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Impact of the Medicine Hydroxyurea on Reproduction and Development, Using Drosophila Melanogaster as a Model Organism

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Abstract: The clinical efficacy of oral hydroxyurea (HU) in the treatment of the sickle cell disease (SCD) is already proven. However, side-effects on reproduction and development still cause concerns, mainly in long term treatments. We used *Drosophila melanogaster* as a model organism in the study of this subject. This species has been considered favorable for studying human diseases and responses to drugs because both organisms share more than 50% of the genes for disease and exhibit a very similar drug metabolism. In addition, *D. melanogaster* allows laboratory approaches that are not possible in humans. We analyzed the impact of the concentrations HU 0.1 and HU 0.25 on productivity, oviposition rate, emergence period duration, mortality and development time from egg to adult. HU affected all these traits, showing dose dependence. The frequency of mating, and duration of premating and mating times of flies were also analyzed in treatments with HU 0.25. Considering all the traits, the treatments decreased the productivity, oviposition rate and frequency of mating, and increased the emergence period duration, mortality rate from egg to adult and development time. The premating and mating times duration were affected in a more complex way. On the basis of the knowledge that HU affects DNA synthesis and repair, and other data available in the literature, we raised hypotheses to explain the present observations. The results and hypotheses suggested new approaches for further studies of particular and important aspects. In general, this study reinforces the validity of the concern with HU side-effects.

Keywords: Emergence period duration, impaired effect of hydroxyurea, mortality from egg to adult, number of progeny, oviposition rate.

1. INTRODUCTION

Hydroxyurea (HU) ($CH^4N^2O^2$), also referred to as hydroxycarbamide, has been used for decades as a medicine for treatment of several human diseases, including neoplasias, virus diseases and sickle cell disease (SCD) [1- 3]. In spite of the long time lasted from the start of the use of HU [4], the mechanisms underlying its action remain only partially known. HU acts as a cytological toxic, with specific effects on the S phase of cell cycle, blocking DNA synthesis. In neoplasias, the therapeutic effect is due to cell death caused by the blocking. In virus diseases, the lack of sufficient DNA building blocks (also resulting from the toxic effect on the cell cycle), prevents the production of functional viral particles. In both cases the basic medical effect of HU is due to the inhibition of the ribonuclease reductase, an ubiquitous intracellular enzyme that converts ribonucleotides to desoxyribonucleotides, required for DNA synthesis [5, 6]. When DNA synthesis is inhibited by HU, the normal cell division is blocked by the action of checkpoints, a kind of "quality controls" of complex molecular nature (involving several proteins) that are present in different phases of the cell cycle. The first checkpoint is located at the end of the cell cycle's G_1 phase, just before entry into S phase where DNA is duplicated. This checkpoint ensures that mitosis does not continue until DNA synthesis is completed or until the abnormal DNA is repaired. The impossibility to restore DNA prevents cell division and leads the cell to death (apoptosis) [7, 8]. The mechanisms involved in the cell toxicity by HU have been focused in many studies, revealing a variable influence of different gene mutations and accumulation of gene products [9, 10].

In SCD, the medical efficacy of HU is due to action on blood. SCD is a genetic disease due to a single gene mutation in the ß-globin chain of the hemoglobin [11]. In the people bearer of this disease, the S hemoglobin (HbS) has a tendency to polymerize, leading the erythrocytes to assume a sickle shape. The treatment with HU produces an increased synthesis of fetal hemoglobin (HbF) that is translated into a rapid and intense increment in HbF-containing reticulocytes, without significant bone marrow toxicity. This increase inhibits the polymerization of sickle hemoglobin (HbS) that is responsible for clogging the small blood vessels, causing tissue damage, pain and anemia [11, 12]. The basic mechanisms that cause the increased HbF synthesis are still largely unknown. For some authors the process is mediated by the nitric oxide (NO) radical, which is generated by HU *in vivo* and *in vitro* and involves molecular regulation that occurs through complex interactions cis and trans to the beta globin gene locus [13, 14].

