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***In vivo* zinc oxide nanoparticles induces acute hyperglycemic response a dose-dependent and route of administration in healthy and diabetic rats**

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Abstract

In recent years, different studies carried out in experimental diabetes models suggest that zinc oxide nanoparticles (ZnONPs) can be good antidiabetic agents, this evidence was obtained from long-term treatments using repeated doses of zinc oxide nanoparticles. The goal of this work was to evaluate the acute effects during six hours post-administration of ZnONPs on glycemia in healthy and diabetic rats and to compare the effects of oral and intraperitoneal administration. For this study male Wistar rats were used and experimental diabetes was induced by streptozotocin-nicotinamide intraperitoneal administration. At short-term ZnONPs administration induced hyperglycemic response in healthy and diabetic rats, the effect was dose-dependent and administration route. The diabetic rats were more sensitive to ZnONPs effect. In conclusion, this study provides novel information about the acute effects of zinc oxide nanoparticles on the fasting glycemia in experimental diabetes *in vivo* model and healthy rats, these data are important for its future clinical applications.

Keywords: Zinc oxide nanoparticles, diabetes, hyperglycemic response, zinc, nanomedicine, nanoparticles toxicology.

Introduction

The application of nanotechnology to solve human health problems has increased the scientific interest in recent years. Different studies have been conducted to analyze the effects of metal nanoparticles on hyperglycemia in models of experimental diabetes with rodents. It has been reported that metal nanoparticles (silver, gold, zinc oxide) possess antihyperglycemic activity in diabetic rats after daily treatment for a period of time [1-7].

In particular, zinc oxide nanoparticles (ZnONPs) have been suggested as promising antidiabetic agents. Oral administration at dose 1-10 mg/kg during 4 weeks reduced hyperglycemia in type 2 diabetes (D2) and type 1 diabetes (D1), but insulin was not affected with treatment ZnONPs in D1, insulin only increased at dose 10 mg/kg in D2. In the same study single dose ZnONPs at 3-10 mg/kg improved glucose tolerance in D2 while in D1 significant changes were not observed [3]. Other study using 10 mg/kg oral route during 4 weeks in D1 showed reduction in hyperglycemia and increase in insulin level [2]. Oral administration for a long time (1-8 weeks) at dose 1-10 mg/kg ZnONPs also decreased glycemia in D1 and insulin level was increased [8]; treatment for 7 weeks using dose 1-10 mg/kg improved glucose tolerance and insulin in D2 [9]. Despite the important evidence supporting that the oral administration of nanoparticles for several weeks in a dose range of 1-10 mg/kg/day has antidiabetic activity, the immediate effect post-administration of ZnONPs on basal glycemia has not been studied and this knowledge is important for the glycemia control of a diabetic patient. The objective of this investigation was

to evaluate the acute effects during six hours post-administration of ZnONPs on glycemia in healthy and diabetic rats and to compare the effects of oral and intraperitoneal administration.

Experimental

Materials and Methods

Materials

Zinc oxide nanoparticles dispersion was purchased from Sigma-Aldrich catalogue 721077; 0.9% sodium chloride solution sterile was purchased from PISA Pharmaceutical Co. Streptozotocin (cat. S0130, purity \geq 98% HPLC) and nicotinamide (cat. N3376, purity \geq 98% HPLC) were purchased from Sigma-Aldrich.

Animals

Wistar male rats (n=96), three months aged, were used for the experiments. The rats were housed in individual cages with water and food ad libitum (Rodent Laboratory Chow 5001, PMI Nutrition International LLC). They were maintained in room with light-dark cycles (12 h/12 h) and controlled room temperature (25 °C). All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH) and approved by the Bioethics Committee of the University of Colima (Approval number 2018-15).

Experimental design and diabetes induction

The rats were divided into 2 groups: diabetic rats (n = 48) and non-diabetic rats (n = 48). Experimental diabetes in rats was induced by the intraperitoneal administration

of streptozotocin (65 mg/kg body weight) dissolved in citrate buffer pH = 4.5, fifteen minutes before, intraperitoneally nicotinamide was injected (230 mg/kg body weight) dissolved in 0.9% saline solution, this model induces partial cytotoxicity on pancreatic beta cells producing moderate hyperglycemia without loss of body weight or drastic decreases in plasma insulin levels [10]. At seven days, the glycemia was measured in blood samples collected from rat tail using an Accu-chek Performa blood glucose system (Roche Diagnostics); rats with fasting glucose of 126 mg/dL were included in the diabetes group (World Health Organization).

Evaluation of zinc oxide nanoparticles on fasting glycemia values

All rats were left fasting for 8 h (07:00-15:00 h) previous to evaluation. Both groups, diabetic and non-diabetic rats were subdivided to test two dose ZnONPs, 10 and 100 mg/kg body weight by two route of administration, oral and intraperitoneal (ZnONPs dispersion was prepared in 0.9% saline solution sterile). At time 0 and 15, 30, 60, 90, 120, 240 and 360 minutes post-administration of ZnONPs glycemia value was evaluated using an Accu-chek Performa blood glucose system. The blood sample was taken as described below: the distal part of the tail of the rat is cleaned with an alcohol swab, then a small cut is made in the distal portion of the tail with scissors and the drop of water. Blood obtained is deposited on the test strip placed on the digital glucometer. The clot is removed from the tail to obtain a drop of fresh blood and thus be able to perform the glucose measurement, this procedure was repeated in each measurement.

