Original Articles

Teratogenic Evaluation of Metronidazole and Ornidazole Using *Drosophila melanogaster* as an Experimental Model

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Received 9 September 2003; Accepted 14 January 2004

BACKGROUND: *Drosophila* and vertebrates show similarities that suggest that the mechanisms involved in the induction of developmental defects may be similar in both. Therefore, *Drosophila* has been proposed as a useful, rapid, and economical model in the preliminary screening for teratology studies. The objective of the present study was to investigate the effect of metronidazole (MTZ) and ornidazole (ONZ) on the developmental stages of *Drosophila melanogaster*. **METHODS:** Samarkand wild-type females were allowed to lay eggs for 24 hr in media containing MTZ or ONZ at concentrations of 0, 500, 1000, and 2000 μg/ml. When larvae completed their development, the emerging flies were counted and examined for morphological abnormalities. **RESULTS:** After the analysis of 400–1000 flies for each concentration, ONZ-treated flies did not show an incidence of malformations above control values, although a significant high number of individuals with reduced body size was observed ($p < 0.005$, χ^2 test). On the other hand, the 1000- and 2000-μg/ml MTZ-treated series presented higher frequencies of total abnormalities than did concurrent and historic controls ($p < 0.05$, χ^2 test), indicating an MTZ effect during developmental morphogenesis. **CONCLUSIONS:** These findings contribute to the characterization of both nitroimidazoles, which are widely used, especially in underdeveloped countries. At the same time, this *Drosophila* bioassay is sensitive enough to detect differential effects of MTZ and ONZ (abnormalities vs. growth effects), showing specificity and selectivity. *Birth Defects Research (Part A) 70:157–162, 2004.*

INTRODUCTION

Developmental biology, genetics, and prenatal toxicology have shown that system models, even those far removed from mammals and humans in the zoological scale, can be useful tools for understanding pathogenic mechanisms in developmental toxicology (Ricciardi, 1997). *Drosophila* and vertebrates show similarities, suggesting that mechanisms involved in the induction of developmental defects may be similar (Petersen, 1990). Therefore, *Drosophila* has been proposed as a useful, rapid, and economical model for the preliminary screening of developmental toxicants (Schuler et al., 1982, 1985; Ranganathan et al., 1987; Lynch et al., 1991).

Imidazole derivatives are widely used in human and animal therapy, as well as in fungicides in plants; therefore, it is important to characterize any possible undesired effects in different experimental models. In particular, the nitrogen group present in nitroimidazole derivatives is

Grant sponsor: PICT; Grant number: 01629/1997-2000; Grant sponsor: UBACyT; Grant number: TB15/1998-2001.
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Published online 2 April 2004 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/bdra.20008

