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ORIGINAL RESEARCH

Exposure of pregnant rats to shortwave electromagnetic athermal radiation does not adversely affect their fetuses

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BACKGROUND: The aim of this study was to determine whether shortwave electromagnetic radiation in pulsed mode with a frequency of 45 Hz, promotes teratogenic changes, stillbirths and changes in organ weight at birth in the fetuses of pregnant rats exposed to this radiation.

METHOD: Ten black Macole pregnant female rats were studied, 5 in a test group, subjected to electromagnetic radiation with an average power of 4.5 W for 15 minutes, on a daily basis, during the entire period of pregnancy and 5 in a control group that was not exposed to radiation. At day 21, the animals were euthanized for analysis of fetuses. To assess the thermal effect of the radiation the abdominal temperature was measured before and after application, together with prevailing ambient temperature. The fetuses were evaluated through the Galant reflex to assess the existence of stillbirths. The fetuses were removed and weighed; through a midline laparotomy their hearts, stomachs, kidneys and livers were excised and weighed, wet and dry. Twenty-nine fetuses were harvested in the control group and 59 in the test group, with no cases of stillbirth, teratogenesis or malformation of internal organs.

RESULTS: The analysis found no differences in birthweight and weight of dehydrated organs when compared to the control group.

CONCLUSION: The results in our findings suggest that exposure to shortwave therapy without significant heat buildup, does not lead to teratogenic changes and did not affect the mass and weight of dehydrated internal organs.

KEYWORDS: radio waves; pregnancy; teratogenesis; adverse effects.

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INTRODUCTION

With the introduction of short wave equipment for therapeutic use, questions were raised about its possible side effects. Its indications are varied, such as gain of tissue extensibility favoring the gain range of motion of joints, vasodilation, edema, reabsorption of hematoma and analgesia. However, in addition to the physiological effects, side effects may occur, including, embryonic development disorders in pregnant women.[1,2,3]

There are no clear published data on whether pulsed athermic shortwave therapy generates the same harmful consequences as does continuous thermal therapy.[4,5] The risks of prenatal exposure to ionizing radiation vary, depending upon the stage of fetal development.[3,6]

An embryo is most susceptible to the effects of radiation during organogenesis (2–7 weeks after conception) and in the early fetal period (8–15 weeks after conception).[4] The effects of exposure can be teratogenic, carcinogenic, or mutagenic and are directly related to the level of radiation exposure. Risk estimates for teratogenic (non-cancerous) and carcinogenic effects have been calculated in survivors of atomic bomb and nuclear fallout, as well as from early use of diagnostic imaging in pregnant women, and from animal studies.[7]

When pulsed shortwave with low average power is used, low concentrations of electromagnetic field are created. Therefore, the treatment is relatively athermal because higher frequency longer pulse is necessary to generate heat.

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This contraindication of continuous shortwave therapy must be taken into account.[1,8]

Kállen et al.[9] emphasized increased incidence of death and malformations in children of women who made use of shortwave equipment. Lermam et al.[5] made a prevalence study among 933 Israeli therapists, who became pregnant during their professional activity and indirectly received shortwave; they suggested that the short waves have potential harmful effect on pregnancy, specifically causing low fetal weight.

Larsen et al.[10] applied a questionnaire by telephone to all pregnant physiotherapists registered in Denmark who were indirectly exposed to electromagnetic waves in their workplace, during the first month of pregnancy, noting that there were no significant changes in the children born under these conditions.

Lary et al.[11] reported that the thermal application of short waves in pregnant rats was associated with teratogenic changes, low weight, size change, and fetal reabsorption or death. The application force was 55 A/m electric field strength of 300 V/m, lasting for 20–40 minutes. Edwards et al.[4] reported that heat applied directly to embryos in early stages of pregnancy, causing a temperature rise of 2 degrees or more, resulted in teratogenic effects, mainly by apoptotic cell death, particularly in the central nervous system.

Rosário[12] irradiated pregnant rats during embryonic development with a single 10 min application of a continuous focus shortwave, with a power of 60 W and found teratogenic alterations in the irradiated fetuses.