Statistics

All data were expressed as mean \pm error standard. Experimental groups were compared using one-way ANOVA and post hoc test (Bonferroni) to analyze each pair. Differences were considered significant at $p < 0.05$.

Results and Discussion

When the effects of ZnONPs were evaluated by intraperitoneal route with high single dose (100 mg/kg), the glycemia increased significantly compared with control group ($p < 0.05$) reaching a maximum peak at 30 minutes in healthy rats and 60 minutes in diabetic rats and these effects were reversed until 6 hours post-administration (Figure 1B and Figure 2B). This effect was higher in diabetic when compared with healthy rats ($p < 0.05$). In the case of dose at 10 mg/kg route i.p., post-administration ZnONPs in healthy and diabetic rats glycemia was not affected by 6 hours ($p > 0.05$).

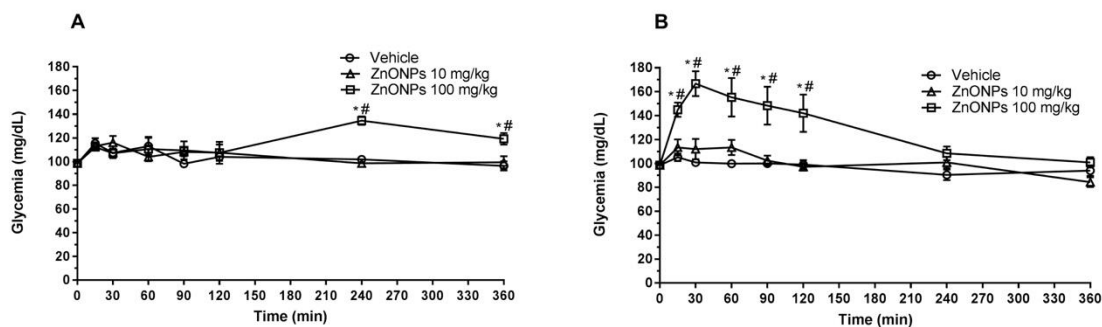


Figure 1: Short-term effect of zinc oxide nanoparticles by two routes of administration on glycemia in fasting healthy rats. A) Oral administration (p.o). B) Intraperitoneal route (i.p). Vehicle (received 0.9% saline solution sterile). * significant respect to vehicle ($p < 0.05$), # significant respect to group treated with 10 mg/kg.

In the case of ZnONPs oral administration, at dose 100 mg/kg the glycemia increased to the 2 hour post-administration and it was kept high for four hours, in both healthy and diabetic rats (Figure 1A and Figure 2A).

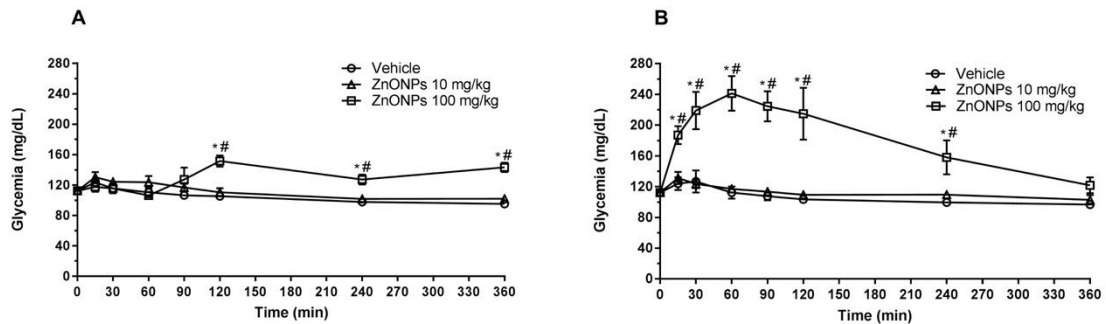


Figure 2: Short-term effect of zinc oxide nanoparticles by two routes of administration on glycemia in fasting diabetic rats. **A)** Oral administration (p.o). **B)** Intraperitoneal route (i.p). Vehicle (received 0.9% saline solution sterile). * significant respect to vehicle ($p < 0.05$), # significant respect to group treated with 10 mg/kg.

Comparative analysis at the highest dose that was administered to diabetic rats showed that the intraperitoneal administration induces a greater hyperglycemic response than oral supplementation of ZnONPs, however at six hours post-administration, the i.p group reversed the hyperglycemia while that the oral administration group maintained hyperglycemia (Figure 3).