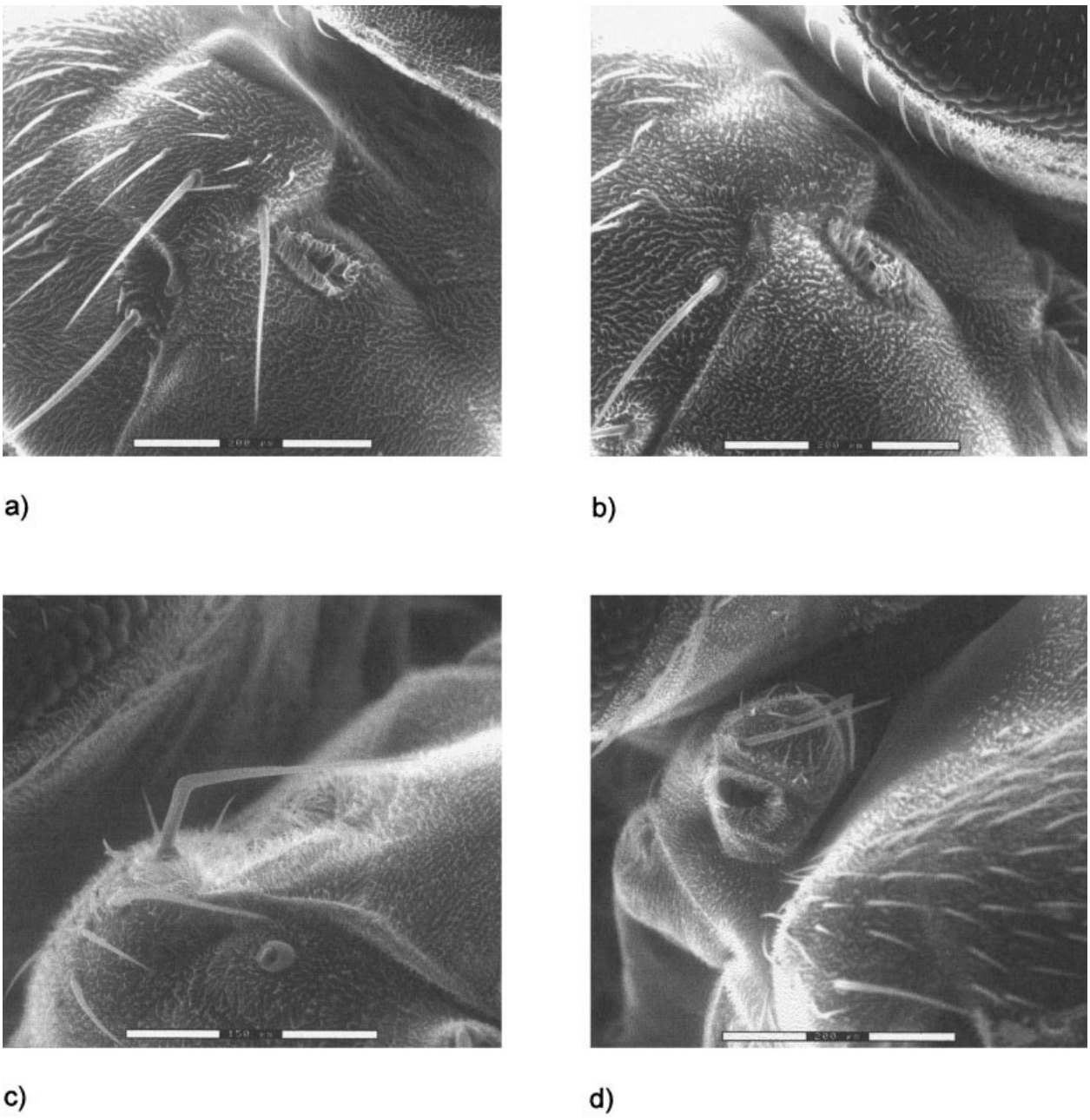

Figure 1. Effects of MTZ treatment on humeral bristles and/or thorax malformations. **a:** Normal humeral bristles (control). **b:** Missing humeral bristles (2000 μg/ml MTZ-treated). **c:** Bent humeral bristles (1000 μg/ml MTZ-treated). **d:** Bent humeral bristles and severe malformation in thorax (2000 μg/ml MTZ-treated).

considered responsible for the mutagenicity of these compounds (Dobiáš et al., 1994). Our previous work at different levels of complexity (from cell to tissue) supported a new characterization of these compounds at the organism level. Using bone marrow cells from different strains of mice, Chinese hamster ovary (CHO) cell lines, or human peripheral blood lymphocytes, we could demonstrate the detrimental effects of some nitroimidazoles (Mudry et al., 1994, 1995; Carballo et al., 2001; Rodriguez et al., 2002; López Nigro et al., 2003).

Although different short-term tests have been applied to characterize the genotoxicity of nitroimidazole effects in different animal or human systems, data on the teratology or developmental toxicology of these compounds derivatives are scarce (Piper et al., 1993; Dobiáš et al., 1994; Mudry et al., 2001). As mentioned previously, *Drosophila melanogaster* has been proposed as a model system for screening developmental toxicity of xenobiotics; thus the present study aimed to provide new data on the effect of two 5-nitroimidazoles, metronidazole (MTZ) [1-(2-hy-

