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Micronuclei in diabetes: Folate supplementation diminishes micronuclei in diabetic patients but not in an animal model

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Abstract

Diabetes mellitus (DM) is associated with a high risk of health complications, mainly due to excessive free radical (FRs) production that could result in an increased frequency of micronuclei. The consumption of antioxidants, like folic acid (FA), may mitigate the effects of the FRs. In the present study, micronucleated polychromatic erythrocyte (MNPCE) frequencies were determined in blood sampled weekly from the tails of pregnant female Wistar rats and pregnant Wistar rats with experimental diabetes that were given unsupplemented diets and diets supplemented with FA. At birth, the pups were sampled to analyze micronucleated erythrocyte (MNE) and MNPCE frequencies. Moreover micronucleated cells (MNCs) were evaluated in buccal mucosa samples taken from 81 healthy adult subjects, 48 patients with DM, and 30 DM patients who were sampled before and after FA treatment. Increases in MNPCE frequencies were significant only at the first sampling ($P < 0.01$ and $P < 0.03$) in pregnant rats with experimental diabetes. In addition, the pups from the diabetic group and from diabetic group treated with FA had higher frequencies of MNEs ($P < 0.03$ and $P < 0.001$, respectively) and MNPCEs ($P < 0.009$ and $P < 0.05$, respectively) than the controls. No differences were found in diabetic rats and newborn rats born to diabetic mothers treated with FA compared with untreated animals. Patients with DM had a higher frequency of MNCs compared with healthy subjects ($P < 0.001$). Also FA reduced the frequency of MNCs in DM patients ($P < 0.001$). The results of this study indicate that diabetes results in elevated frequencies of micronuclei, and that, at least in humans, FA can protect against the elevation.

Keywords: Micronuclei; Diabetes; Folic acid; Free radicals; Human; Rat

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1. Introduction

Diabetes mellitus (DM) is characterized by an elevation in blood glucose concentration. The disease is progressive and is associated with the development of complications, like atherosclerosis, renal and neuronal damage, and blindness [1–3]. Experimental evidence indicates that these complications are due mainly to the production of excessive concentrations of free radicals (FRs), which result in oxidative damage to biomolecules [1,4–9]. Oxidative damage to the genetic material could cause DNA strand breaks [5,8,10–13] and micronuclei (MN), and these types of damage could have teratogenic or carcinogenic consequences [14–17].

Elevated frequencies of micronucleated erythrocytes (MNEs) have been measured in premature children born to mothers with pathologies related to oxidative stress [16], like arterial hypertension and DM. Also, increases in micronucleated cells (MNCs) have been observed in buccal mucosa of patients with other pathologies characterized by increases in FRs production, like rheumatoid arthritis [18,19].

The damage caused by FRs can be mitigated by antioxidant defence systems, which in the case of DM, become overwhelmed by FRs generated by the disease processes [4,7,8]. Antioxidant support systems can in theory be supplemented by using antioxidants like folic acid (FA) [20] that have the capacity to resist (or to neutralize) the effects of FRs [12,21–25]. FA deficiency increases spontaneous chromosomal damage by massive incorporation of uracil within DNA, which produces chromosomal breakage and MN formation [26], and can influence the genotoxic responses to other compounds [27,28]. The use of supplemental FA by women pre- and post-conception diminishes the occurrence of neural tube defects in their offspring [29]. Observations such as these illustrate the health advantages produced by the intake of this vitamin [30].

Previous studies indicate that FA supplementation decreases the frequency of MN in humans and experimental systems [14,20,31]. In the present study, we have evaluated the effect of DM on MN frequency, and the effect of FA supplementation on DM-associated MN.

2. Materials and methods

2.1. Rat study

2.1.1. Animals

The study was approved by our Institutional Research Committee (register number 2002249018) and by a local Animal Care Committee. All experiments were performed according to the guidelines for the care and use of experimental animals at the Centro de Investigación Biomédica de Occidente, which are in compliance with those given in national (México; NOM-062-ZOO-2001) and international guidelines for the humane treatment of research animals.

Twenty-one 3.5-month-old female Wistar rats were housed individually in polycarbonate cages, and given water and food ad libitum. All animals used in the study were supplied by the laboratory animal facility of the Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, México. In addition, 48 pups of undetermined sex, born to these rats, were used during the course of the experiments.

2.1.2. Adults rats

2.1.2.1. Study groups. Three groups of seven adult female rats were formed. Group 1 (negative control) received a single intraperitoneal (i.p.) injection of 0.3 ml injectable distilled water, and then were mated with males of the same strain. Pregnancy was determined by the presence of sperm in a vaginal smear [14], and when pregnancy was confirmed, the rats were given daily orally by gavages doses of 0.5 ml water until delivery. Group 2 and 3 rats were first made diabetic as described below. Group 2 rats (diabetes without FA) were mated and treated with water as above. Group 3 animals (diabetes with FA) underwent the same procedure as Group 2, except that they received daily 0.5 ml doses of 0.7 mg of FA/kg (Sigma, St. Louis, MO; CAS No. 59-30-3) orally by gavages (in water) from the first day of pregnancy until the birth. The dose of FA was based on the therapeutic dose recommended for pregnant women (5 mg/day), using an average human body weight of 70 kg and multiplying by 10, because is established that on a body weight basis, humans are generally more vulnerable than are experimental animals, probably by a factor of about 10 [32].

2.1.2.2. Experimental diabetes induction in rats. Fourteen rats (Groups 2 and 3) received i.p. injections of 65 mg streptozotocin (STZ)/kg (Sigma, St. Louis, MO; CAS No. 18883-66-4) to induce DM [33,34], at least 3 days previously to be mating. The diabetes was confirmed with a glucometer (One Touch Ultra; Reg. Not 1691E2002, S.S.A. Johnson & Johnson, México, S.A. of C.V.). To consider that an experimental diabetes in the rat was established, it was required that the rat maintains values greater than 250 mg/dl of blood glucose after the induction. In order to assure that the hyperglycaemic state was maintained, glucose levels were monitored every 7 days during the experiment.

2.1.2.3. Sample preparation and MNE analysis. A total of five samples of blood were taken of each rat during the experiment. The basal sample was taken previously to mating and the next samples were taken every 7 days during the pregnancy at days 7, 14 and 21 of gestation, and at 7 days after the delivery, and two smears were made of each sample time on pre-cleaned