

DENTAL GERM FORMATION AS INFLUENCED BY 5-FLUOROURACIL (5-FU) IN NEWBORN RATS

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ABSTRACT - It was studied the influence of the antimetabolic drug 5-fluorouracil (5-FU), administered to pregnant rats, on dental germ formation of newborn rats. A total of 125 female aged 90-100 days and 15 male adult rats (*Rattus rattus norvegicus*, *albinus*, Wistar) were used. The drug was injected intraperitoneally in single doses to females at the 9th, 10th, 11th, 12th and 13th days of pregnancy. The animals were divided in four groups according to the doses administered: 10, 20, 30 and 40 mg/kg. Results show that the drug induced no toxicity to mothers, other than loss of body weight with higher doses (30-40 mg/kg), but caused in the fetuses dental germ anomalies and body external malformations. The proportion of such anomalies were, besides dose dependent, influenced by the day of pregnancy when the drug was administered to the mothers: there was a greater sensitivity at the 9th day and a lesser one at the 10th day. Higher doses (30-40 mg/kg) caused frequently embryonic death or fetal uterine reabsorption. Agenesis of the dental germ was frequently found.

Key Words: antimetabolic drugs; 5-fluorouracil; dental germ; fetal malformation.

RESUMO - Foi estudada a influência da droga antimetabólica 5-fluorouracil (5-FU), administrada a ratas prenhes sobre a formação do germe dental de ratos recém nascidos. Foram utilizadas 125 fêmeas com 90-100 dias de idade e 15 machos adultos (*Rattus rattus norvegicus*, *albinus*, Wistar). A droga foi injetada por via intraperitoneal a ratas grávidas, em doses únicas, aos 9^o, 10^o, 11^o, 12^o ou 13^o dias de gestação. Os animais foram divididos em quatro subgrupos de acordo com a dose administrada: 10, 20, 30 e 40 mg/kg. Os resultados mostraram que nessas doses, o 5-FU não teve efeito tóxico nas ratas prenhes, além de perda de peso com doses mais elevadas (30-40 mg/kg), enquanto causou anomalias dentais nos embriões, bem como malformações externas. A proporção de tais anomalias foi, além de dose dependente, influenciada pelo dia de gestação no qual o antimetabólico foi administrado às mães: foi observada maior sensibilidade ao 9^o dia e menor ao 10^o dia. Doses mais elevadas (30-40 mg/kg) causaram frequentemente morte do embrião e reabsorção fetal. Agenesia do germe dentário foi frequentemente observada.

Palavras-Chave: drogas antimetabólicas; 5-fluorouracil; germe dentário; malformações fetais.

Introduction

Antimetabolic drugs are useful for the treatment of cancer due to its toxic action to highly proliferating tumor cells. The limitation of its use is that they are also toxic to cells of normal tissues in the mitotic phase, so that the proliferating normal tissue is sensitive to these drugs in proportion to its mitotic activity, what means severe unwanted side effects. Many observations have been made in drug treated cancerous pregnant women and in experimental animals given antimetabolic drugs. Results show not predictable unwanted effects on embryos or newborn. It was reported in pregnant women (STADLER and KNOWLES, 1971; BLATT *et al.*, 1980; STEPHENS *et al.*, 1980; TOBIAS and BLOOM, 1980; GILLILLAND and WEINSTEIN, 1983;) that some chemotherapeutic drugs such as adriamycin, actinomycin D and vincristin, administered before and within the first 3 months of pregnancy caused no problems to the newborn.

However, the authors refer to fetal malformations due to alkylating (cyclophosphamide, busulfan and chlorambucil) and antimetabolic agents (aminopterin, thioguanine and 5-fluorouracil) given to women during the 3 first months of pregnancy. STEPHENS *et al.* (1980) refer to abortion of an abnormal fetus, from a mother given 5-fluorouracil during the 11st-12nd weeks of pregnancy. Experimental studies in rodents, rabbits and monkeys have shown embryotoxic and teratological effects of antimetabolic drugs. Studies have been carried out with 5-fluorouracil (DAGG, 1960; WILSON, 1971; SHAH and MACKAY, 1978; GRAFTON *et al.*, 1987), adriamycin and daunomicyn (THOMPSON *et al.*, 1976), 3,3-dimethyl-1-phenyltriazene (FRANK *et al.*, 1989). For further pharmacological references on antimetabolic drugs, see CHABNER *et al.*, (1996). The influence of anticancer drugs on dental development has been widely studied. The rat has a special experimental interest due to the