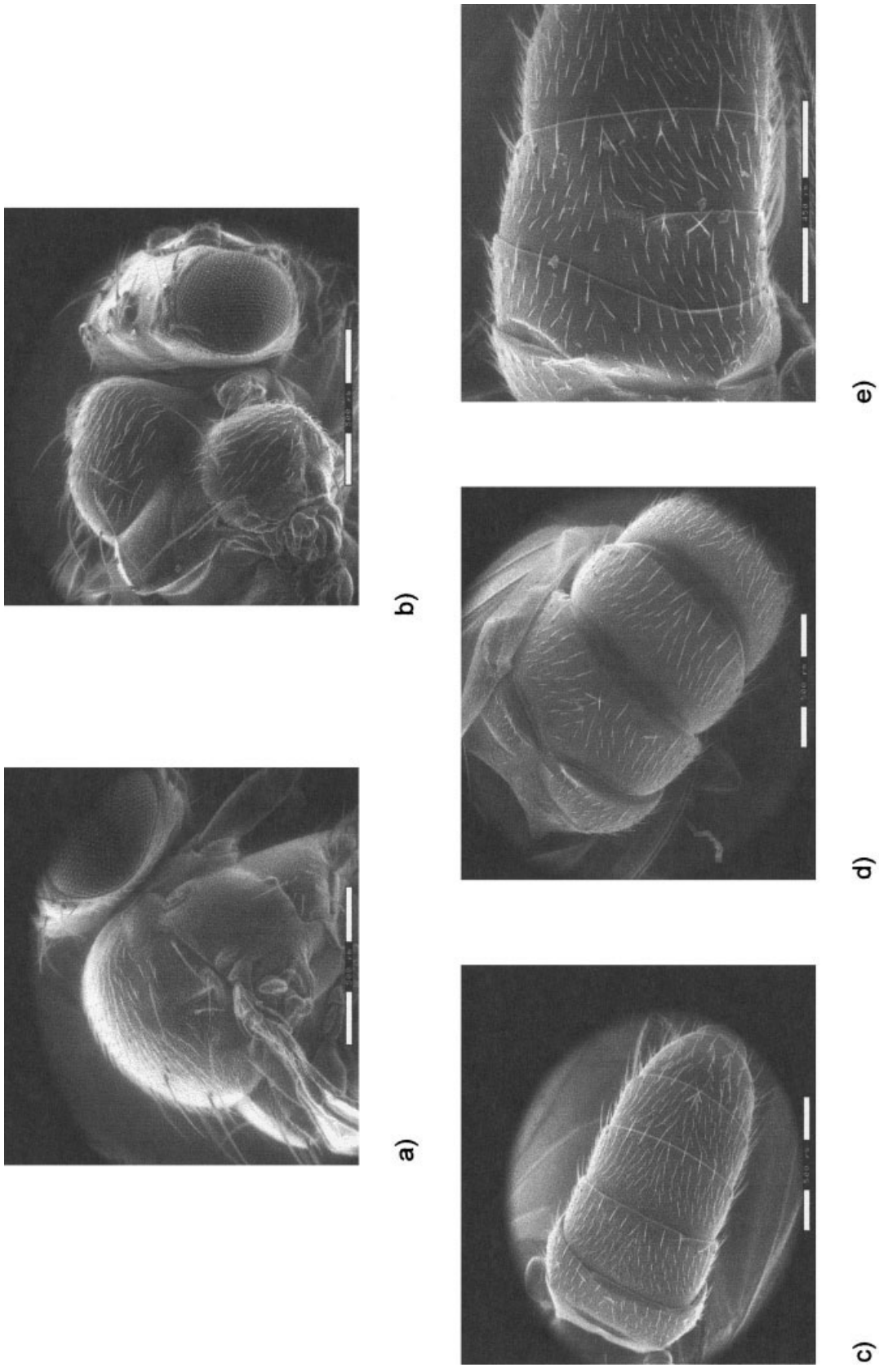

Figure 2. Effects of MTZ or ONZ treatment on thorax or abdomen. **a:** Normal thorax (control). **b:** Thoracic malformations (1000 μg/ml MTZ-treated). **c:** Normal abdomen (control). **d:** Interrupted abdominal tergites (2000 μg/ml ONZ-treated). **e:** Fused abdominal tergites (1000 μg/ml MTZ-treated).