In addition to the interference in the syntheses of HbF, HU reduces till three times the synthesis of endothelin-1, a

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vasoconstrictor possibly involved in the adherence of sickle cells to the endothelium that is the cause of vase-occlusion and crisis. Other HU effects on the properties of erythrocytes may also be important for therapeutic efficacy [15-17].

Over time, other therapies were developed and are also used with success for neoplasias and virus diseases treatment. However, for SCD, HU remains the predominant form of preventive treatment of the painful symptoms and complications caused by the disease. Currently, bone marrow transplant offers the only potential cure for SCD, but finding a donor is difficult, the process is expensive and the procedure has serious risks for patient's life due mainly to severe infections and immune system problems [18, 19].

The introduction of HU in the treatment of SCD was considered a turning point because it increases greatly the prognostic, allowing a high number of patients to live longer, enjoying an improved life quality. However, HU treatment presents side effects that cause medical concern, mainly in long-term treatments. In addition to possible teratogenic, mutagenic and carcinogenic effects [20, 21], there are concerns about decrease of reproductive capacity, and developmental problems [22, 23]. This concern is reinforced by the fact that HU was included in the list of substances to be evaluated as to the potential for adverse effects on reproduction or development of the National Toxicology Program from US Department of Health and Human Services [24].

It is clear that as the knowledge about the side effects of HU becomes greater, the possibility to monitor and eventually counteract such effects increases. However, many of the HU side effects are difficult to study in humans due to methodological reasons and thus they are lacking information.

Drosophila melanogaster has been a successful model for the study of many human diseases, including cancer, neurodegenerative diseases, immune system problems, vision and hearing pathologies, heart diseases and many others [25-28]. Besides the easy maintenance in the laboratory and the extensive knowledge on its biology (accumulated in more than a hundred years of study), D. melanogaster presents high homology with man with regard to disease-causing genes, less complex processes that trigger the diseases and ability to respond to drugs similarly to man [29-31].

We treated *Drosophila melanogaster* with HU with the aim to detect adverse side effects in aspects of the reproduction and development. We also expected that eventually we could find possibilities for further studies of these biological traits that in some way could benefit humans.

2. MATERIALS AND METHODS

2.1. Species and Culture Medium

Drosophila melanogaster was collected in São José do Rio Preto, SP, Brazil (Lat. 20° 49' 11" Long. + 49° 22' 46" + = 3h 17m 31s, H 489). These flies have been kept in the stocks of the Department of Biology from IBILCE – UNESP, in the standard culture medium of banana-agar and 20 ± 1°C temperature. The same conditions were used for maintaining the experiments.

2.2. Procedures Used in the Experiments

Basically the study involved the treatment of *D. melanogaster* with HU in six consecutive generations. The parents of the first generation were 45 pair-mating crosses of five days old virgin flies, individually prepared in tubes, for the control (C) and the treated (T) experiments, aiming to be sure to start with a reasonable number of crosses yielding descendants. The parents for the consecutive five generations were 45 virgin females and males taken from the previous generation. In C and T experiments, the 45 couples were divided into three replicates (15 couples each), prepared in bottles. Three transferences to fresh culture medium were done at intervals of four days, in every generation.

Hydroxyurea ($CH_4N_2O_2$) is a white, crystalline and soluble in water powder which spreads equally by the body fluids [32]. The treated experiments received HU in the concentrations 0.1 or 0.25 μg/mL of culture medium (HU 0.1 and HU 0.25, respectively). The two concentrations were used in order to detect dose dependence effects. HU was dissolved in the warm culture medium and then put into the vials. Bottles and tubes received 40 ml and 4 ml of culture medium for raising the flies, respectively. Hydroxyurea was provided by the Center of Hematology, Faculty of Medicine from São José do Rio Preto (FARME).

2.3. Characteristics Studied

We studied the effect of HU on the characteristics productivity (number of progeny), oviposition rate (number of eggs), emergence period duration (time lasted from the first to the last adult emerged), mortality and development time from egg to adult, frequency of mating and duration of premating and mating times.

The characteristic productivity was evaluated in the six generations. The other traits were analyzed in the progeny of F3 or F4 generations.

