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# Evaluation of potential embryo toxicity of albendazole sulphoxide in CF1 mice

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**Key words:** benzimidazole, early pregnancy, developmental exposure.

**ABSTRACT:** Benzimidazole compounds are used in both humans and animals for controlling helminth parasites. Albendazole has teratogenic effects attributed to its active metabolite albendazole sulphoxide. The aim of this work was to evaluate the effect of the latter compound when administered to pregnant CF1 mice during the preimplantation period. Females were superovulated by intraperitoneal injection of 10 IU of eCG and 10 IU of hCG (48h later) and were paired with males of proven fertility. Albendazole sulphoxide (200 mg/kg) was orally administered by gavages at day 1, 2 or 3 of pregnancy; the control group received only the vehicle (carboxymethylcellulose). Females were killed by cervical dislocation at day 4 of pregnancy and embryos were flushed from uteri with Ham F10 media supplemented with bovine serum albumin (0.4%). Number of collected embryos per female, percentage of morphologically normal embryos, differentiation rate and number of cells per embryos were recorded. The variables were analyzed on a per litter basis by Kruskal-Wallis test. There was no effect of albendazole sulphoxide on parameters evaluated ($P>0.05$). We conclude that the preimplantation mouse embryo development was not significantly affected by albendazole sulphoxide.

## Introduction

Mammalian preimplantational development involves cellular processes that transform the zygote, a totipotent cell, in a blastocyst, i.e., in an embryo consisting of differentiated cells. These stages can be modified by certain agents that give rise to anomalies. The action of teratogens depends on multiple factors as the maternal and embryonic genotype, stage of development, dose and time of exposition to them.

Among the agents that have demonstrated teratogenic effects, there are drugs utilized in parasitic control in farm animals whose presence causes important sanitary problems and economic losses. The need of controlling them has promoted the development of molecules with anthelmintic activity, being benzimidazole a broad-spectrum anthelmintic agent used in veterinary medicine.

Benzimidazole presents a broad spectrum of activity as an anthelmintic agent, with high effectiveness and safety (Campbell, 1990), however administered during gestation, they have shown teratogenic effects such as external, skeletal and vascular abnormalities (Cristòfol *et al*., 1997; Navarro *et al*., 1998, 1999; Teruel *et al*., 2003; 2009a).

Benzimidazole anthelmintics inhibit the energetic metabolism of parasites and bind with tubulin, prevent-

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ing its polymerization to microtubules, thus interfering with mitotic activity (Lacey, 1988; 1990). Several studies suggest that the capacity of benzimidazolic drugs to bind with the tubulin of cellules is responsible for the toxic effects observed during the gestation (Delatour and Parish, 1986; Piscopo and Smoak, 1997). Albendazole is an anthelmintic agent belonging to the benzimidazole class and its efficacy is attributed to its active albendazole sulphoxide metabolite, owing to its higher systemic availability compared to ABZ (Delatour *et al*., 1984).

While the effects of albendazole sulphoxide at post implantation pregnancy are known, limited data on possible effects on earlier development are available. For this reason, albendazole sulphoxide was chosen as a model metabolite for a study *in vivo* to obtain information about the possible embryonic alterations induced by this anthelmintic drug after its administration to pregnant CF1 mice.

## Material and Methods

### *Animals*

Female and male CF1 mice were kept on a lighting regimen of 14 hours light alternating with 10 hours darkness for seven days before use, allotted in plastic boxes with free access to tap water and used according to the Animal Welfare Act (Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires).

Thirty 5-8 week old female were superovulated by intraperitoneal injection of 10 IU of equine chorionic gonadotropin (eCG, Novormon®, Laboratorios Syntex S.A. Argentina). Forty eight hours later they received 10 IU of human chorionic gonadotropin (hCG Ovusyn®, Laboratorios Syntex S.A. Argentina) and were paired with males of proven fertility. Day one of pregnancy was the day on which vaginal plugs were found.