Table 1
Frequency of Malformations after MTZ Treatment

Treatment	Number of malformations					% (malformed/ total flies)
	Wing	Thorax	HB	Abdomen	Other	
Historic control	–	2	24	4	–	1.7% (30/1766)
Control	–	2	5	3	–	1.6% (10/645)
MTZ 500 μg/ml	–	–	6	2	1	2.8% (9/321)
MTZ 1000 μg/ml	2	2	9	2	2	3.9%** (17/439)
MTZ 2000 μg/ml	4	2	8	2	1	3.2%* (17/549)

*$p < 0.05$, **$p < 0.005$, χ^2 test (1 tail).
HB, humeral bristles.

droxyethyl)-2-methyl-5-nitroimidazole] and ornidazole (ONZ) [1-(3-chloro-2-hydroxy)propyl-2-methyl-5-nitroimidazole] on the developmental stages of *D. melanogaster* from the egg through the three larval stages to the pupa.

MATERIALS AND METHODS

Samarkand wild-type *D. melanogaster* were used in all the experiments. The stock was obtained from the collection of the Drosophila Laboratory of the National Agency of Atomic Energy, Buenos Aires, Argentina, where it had been maintained for more than 30 years. Therefore, this stock is highly inbred and homozygous, so it is very useful when one must avoid the appearance of defects due to loss of heterozygosity for recessive alleles.

A total of 10 females were crossed to 15 males for five days, then they were permitted to lay eggs for 24 hr in vials containing a mashed potato medium prepared with a solution of 500, 1000, and 2000 μg/ml MTZ or ONZ. Each vial represents the eggs from 10 females, and each treatment group consisted of triplicate vials of media supplemented with the corresponding concentration of the parenteral solutions of MTZ or ONZ that were used for each dose. Triplicate vials of concurrent controls were run for each experiment.

The medium contained 1 gm of dry instant mashed potato powder per 5 ml of the solution of each drug to be tested in water, with the addition of an alcoholic solution of nipagin (3.6 ml/100 ml of water). The emerging larvae were fed on this medium until pupation. Concurrent control series, in water plus nipagin, were run for each experiment. All series were kept at 25 ± 1°C until full development of the larvae.

Adults were examined for possible morphological alterations by use of a stereoscopic microscope Wild M8 Leica (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). The malformations were classified according to the organ affected, but the evaluation was of the overall frequency of abnormalities, which reflects an overall effect on the population of descendants.

During the systematic observations, special attention was paid to malformations in humeral bristles and on the presence of a "notch" in the wing, because these characters were proposed by Lynch et al. (1991) as good indicators of developmental toxicity. Individuals classified as having reduced body size were those that had half the body size of normal individuals of the same sex and age.

Some of the observed anomalies were further analyzed with a 2010 ElectroScan Environmental Scanning Electron Microscope (ESEM; ElectroScan, Wilmington, MA), which permitted a detailed observation of the alterations and the photographic register presented in results.

Statistical evaluation was performed by means of the χ^2 test for proportions (1 tail).

RESULTS

Most of the malformations that appeared in the offspring affected humeral bristles (Fig. 1) or the thorax or abdomen (Fig. 2). Some of these defects, as mentioned above, were observed and registered by means of the ESEM and are shown in the figures.

No differential mortality was observed in larvae grown in MTZ- or ONZ-supplemented media. None of the nitroimidazole treatments were toxic for adults after 24 hr of feeding. The onset of pupation was the same as in controls in both treatments, although in the 2000-μg/ml ONZ-treated series, a delay in the emergence of adults was observed.

Different responses in individuals treated with MTZ or ONZ were seen when the induction of malformations was studied. The administration of MTZ in the food during the

Table 2
Frequency of Malformations after Treatment with ONZ

Treatment	Number of malformations					% (malformed/ total flies)
	Wing	Thorax	HB	Abdomen	Other	
Historic control	–	2	24	4	–	1.7% (30/1766)
Control	–	–	19	1	–	1.8% (20/1121)
ONZ 500 μg/ml	1	–	13	3	–	1.8% (17/959)
ONZ 1000 μg/ml	–	–	12	1	–	1.8% (13/739)
ONZ 2000 μg/ml	1	–	8	7	–	1.7% (16/946)

χ^2 test (1 tail) not significant.
HB, humeral bristles.