There are few reports on teratogenic alterations caused by short wave athermal applications.[2–5] However, determining what may be the ideal athermic therapy is not so simple, because the variables involved in this process are numerous: they include the size of the plates, the power of the apparatus, the method of application of the electrodes, the total time of application and the frequency of treatment. Therefore, this study aimed to verify the effect of athermic electromagnetic waves (short waves in pulsed mode) on the fetuses of pregnant rats.

METHODS

General

This experimental study followed the standards established in the CIOMS (Council for International Organization of Medical Sciences) Ethical Code for Animal Experimentation[13] and the precepts of the Brazilian College of Animal Experimentation – COBEA. The study was approved by the Ethics Committee of the Universidade São Marcos.

Sample

Ten adults female and ten adult male rats (Rattus norvegicus – Black Macole breed), were used. They were fed "ad libitum" and kept in a light-dark cycle of 12 hours at room temperature of 20 ± 2° C in individual cages.

The animals were randomly divided into two groups: the test group (n = 5), which was subjected to irradiation and the control group (n = 5) who received placebo (therapy of unit shortwave off).

Vaginal smears was performed to determine the estrous cycle of rats with a sterile pipette containing 0.9% NaCl. When in estrus, the females were placed overnight (11:00 PM–6:30 AM) for copulation one by one (one male for every female). Vaginal smears were collected and, when positive for sperm, day zero of the pregnancy was established. This procedure was adopted to ensure that short wave was applied from the first day of pregnancy. The abdomen was shaved on this day to avoid interference with the measurement of temperature.

Females of the test group were exposed daily (between 07:00 and 08:30 AM) from day 1 to 20 of the pregnancy to a shortwave electromagnetic field; radiation was turned on for 15 minutes for each rat. The control group underwent a simulation of the procedure, without suffering any radiation, and can thus be considered as the placebo group.

Equipment

The shortwave apparatus used in the study was DIATERMED II, with 12 × 17 cm plate, reference number 4022S (Carci®), operated at a frequency of 27.12 MHz, with 100% intensity peak (maximum intensity of 250 W) type electrode. The apparatus was used in pulsed fashion, with pulse duration of 400 μsec and frequency of 45 Hz for 15 minutes per day, generating an average power of 4.5 W. Thus the dosage was Schliephake I, which is accepted as athermic therapy.

Procedures

The animals were kept in cages made of non-insulating material (wood without nails), 15 cm high, 20.5 cm long and 20 cm wide; for shortwave treatment, they were placed one by one between the plates of the emission of the short wave equipment, with a distance of approximately 5 cm from each side of the animal. The temperature in the room where the shortwave applications were performed was monitored (Environment Thermometer, Western®). The animals were allowed to acclimatize to the room temperature for 10 minutes before a pre-exposure measurement of abdominal temperature was obtained (Versatile Thermometer Plus, Model TS-201, Techlini®). After the application of the therapeutic shortwave procedure, the abdominal temperature was again measured. Temperature readings were taken in triplicate and the average was recorded. The rats in the control group underwent the same procedure.

On the twentieth day of gestation, animals were anesthetized by inhalation of diethyl ether; when completely anesthetized, animals were sacrificed using a guillotine. A caesarean section was immediately performed through a midline laparotomy to remove the fetuses, which were immediately submitted to the Galant Test to evaluate the existence of stillborn; the fetuses were then weighed.

The fetuses were dissected through a midline laparotomy, and the weight of their liver, stomach, heart, left and right kidneys was determined (wet weight). The organs were then placed in crucibles and dried in a stove (Adontobrás®, digital ECB 1.2. model) at 37°C for 24 hours; after this, the organs were weighed again (dehydrated weight).

Statistical Analysis

For comparison between groups, the average of all the values recorded daily in each rat (control group, the test group and the ambient temperature) were recorded. We also calculated the mean and standard deviation for each group (exposed and non-exposed) for each subject and the environment.

Student's T test was used for all groups. A significance level of $p < 5\%$ was adopted. The Pearson correlation coefficient also was determined for the test group.