continuous growth of incisive teeth, lasting for the whole life of the animal. At any time, one can observe, in each tooth, the whole cycle of the organ development: growth, calcification and eruption (FARRIS and GRIFFITH, 1971; NISHMURA and TANIMURA, 1976; DAHL and KOPPANG, 1985). Dental anomalies in rats and mice related to disturbance of dental growth and tissular alterations were observed with antimetabolic drugs: cyclophosphamide (NORDLINDER, 1971; KOPPANG, 1973, 1978; MASSINI, 1975; VAHLSING, 1975, 1977; MATHEUS and HETEM, 1990; MATHEUS *et al.*, 1994) colchicine (DIAS COSTA, 1978; NOGUEIRA *et al.*, 1980, 1981), doxorubicin (DAHL and KOPPANG, 1985), 5-fluorouracil (DE DEUS and HAN, 1976). BASTOS-RAMOS *et al.*, (1992) showed that in adult rats 5-fluorouracil did not disturb dental growth, unless when very high near to lethal doses were used. The present research intends to study the influence of the very potent anticancer drug 5-fluorouracil, given to mothers in early phases of pregnancy, in increasing doses, on the dental germ of their offsprings as well its embryotoxic and general teratogenic effects.

Material and Methods

A total of 140 adult rats (*Rattus rattus norvaegicus*, *albinus*, Wistar), in good controlled health conditions were used. 125 were virgin females, aged 90-100 days and 15 were adult male rats. Males and females were put together and the fertilized females were used for the experiment. They were divided in four groups, according to the doses of 5-fluorouracil administered. A control group injected with saline was run in parallel. The drug was administered intraperitoneally, in single doses, at the 9th, 10th, 11th, 12th and 13th days of pregnancy. Four doses of 5-fluorouracil were used, as follows: 10 mg/kg; 20 mg/kg; 30 mg/kg and 40 mg/kg. At the 21st day of pregnancy, the rats were anesthetized with ethyl ether, the uterus exposed by laparoscopy, the embryos were taken out for the observation of an earlier fetuses reabsorption. The newborn rats were weighed and observed under lens (4 x magnification) regarding their external characteristics, to check possible malformations. For microscopic studies, the heads were cut off, fixed in 10% formol solution for 24 h, cut at medial

sagittal plane, hydrated, dehydrated, diaphanized and prepared in paraffin to histology. The tissues were examined by light microscopy. In every group, control experiments with saline treated rats were run.

For statistical analysis of the results, Tuckey test, variance analysis and X² test were used.

Results

1. *Toxicity of 5-fluorouracil to the pregnant rats.* No important sign of intoxication was observed in 5-FU treated mothers, but a poor gain or even loss of body weight in the ones injected with higher doses (30-40 mg/kg). In these animals, a decrease of 30% of successful pregnancy, as compared to controls (100%), was observed. However, when these doses were given at the 13th day fertilization, the pregnancy was less affected. It was not affected at all with doses of 10 or 20 mg/kg injected in any time of pregnancy. 2. *Toxicity of 5-FU to fetal uterine implantation.* The number of uterine embryonic implantation was significantly reduced when the drug was injected at the 9th day of pregnancy, and it was expressive with doses of 30 and 40mg/kg. By the time of birth 100% of the offspring from control not treated mothers were alive. 3. *Fetal dental germ abnormalities.* The abnormalities detected at birth were of different kind, as follows: absence of dental germ; hyperchromatic and roundish nucleus pulp cells; inflammatory processes; reduced bone tissue; cleft at the edging of the germ. The proportion of abnormalities increased with the doses of 5-FU given to mothers and were related to the period of pregnancy it was given. With higher doses (30 40 mg/kg) it was poorly observed due to frequent uterine reabsorption. No abnormality was found in offspring from control mothers. Results are summarized in Table 1. 4. *Fetal gross abnormalities.* Somatic general abnormalities were observed in newborn from 5-FU treated mothers, most of which showed also dental germ problems. The abnormalities were: labial cleft; cephalic extrusion; cephalic hemorrhage; total or partial absence of limbs, fingers or tail; diffuse edema. The malformations increased with doses of 5-FU and was related to the day of pregnancy the drug was administered to the mothers. Results are summarized in Table 1.

Table 1. Influence of 5-fluorouracil (5-FU), administered to pregnant mothers, on offspring rats: gross somatic malformations* and dental abnormalities**.