Table 3
Frequency of Adults with Reduced Body Size after ONZ Treatment

Treatment	Flies with reduced body size/total flies	%	p^a
Control	16/1121	1.4	–
ONZ 500 μg/ml	34/959	3.5	<0.005
ONZ 1000 μg/ml	35/739	4.7	$<10^{-4}$
ONZ 2000 μg/ml	96/946	10.1	$<10^{-4}$

[a]χ^2 test (1 tail).

larval stages raised the frequency of abnormalities observed in the adult flies above control values (Table 1) at all three concentrations tested (500, 1000, and 2000 μg/ml); differences were significant at the two higher concentrations (χ^2 test, 1 tail). On the other hand, ONZ did not modify the incidence of malformations observed in concurrent or historic controls (Table 2). It should be noted that the experiments with ONZ were repeated in order to confirm these negative results. In addition, significant differences in the frequency of individuals with reduced body size were observed after ONZ treatment (Table 3), but not after MTZ administration.

DISCUSSION

Metronidazole, a systemically active trichomonicide introduced in the mid-1950s, is one of the most widely used drugs during pregnancy, although it passes freely through the placental barrier. Some studies seem to show that MTZ does not pose any serious embryotoxic or teratogenic hazards to humans (Morgan, 1979; Roe, 1985; Piper et al., 1993, Diav-Citrin et al., 2000), and that the deleterious MTZ effects observed during human development may be closely associated with the lifestyle, sexual promiscuity, and alcohol intake of the mother (Dobiáš et al., 1994). Previous experimental in vivo evaluation in adult female rats mated after MTZ treatment showed different types of damage, increasing the frequency of post-implantation death and dominant lethals (Mudry el al., 2001). In the present experiments with wild-type *Drosophila*, MTZ values of malformation frequencies were increased for higher doses (1000 and 2000 μg/ml; $p < 0.05$; χ^2 test). These results are in accordance with data reported by Ivanov (1969) in guinea pigs (cited in Dobiáš et al., 1994), although as mentioned earlier, other studies of the offspring of women who had been treated with MTZ during pregnancy do not clearly indicate that it is teratogenic or embryotoxic. In nine cases of cleft lip, Czeizel and Rockenbauer (1998) found a higher than expected number of mothers treated with MTZ during the second and third months of gestation, but they concluded that the drug presented no clinical association with congenital abnormalities. Nevertheless, in a retrospective cohort study to evaluate the role of in uterine exposure of MTZ and the risk of subsequent cancer, Thapa et al. (1998) observed an increased incidence for neuroblastomas that required further evaluation.

ONZ had excellent tolerability in humans when administered during pregnancy, and the children born to the treated patients showed normal development and growth (Bourget et al., 1995). Notwithstanding, reproduction studies of ONZ in rats showed that male, but not female, fertility was affected by the drug; spermatotoxicity was the main effect detected (McClain and Downing, 1988; Linder et al., 1992; Bone et al., 1997; Cooper et al. 1997). In our experiments, the flies that had been treated with ONZ during their larval stages did not show significant differences in the incidence of defects observed, confirming in *Drosophila* the results reported in other animal models. The only consequence of the treatments was a remarkable dose-dependent increase in the frequency of reduced body size in individuals (Table 3).

As mentioned previously, *Drosophila* has frequently been used as a valuable model for screening of developmental toxicants, not only in vivo but also in vitro (Bournias-Vardiabasis et al., 1983). We believe that the use of this organism as an experimental toxicology model is promising because it is economical and time-efficient to use in the screening of potential developmental toxicity of chemicals or environmental contaminants.

In the present study, the assays were performed with a highly inbred and highly homozygous wild-type strain of *D. melanogaster*. Thus, the anomalies observed are not the consequence of loss of heterozygosity for recessive genes due to somatic mutational events (such as deletions or chromosome losses), but to developmental morphogenesis alterations.

In addition, the assay was sensitive enough to distinguish between the differential effects of MTZ and ONZ, one inducing developmental abnormalities, and the other causing growth effects. This finding strengthens the utility of this assay because it suggests specificity and selectivity.

ACKNOWLEDGMENTS

We thank R. Vázquez and N. Andrioli for the technical assistance during their training in Drosophila assays in the GIBE (Facultad de Ciencias Exactas – UBA).

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