RESULTS

A total of 88 fetuses born to 10 females were included in this study, 29 fetuses in the control group and 59 in the test group. In both groups, all fetuses tested positive to the Galant Reflex, indicating the absence of stillbirths. Macroscopic evaluation of the fetuses showed no teratogenic changes, both externally and in the studied internal organs.

In every rat of the test group, a temperature increase ranging from 0.27°C to 0.52°C, was detected. The average increase in this group was 0.34°C, which proved to be significant ($p < 0.05$), as shown in Table 1. No differences occurred in the control group.

Comparison between groups revealed no significant differences between tests and controls in the weight of the fetus. Wet weight comparisons for organs are displayed in Table 2; variances were found to be equivalent; there was no significant difference between control and test fetuses for the liver. For other organs the organ weights of the irradiated fetuses was significantly smaller vs. controls, significant at the 5% level.

The weight of the dehydrated organs is shown in Table 3. No significant differences were found for any of the orgeans between test and control rats.

DISCUSSION

According to Low and Reed[14] the application of short wave in continuous or pulsed fashion has indications during pregnancy. The continuous mode is considered thermal because it generates warming, whereas the pulsed mode is considered athermal. This generates the question of what constitutes a teratogenic agent, because it may be the heat generated, the electromagnetic wave or the interaction of these two factors.

The major problem in the therapeutic application technique is to determine exactly when there is heat buildup and whether this significantly alters the local temperature and can therefore be called thermal therapy. This is because some variables are involved, and these include the average power, the feeling of the patient and the Specific Absorption Rate.

Regarding the thermal issue, i.e, application of electromagnetic waves with heat buildup, a teratogenic effect on fetuses during pregnancy has been well described. The intent of this work was to seek answers to the question of the isolated effect of electromagnetic waves in embryonic formation. Therefore, we used an average power of 4.5 W, considered athermic, with a dose in the range of Schliephake I; abdominal temperature was measured before and after application of the radiation, which showed a slight variation in surface temperature tested (+0.34°C). This is assumed to be a Schliephake dose I because only a temperature elevation is 2°C in the region exposed to the application of shortwave is considered thermal.

Bossini et al.[15] demonstrated that athermic application (mean dose 4.8 W) in a single day, for 10 minutes, at the end of the first third of pregnancy (seventh day) caused a difference body mass at birth, compared with the control group; this difference was not observed in the weight of the dehydrated bodies, which raises doubt as to the method of weighing the fetus immediately after birth, i.e the analysis of mass body of fetuses made on the wet weight. This may explain the differences in our findings, which showed no statistically significant differences between fetal body weight, no differences in dry organ weights (liver, heart, kidneys and stomach) and no teratogenic changes in fetuses of both groups.

Tofani et al.[16] evaluated the application of short wave athermic therapy (50 Hz) measuring the specific absorption rate (SAR) 0.1 m in W/cm^2 and 27.12 MHz, applied on different days of pregnancy. Three groups were established: group A was control, group B was exposed throughout pregnancy (20 days), group C exposed from day 0 to day 6 and group D exposed from day 6 to 15 of pregnancy. Through Dunnett's Multiple comparsion test, changes such as poor low weight and cranial malformation were found, thereby suggesting that the time of exposure, even at low doses, may induce deleterious effects on embryonic function.

More studies are needed to determine risk-free application dosage for human fetus because there is evidence that athermic shortwave may cause changes during the embryonic phase, even with minimal SAR, when the application time is prolonged.

CONCLUSION

According to the findings in this study, therapeutic shortwave applied in pulsed mode with reduced frequency, not accumulating heat for a period of 15 minutes a day, did not cause changes of macroscopically evident teratogenic nature; neither were changes in body mass and weight of dehydrated organs in rats detected.

Table 1 - Temperature of the pregnant rats and the ambient temperature throughout pregnancy.

