradipine, of the dihydropiridine group, in order to compare results with those obtained with verapamil, of the phenylalkylamine group.

Material and Methods

To the experiments, 44 Wistar rats (*Rattus norvegicus*, var. albinus) in controlled good health conditions were used. Thirty-two were virgin females, aged 90-100 days at the onset of the experiments and 12 were reproductive male rats. The female were divided in two groups: treated with isradipine and not treated, running in parallel (control). Isradipine was administered during 35 days before mating and 21 days during pregnancy. The drug was added to the drinking water in doses of 1.0 mg/rat/day. All the animals were weekly weighted and behavior observed. In a group of rats the administration of the drug continued for 30 days after the natural delivery. After 7 weeks of treatment and the estrous phase

determined, female and male rats were put together overnight for mating; diagnosis of pregnancy was determined by presence of spermatozoa in the vaginal secretion. At the 20th day of pregnancy, the uterus of mothers were exposed by caesarean surgery under anesthesia (ketamine, 100 mg/Kg and xylazine, 3.0 mg/Kg) and observed both the number of alive newborn and dead fetuses, and the embryonic reabsorption and uterine embryonic implantation (= number of embryos + number of embryonic reabsorption). The ovary was removed and the number of corresponding ovules released during fertilization was counted. The newborn rats were cleaned, weighed and somatic external characteristics examined under 3 X magnification. Ears and eyes implantation, palate, labial cleft, anterior and posterior limbs and tail were examined. Blood samples were taken in heparin from mothers and newborn. Mother's femoral bone was removed and freed of adherent soft tissues; length, fresh and dry weight measured. Biochemical determination of calcium, inorganic phosphate and total proteins were carried out in plasma obtained from mothers and new-born, using a Beckman-Du 600 Spectrophotometer. Plasmatic calcium was determined by complexiметric method of O-cresolphtalein-complexon using Merck Reagents. To plasmatic inorganic phosphate determination, FISKE and SUBAROW (1925) method was used. Total proteins were determined by Biuret method, using a "Bioclin" kit reagents. Dental eruption in young was observed by thickening of gingival epithelium in "U" shape. Significance of the difference between groups was assessed by Student "t" test.

Results

1 - *Fetal uterine implantation and fetal reabsorption*. The mean number of fetal uterine implantation in control mothers was of 11.51(±1.19). In treated mothers this mean significantly raised to 13.3(±1.3). This uterine implantation was correspondent to the number of ovarian luteinizing bodies. No uterine reabsorption was observed in the control mothers but in the treated ones, fetal reabsorption of 27 % was observed.

2 - *Alive offspring and somatic characteristics*. In control rats, alive newborn corresponded to 100% of uterine implantation. However, in isradipine treated group, the alive offsprings was significantly reduced in 27 %. The body weight of offspring born from the treated mothers was at birth, 12 % lesser than in the control and 30 days after the birth it continued to be significantly lower as compared to control. No macroscopic malformations in control and treated newborn were observed and no sign of intoxication or behavioral disturbance occurred in the isradipine treated mothers as well as in their newborns up to 30 days after birth (TABLES 1 and 2).

TABLE 1 – UTERINE IMPLANTATION, FETAL REABSORPTION AND ALIVE OFFSPRING (MEAN VALUES) OF CONTROL AND ISRADIPINE (1.0MG/DAY/RAT) TREATED RATS FOR 56 DAYS.

	Control (n=8)	Isradipine (n=8)
Uterine implantation	11.5 (± 1.19)	13.0 (± 1.30)
Fetal reabsorption	Zero	3.5 (± 3.02)
Alive offspring	11.5 (± 1.19)	9.5 (± 3.96)

TABLE 2 – BODY WEIGHT (G) OF ALIVE NEWBORN AT BIRTH (CESAREAN BORN-ZERO DAYS OF LIFE) AND AT THE 30TH DAY OF LIFE (NATURAL DELIVERANCE). CONTROL AND ISRADIPINE (1.0 MG/DAY/RAT) TREATED MOTHERS.

	Control(n=8)	Isradipine (n=8)
At birth (zero days)	6.73 (± 0.44)	5.93 (± 0.74)
30th days of life	75.67 (± 6.33)	60.71 (± 3.06)

3 - *Femoral bone length, fresh and dry weights*. The length, fresh and dry weight of femoral bone was not significantly influenced by isradipine treatment (TABLE 3).

4 - *Dental eruption*. Dental eruption period did not differ between control and isradipine treated animals, occurring at the 9th day of birth.

5 - *Plasmatic calcium, phosphate and proteins*. Plasmatic calcium, inorganic phosphate and total protein were not changed in mothers treated with isradipine. Regarding to the newborn rats, the values of plasmatic inorganic phosphate was 102 % higher at birth as compared to their respective mothers and returned to normal values at the 30th day of age (TABLE 4).

TABLE 3 – LENGTH (MM), FRESH AND DRY FEMORAL WEIGHTS (MG) OF MOTHER RATS: CONTROL AND TREATED WITH ISRADIPINE (1.0 MG/DAY/RAT) FOR 56 DAYS (A) AND 86 DAYS (B).

	Control (n=8)	Isradipine (n= 8)
(A)		
Length	26.00 (± 4.07)	26.75 (± 3.65)
Fresh weight	680.62 (± 55.19)	691.87 (± 55.54)
Dry weight	370.00 (± 37.45)	376.25 (± 40.68)
(B)		
Length	30.37 (± 5.84)	28.37 (± 6.81)
Fresh weight	718.12 (±107.96)	724.37 (± 87.80)
Dry weight	347.50 (± 42.67)	350.00 (± 46.59)

TABLE 4 – PLASMATIC VALUES (MEAN) OF CALCIUM, INORGANIC PHOSPHATE (MG/100ML) AND TOTAL PROTEINS (G/100 ML) OF MOTHER RATS (A) AND OFFSPRING (B) OF CONTROL AND ISRADIPINE (1.0MG/DAY/RAT) TREATED FOR 56 (CESAREAN) AND 84 DAYS (NATURAL BIRTH).