### *Albendazole sulphoxide administration*

Albendazole sulphoxide (racemic mixture, batch number 6-0011, Microsules Argentina S.A.) was orally administered by gavages at a dose of 200 mg/kg body weight on day 1 ($n$=7), 2 ($n$=6), or 3 of pregnancy ($n$=9); the control group ($n$=8) was administered the same volume of carboxymethylcellulose vehicle.

### *Embryo collection*

Pregnant females were killed by cervical dislocation at day 4 of pregnancy and 560 embryos were flushed from uteri using Ham F10 media (Gibco BRL, Life Technologies NY, USA) supplemented with 0.4% of bovine serum albumin (BSA - Sigma Chemical Company, St. Louis, MO, USA) using a 30-gauge needle on a 1-mL syringe.

### *Evaluated parameters*

Preimplantational development was evaluated considering the total number of collected embryos per female; the percentage of embryos morphologically normal determined by the relation between the number of

**TABLE 1.**

Effect of albendazole sulphoxide on preimplantational development in CF1 mice administered on day 1, 2 or 3 of pregnancy (mean ± SD).

| Variable | Control group | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| **Number of embryos per female** | 19.0 ± 12.0 | 23.3 ± 11.6 | 20.8 ± 16.5 | 11.44 ± 4.3 |
| **Morphologically normal embryos (%)** | 93.1 ± 13.0 | 91.4 ± 16.5 | 87.8 ± 20.7 | 86.9 ± 22.4 |
| **Differentiation (%)** | 15.5 ± 19.6 | 25.8 ± 35.6 | 27.8 ± 28.5 | 17.6 ± 23.6 |

normal embryos and the total collected embryos per cent (embryos morphologically normal were considered those with intact pellucida zone and with no asymmetry, blastomeres separated from the inner cell mass, with cytoplasm granulations or irregular shapes); the differentiation rate determined by the relation between the number of blastocyst and the total of morphologically normal embryos collected per cent; and the cleavage rate determined by counting the nuclei according to the air-drying technique of Tarkowski (1966). Briefly, the embryos were swollen in drops of 1% sodium citrate for 8-10 min at 37°C, transferred to cleaned slides and fixed for 30 s by dropping methanol: acetic acid (3:1 v/v) over the embryos. The cells were stained with Giemsa solution (2%) for 10 min. The nuclei were examined under light microscopy. Additionally, the blastocysts were classified according to blastocoel development, as early blastocysts, medium-sized blastocysts, and expanded blastocysts.

The variables were analyzed on a per litter basis using the Kruskal-Wallis test. Significance level was established at $P<0.05$.

## Results

No statistically significant differences were found in the number of embryos collected by female after the administration of 200 mg/kg of albendazole sulphoxide on day 1, 2 or 3 of pregnancy compared to the untreated control group ($P>0.05$; Table 1). Experimental and control embryos showed normal morphological characteristics ($P>0.05$; Table 1).

The differentiation rate was not affected by albendazole sulphoxide administered on different days of pregnancy ($P>0.05$; Table 1). According to blastocoel development, the proportion of early blastocysts ($56.1 \pm 36.3$) and medium-sized blastocysts ($63.7 \pm 31.1$) did not show differences between groups ($P>0.05$). The percentage of expanded blastocysts was $14.6 \pm 8.6$ corresponding to means of control, day 1 and day 2 groups. Expanded blastocysts were not collected from females from day 3 group.

The cleavage rate was not affected by albendazole sulphoxide administered on different days of pregnancy no matter the developmental stage (morulae or blastocyst) ($P>0.05$; Table 2).

## Discussion

Anthelmintic benzimidazoles are used worldwide in human and veterinary medicine, however they induce embyotoxic effects during pregnancy in domestic (Delatour *et al*., 1981; Marriner, 1986; Fabre *et al*., 1989; McKellar and Scott, 1990) and laboratory animals (Mantovani *et al*., 1995; Cristòfol *et al*., 1997; Navarro *et al*., 1999; Teruel *et al*., 2003; 2009a).