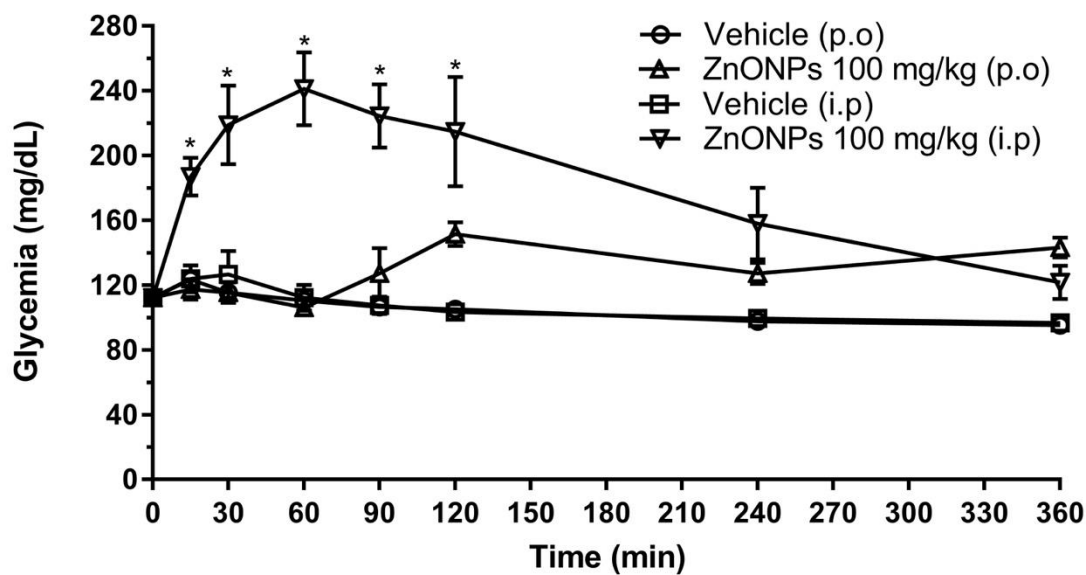


Figure 3: Comparative analysis of short-term effects of zinc oxide nanoparticles on the glycemia of fasting diabetic rats, oral administration (p.o) versus intraperitoneal route (i.p). * significant between treated groups with ZnONPs ($p < 0.05$).

To our knowledge it is the first report that shows that ZnONPs induce hyperglycemic response in the short term. The mechanism through which it induces this effect is unknown. In the long term, it is proposed that the anti-diabetic activity of ZnONPs is carried out due to an increase in insulin level, glucokinase activity, increased expression of insulin, insulin receptor A, GLUT-2 and glucokinase [2], reduction in oxidative stress [3,8], less damage to the pancreatic structure [11,12] and microRNA-103 and microRNA-143 decreased expression [9]. Also, in vitro experiments it has demonstrated that ZnONPs could attenuate hyperglycemia through a mechanism that involves inhibition of α -amylase and α -glucosidase

activity [13]. Moreover, in vitro it has showed that ZnONPs induces GLUT-4 translocation and increased beta-cell proliferation [14].

In contrast, the lack of knowledge about the mechanism involved in the short-term hyperglycemic response induced by ZnONPs generates new research questions for future work. It is widely known that the liver is the main organ to produce glucose and is an essential point in antidiabetic therapies [15]. The hyperglycemic effect reported in the present work could be the result of a direct action of high concentration of zinc ions on the hepatic metabolism; It is known that in hepatocytes zinc at high concentrations induces an increase in glucose production through glycogenolysis [16]. In vivo, a study with zinc supplementation in rats reported a hyperglycemic response, an increase in glucagon, decreased insulin, depletion of hepatic glycogen and attenuation of hyperglycemia when the adrenal glands were previously removed [17]. Despite these studies, the evidence of the mechanism of action by which zinc supplementation induces hyperglycemic response in the short term is scant, and the results shown in this work with ZnONPs increases the interest to carry out future research before its clinical application as antidiabetic agents.

ZnONPs supplementation could to short-term induce hyperglycemic response by inhibition of insulin secretion. It has been reported in beta-cell islets that zinc inhibits concentration-dependent insulin secretion [18] and recent studies has showed that zinc is key for synthesis and insulin secretion in beta cell [19]; ZnONPs could be dysregulating any step in the action mechanism for insulin release from pancreatic beta cell, however new studies must be carried out to test these hypotheses.

In general, the importance of the results obtained in this research is that the hyperglycemic response induced by zinc oxide nanoparticles in a diabetic patient with high glucose would increase the risk of producing a diabetic coma and compromising their life.

Perspectives. To better understand the acute hyperglycemic effect induced by the zinc oxide nanoparticles, it would be important to measure in another study insulin levels in vivo after the administration of nanoparticles (0-6 h) and explore the effects on liver metabolism, this would allow to know if the zinc oxide nanoparticles are carrying out their effect by decreasing insulin release or increasing hepatic glycogenolysis.

Conclusions

In the short term, zinc oxide nanoparticles induces hyperglycemic response in healthy and diabetic rats, the magnitude of the effect was dependent on the dose and administration route. In addition, the hyperglycemic response was greater in diabetic animals. This study provides novel information about of acute effects of zinc oxide nanoparticles on the circulating blood glucose levels that could limit its therapeutic application in diabetic patients. Nevertheless, more investigations are necessary to elucidate its action mechanism.

Disclosure statement

The authors declare no conflict of interests

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