	56 days (n=8)	(cesarean)	84 days (n=8)	(natural birth)
	Control	Isradipine	Control	Isradipine
(A)				
Calcium	6.04 (± 1.28)	5.93 (± 1.16)	5.04 (± 0.88)	4.91 (± 0.70)
Phosphate	3.81 (± 1.16)	3.75 (± 2.59)	3.13 (± 0.41)	3.69 (± 2.47)
Total protein	6.54 (± 0.88)	6.17 (± 2.01)	5.82 (± 0.75)	5.60 (± 0.70)
(B)				
Calcium	5.08 (±1.08)	6.60 (± 2.50)	4.83 (± 0.44)	4.52 (± 0.90)
Phosphate	7.68 (± 2.36)	7.72 (± 3.19)	3.07 (± 0.49)	4.00 (± 0.51)
Total protein	5.69 (± 1.00)	4.96 (± 2.18)	5.51 (± 0.73)	5.03 (± 0.63)

Discussion

There is a great medical, scientific and social interest in the teratogenic effect of drugs given to mothers during pregnancy. Regarding to calcium channel blockers, usually given in long term treatments, an adding interest refers to the possibility of such drugs to be a risk factor for the development or aggravation of osteoporosis, a crippling bone disease that poses as a major public health problem. Although studies about a role of calcium blocking channels in bone cells and tissue have been carried out (HERMANN-ERLEE *et al.*, 1977; LERNER and GUSTAFSON, 1982; SIMEKOVA *et al.,*1987; CHAGNAC *et al.,* 1989; ZAIDI *et al.,* 1989, 1990; DURRIEZ *et al.,* 1990; DATTA *et al.,* 1990; SAMNEGARD and SJÖDEN, 1992; RITCHIE *et al.,* 1994;), the possible influence of these agents on the mineralization of the teeth has been described by VILELA-GOULART *et al.*, (1999 b), using verapamil. Being aware of the effects of these substances on some bone cells calcium cytosol, we decided to study the influence of isradipine, an important widely used calcium blocking agent on pregnancy, on bone mineralization, on dental eruption and the possible repercussion on offspring born from treated mothers. We previously observed (VILELA-GOULART *et al.,* 1999 b) that in pregnant rats, the femur became more densely *mineralized* in verapamil treated rats and dental germ eruption was significantly delayed. Our result could be compared with

those of FOX and DELLA-SANTINA (1989) and SAMNEGARD and SJÖDEN (1992) who reported in rats given verapamil an increase in tibial bone mineralization (as ash per volume) in male rats. However, the latter authors, studying the influence of sex, found an opposite results to female, that is, bone mineralization was significantly reduced by verapamil. They attributed such differences to a possible action of the drug in stimulating androgen secretion, absent in the female. In our experiments with verapamil it was observed a different result: the drug increased bone mineralization in the females, a result we attributed to the rats being pregnant and in this condition, there is a physiological higher absorption (BROMMAGE *et al.,* 1990) which could induce a higher mineralization. However, calcium plasmatic values were not changed by isradipine and the drug failed in increasing bone growth and mineralization, a result that coud be attributed to the doses used or to isradipine itself. Isradipine, different of what was observed with verapamil, did not retard dental eruption in newborn, suggesting no influence on the organ organization. The doses of the drug used in our experiments can be considered adequate, because during the long term treatment, i.e. 56 or 86 days, the rats seemed to thrive and increase their body weight and no disturbed behavior was observed.

Calcium-phosphate balance is essential to rat normal bone mineralization (McCOY, 1949). The dietary calcium-phosphate was adequate as shown by normal growth of control and treated rats. It is well known that the ratio of calcium to phosphorous in the diet exerts a marked effect upon growth and calcium and phosphorous content in the blood serum. No sign of rickets were observed. The daily dietary calcium phosphate was within the range proposed by McCOY (1949) to normal development of the rat. Isradipine did not influence plasmatic calcium and phosphate either in mothers or in offsprings, results quite different of those obtained with verapamil (VILELA-GOULART *et al.,* 1999a). However, in control and treated groups, phosphate was significantly higher, (about 100 %) in offspring at birth, values returning to normal, being comparable to their mothers at the 30th days of age. The increase in ovulation and embryonic uterine implantation in verapamil treated rats was a very consistent finding. However, such implantation did not correspond to the fetuses at term, since a high degree of uterine reabsorption occurred. Results are suggestive in indicating that isradipine stimulated ovarian follicles, liberating immature ovules, which were fertilized but did not succeed in giving rise to functional ovum and fetus. One can speculate whether isradipine enhanced ovulation by itself, influencing calcium-dependent mechanisms or acted, like verapamil, by indirectly stimulating luteinizing hormone or follicle stimulating hormone, which respond to ovulation and corpora lutea formation. Or the drug could act by inhibiting atresia of ovarian follicles not destined to produce functional ovum. One must consider that the present results, although consistent and significant, are not sufficient to a further discussion, but are suggestive to inspire future research. As observed with verapamil (VILELA-GOULART *et al.,* (1999a), isradipine induced significant uterine reabsorption. One can speculate that the drug acted during blastogenesis, which occur in the rat from the 1st to the 6th day, a susceptible period when toxic action of drugs leads to embryo death and fetal reabsorption. The drug did not certainly acted during organogenesis (after the 6th day), because in doing so, it could lead to fetal malformations, a response not observed with isradipine.

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