Albendazole sulphoxide was detected in fetuses of ewes and rats (Capece *et al*., 2002; 2003) after crossing the placental barrier, however for the early embryo development experimental model proposed in this work, a possible effect of albendazole sulphoxide could be feasible after reaching the embryo through the oviductal or uterine fluids so long as there are no placental structures.

The administration of albendazole sulphoxide at a dose of 200 mg/kg of body weight to CF1 females during the first, second or third day of pregnancy does not modify significantly the preimplantational mouse embryo development. These results suggest that the maternal environment where the embryos were developed did not modify their passage from oviduct to uterus. Furthermore, the fact that the proportion of normal

**TABLE 2.**

Cleavage rate expressed as number of cells in CF1 mouse embryos from mothers treated with albendazole sulphoxide on days 1, 2 or 3 of pregnancy (mean ± SD).

| Number of cells | Control group | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| **All embryos** | 26.03 ± 4.2 | 22.38 ± 6.4 | 23.38 ± 5.9 | 22.48± 5.0 |
| **Morulae** | 25.32 ± 5.6 | 21.21 ± 5.2 | 22.10 ± 5.3 | 21.85 ± 4.7 |
| **Blastocysts** | 28.33 ± 2.1 | 26.58 ± 5.1 | 24.67 ± 5.3 | 29.35 ± 6.9 |

morulae and blastocyst was similar in experimental and control groups, leads the hypothesis that major events in the continuity of development such as compaction, maternal genome activation and morulae formation would not be affected by the anthelmintic albendazole sulphoxide.

The differentiation rate was not different for females administered with albendazole sulphoxide which suggests the normality of previous steps such as morulae given that the trophectoderm cells derive from outer cells while the inner cell mass derives from inner cells at the morula stage. However, expanded blastocysts were not collected from females administered with albendazole sulphoxide at 200 mg/kg body weight at day 3 of pregnancy which suggests the presence of some differential characteristic in these embryos that only reach medium blastocyst stage when they were collected at day 4 of pregnancy.

With regard to number of cells, no differences were observed in our study, possibly due to the fact that albendazole sulphoxide does not modify the mitosis of segmentation when administered during the first three days of pregnancy. These findings are very interesting considering the direct relation demonstrated between the anthelmintic benzimidazoles and the cell division (Lacey, 1988; 1990). However, there is evidence that *in vitro* produced bovine embryos and *in vivo* produced rat embryos exposed *in vitro* to albendazole sulphoxide, decreased ability to divide in culture. Furthermore, bovine embryos showed gross morphologic abnormalities (Piscopo and Smoak, 1997) perhaps as a consequence of a direct action of the drug in the culture media for rat and bovine embryos or intrinsic differential characteristics for the *in vitro* produced bovine embryos (Crosier *et al*., 2000, 2001). On the other hand, the fact that no embryotoxic effects of benzimidazole drugs have been found in this study, agrees with the results obtained after administration of albendazole (30 mg/kg bw/d) to Charles River CD-1 mice for 10 days during pregnancy (Killeen and Rapp, 1975), and the administration of albendazole sulphoxide (200 mg/kg) to Balb C mice at day 2 of pregnancy (Teruel *et al*., 2009b). The preceding results support the hypothesis that regardless of the mouse strain, the embryo development would not be sensitive neither to albendazole nor albendazole sulphoxide. However, due to the drug ability to bind with tubulin and inhibit its polymerization to microtubules thus interfering with mitotic activity (Lacey and Watson, 1985), and considering that the mitotic spindle, mechanical agent in the karyokinetic process, is compossed of microtubules (Danilchik *et al*., 1998; Schroeder 1973), further research is required to confirm the absence of toxic effects during cleavage, compaction, morulae and blastocyst formation.

We conclude that, under the conditions of this study, the preimplantation mouse embryo development was not significantly affected by the oral administration of albendazole sulphoxide to CF1 mice on early pregnancy. The results suggest that albendazole sulphoxide at doses higher than those used in human and veterinary medicine is not embryotoxic when is administered during early embryo development.

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