	Rats Exposed to SW				Rats Not Exposed to SW		
	Pre	Post	Ambient		Pre	Post	Ambient
Rat a	36,07 ± 0,02	36,43 ± 0,02	21,47 ± 0,10	**Rat a**	36,49 ± 0,10	37,06 ± 0,05	22,68 ± 0,12
Rat b	36,38 ± 0,03	36,62 ± 0,03	21,47 ± 0,12	**Rat b**	36,73 ± 0,09	37,00 ± 0,10	23,50 ± 0,10
Rat c	36,32 ± 0,04	36,61 ± 0,04	20,06 ± 0,13	**Rat c**	36,96 ± 0,07	36,56 ± 0,07	23,5 ± 0,09
Rat d	36,26 ± 0,02	36,78 ± 0,01	19,41 ± 0,10	**Rat d**	36,55 ± 0,05	36,58 ± 0,03	21,00 ± 0,13
Rat e	36,37 ± 0,01	36,65 ± 0,02	20,06 ± 0,09	**Rat e**	36,68 ± 0,05	36,80 ± 0,04	22,67 ± 0,14
Mean ± SD	36,28 ± 0,12*	36,62 ± 0,12*	20,49 ± 0,92	**Mean ± SD**	36,68 ± 0,18	36,80 ± 0,23	22,67 ± 1,02

Paired T Test *$p < 0.05$

Table 2 - Comparison of the weight in milligrams of wet organs.

	Control Group	Experimental Group
Heart	15,92 ± 2,05	10,40 ± 3,05*
Liver	245,66 ± 5,09	235,28 ± 4,05
Stomach	20,85 ± 4,15	8,08 ± 2,17*
Right kidney	7,47 ± 1,14	4,51 ± 1,01*
Left kidney	7,04 ± 0,45	4,51 ± 0,14*

T Test *p < 0,05

Table 3 - Comparison of the weight in milligrams of dehydrated organs.

	Control Group	Experimental Group
Heart	4,44 ± 0,10	4,37 ± 0,12
Liver	97,03 ± 2,56	92,68 ± 2,15
Stomach	3,37 ± 1,56	2,93 ± 1,72
Right kidney	0,76 ± 1,12	1,29 ± 1,15
Left kidney	1,85 ± 1,35	1,66 ± 1,38

EXPOSIÇÃO DE RATAS PRENHES A ONDAS CURTAS DE RADIAÇÃO ELETROMAGNÉTICA ATÉRMICA NÃO AFETA ADVERSAMENTE OS SEUS FETOS

RESUMO

OBJETIVO: O objetivo deste estudo foi determinar se a radiação eletromagnética de ondas curtas em modo pulsado com uma frequência de 45 Hz, promove mudanças teratogênicos, natimortos, mudanças no peso do órgão ao nascimento em fetos de ratas grávidas expostas a essa radiação.

MÉTODO: Foram estudados dez ratas grávidas da raça Marcole, cinco num grupo teste, submetidas a radiação eletromagnética com uma potência média de 4,5 W, durante 15 minutos, diariamente, durante todo o período da gravidez e cinco num grupo controle, não expostas a radiação. No 21° dia, os animais foram sacrificados para análise dos fetos. Para avaliar o efeito da radiação térmica, a temperatura abdominal foi medida antes e depois da aplicação, em conjunto com a temperatura ambiente prevalente. Os fetos foram submetidos ao reflexo de Galant para avaliar a existência de natimortos. Os fetos foram removidos e pesados; através de uma laparotomia mediana seus corações, estômagos, rins e fígados foram extirpados e pesados, frescos e secos.

RESULTADOS: Vinte e nove fetos foram colhidos no grupo controle e 59 no grupo de teste, sem casos de natimortos, teratogênese ou malformação dos órgãos internos. A análise não encontrou diferenças no peso ao nascer e peso de órgãos desidratados quando comparados com o grupo controle.

CONCLUSÃO: Os resultados sugerem que a exposição a terapia de ondas curtas, sem o acúmulo significativo de calor, não levou a alterações teratogênicos e não afetou o peso dos fetos ou o peso dos órgãos internos desidratados.

UNITERMOS: ondas de rádio; a gravidez; teratogênese; efeitos adversos

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