Time of pregnancy (days)	Dose of 5-FU (mg/kg)	Somatic malformation (% of animals)	Dental abnormalities (% of animals)
9 ⁰	10	21% malformations	15% abnormalities
	20	20% malformations	19% abnormalities
	30	100% fetal reabsorption	-----
	40	100% fetal reabsorption	-----
10 ⁰	10	Normal	Normal
	20	Normal	Normal
	30	Normal	Normal
	40	Normal	Normal
11 ⁰	10	Normal	Normal
	20	Normal	Normal
	30	100% fetal reabsorption	-----
	40	100% fetal reabsorption	-----
12 ⁰	10	Normal	Normal
	20	22% malformations	20% agenesis
	30	47% malformations	21% agenesis
	40	97% reabsorption/1 dead	-----
13 ⁰	10	Normal	Normal
	20	Normal	Normal
	30	72% malformations	20% agenesis
	40	37% fetal reabsorption and 63% dead fetuses	-----

*Gross somatic malformations: labial cleft; encephalic extrusion; encephalic hemorrhage; total or partial absence of limbs, finger or tail; diffuse edema. **Dental abnormalities: absence of dental germ; hyperchromatic and roundish pulp cells; inflammatory processes; reduced bone tissue; cleft at the edge of the germ.

Discussion

It is well known that many drugs administered in pregnancy, mainly during the early stages, in human or animals, can be damaging to the embryo or fetus, inducing premature death and absorption or uterine reabsorption. Otherwise, they can cause death at birth, or the newborn survives with morphological or functional abnormalities. Regarding to this, antimetabolic drugs, widely used in the control of cancer, are particularly important, due to their high toxicity to growing tissues, being a real problem if it has to be given to cancerous mother. Why to study the embryonic responses to a powerful anticancer drug like 5-fluorouracil, if it is clearly expected to be damaging to such organisms? It is important because the experimental

results in animals, using this and other anticancer drugs, as well as clinical observations in cancerous pregnant women given antimetotics have shown that there are dissimilar responses, according to the drug, the doses and the period of pregnancy (STADLER and KNOWLES, 1971; TOBIAS e BLOOM, 1980; GILILLAND and WEINSTEIN, 1983; BLATT *et al.*, 1992). As far as to dental growth is concerned, previous experiments in our laboratories (BASTOS-RAMOS *et al.*, 1992) have shown that 5-fluorouracil exerts a reduced influence on the dental development of adult rats, causing some impairment only when very high doses, near to lethal were given. These results allow to suppose that dental growing tissues in rats are somewhat resistant to the antimetabolic action of this drug. These findings induced to study its influence

on embryo's dental germ formation, when given to pregnant mothers, as compared to other embryotoxic or teratogenic effects in the same animal. The initial dose chosen for the experiments - 40 mg/kg, resulted to be very embryotoxic, giving rise to many dead embryos or very high rates of uterine embryonic reabsorption. The mothers showed no important signs of intoxication, other than loss of weight with higher doses or poor weight gain, which could be related to failing in pregnancy in most of them. Using lower doses-20 and 10 mg/kg, no signs of toxicity to mothers were observed but they were appropriated to cause embryonic malformations. One can establish some parallel between the percentage of body malformations and dental anomalies when 10 and 20 mg/kg of 5-FU were used. However, with doses of 30 mg/kg, dental germ anomalies were half to one third less frequent as compared to the proportion of general body malformations, considering the number of fetuses examined. Very important to the results, other than the doses used, were the days of pregnancy in which 5-FU was injected. It was chosen the period from the 9th to the 13th days of pregnancy, as long as it is the most important period to embryonic formation of structures of the rat head. DAGG (1960) refers that the 9th day is very important for the formation of ocular structures. Our results can be considered concordant with this Author's finding. As a matter of fact, when given at the 9th day, the occurrence of malformations was more frequent with doses of 10 and 20 mg/kg and carried out total absorption of embryos with doses of 30 and 40 mg/kg. The high rate of fetal reabsorption is consistent with findings of SHAH and MACKAY (1978) using hamsters and 5-fluorouracil. In our experiments the sensitivity was less evident when the drug was administered at the 10th and 11th days: no malformations were observed with doses of 10 and 20 mg/kg although there was no birth with doses of 30 and 40 mg/kg administered at the 11th day if pregnancy. On the other hand, a high rate of malformations were observed with 5-FU injected at the 12th day of pregnancy, that is 22% with 20 mg/kg and 47% with 30 mg/kg. The 12th day is just the crucial day for the closing of the palate, what could be related to our results. The least sensitivity was observed when 5-Fu was given at the 10th day (100% normal), and at the 13th day of pregnancy: with doses of 10 and 20 mg/kg, all fetuses were normal; with doses of 30 mg/kg, 20 % of the fetuses had agenesis of dental germ and 72%, body malformations. At this time, doses of 40mg/kg, usually leading to total uterine embryo reabsorption, gave rise to dead but completely formed fetuses. The results of this work are poorly explained by the current knowledge about rat

embryology, but are expressive to lead further experiments, to clear the influence of doses of 10 and 20 mg/kg, given to mothers at the 9th day of pregnancy, on the development of dental tissues, from birth to adult age.

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