2.3.1. Productivity

This study involved the daily counting of the progeny in each vial of the replicates, in every generation, separately by sex, from the first till the last fly emerged.

2.3.2. Oviposition Rate

Twenty crosses of virgin, five days old flies were prepared for each experimental group (control, HU 0.1 and HU 0.25). Twenty four hours later, the females of the couples were placed individually into empty bottles which had a tablespoon (fixed in the stopper), containing culture medium prepared with agar-agar and sugar (100 ml water, 0.5 g agar-agar and 2.5 g sugar). Yeast (*Saccharomyces cerevisiae*) suspension was placed over the medium in about half the spoon to activate the oviposition. Spoons containing eggs were replaced by spoons with fresh medium every 24h until the oviposition ceased. The eggs laid in the spoons were counted as soon as they were removed from the tubes.

2.3.3. Emergence Time Duration, Mortality and Developmental Time from Egg to Adult

This study started with 200 eggs per experiment (C, HU 0.1 and HU 0.25), laid in spoons containing culture medium like in the tests for oviposition rate. The age of the eggs used

was as close as possible. The 200 eggs were divided into eight bottles containing the same culture media of the experiment from which the eggs were taken. The flies were counted daily until the last emerged, in order to detect the distribution of emergence in days. The difference between the number of eggs that started the experiments and the number of adults produced allowed to evaluate the mortality during development, and the time lasted between the oviposition and the emergence of the adult flies gave a measure of the developmental time duration.

2.3.4. Premating and Mating Time Duration, and Frequency of Mating

In this study only HU 0.25 concentration was used in the treatment of flies. Four types of experiments were prepared with five days old, virgin couples individually put into empty tubes as follows: (1) C males and females, (2) HU 0.25 males and females, (3) C males and HU 0.25 females and (4) HU 0.25 males and C females. The behavior of the couples was observed for one hour, in the morning (at 10:00h; 24°C), and in the afternoon (at 15:00h; 26°C). Ten couples of each combination were analyzed for type of experiment, in each period of the day.

2.3.5. Statistical Analysis

The statistical analysis of data involved the use of ANOVA (analysis of variance) and nonparametric tests of Kruskal-Wallis and Mann-Whitney [33, 34]. The software used was the Minitab Release Package 14.

3. RESULTS

3.1. Productivity

Table **1** shows data on the mean productivity per parental couple, in the control and treated mediums (HU 0.1 and HU 0.25), computing separately male and female offspring and their sum (total), for each generation.

Comparisons by Student's t-test showed that female and male progeny proportions did not differ in the three groups of experiments, in every generation. Considering together the six generations, the total number of males (M) and females (F) produced per experimental group was also very close (C: F= 8,848, M= 8,640; HU 0.1: F= 6,507, M= 6,499; and HU 0.25: F= 4,928, M= 4,942).

ANOVA for comparison of the mean productivity among groups and pairwise multiple comparisons in each generation showed significant differences only in F5 generation (F = 21.5; P = 0.002), involving the comparisons between C and each of the treatments HU 0.1 and HU 0.25. The confidence interval for difference of means (IC [μ_1- μ_2; 95%]) showed: C *versus* HU 0.1= -55.1, -16.2; C versus HU 0.25 = -55.6, -16.8). However, in the light of the numbers, the productivity of couples was always higher in C than in T experiments. These in turn showed that, in all generations except the first, the flies from the experimental group HU 0.25 had lower productivity than those from HU 0.1.

3.2. Oviposition Rate

The number of eggs laid in C and T culture media were daily computed until the cessation of oviposition, which occurred in the tenth day, in the three groups (Table **2**). The total number of eggs obtained in C, HU 0.1 and HU 0.25 showed 55% and 48% decrease in the comparison of C with HU 0.25 and HU 0.1 with HU 0.25, respectively. Using the nonparametric statistics Kruskal-Wallis (H = 7.94; P = 0.019), followed by Mann-Whitney test for pairwise comparisons, significant differences were obtained in C *versus* HU 0.25 (W=109.5; P=0.038) and HU 0.1 *versus* HU 0.25 (W=116.0; P= 0.008).

In the three groups, the fourth day showed the greatest number of eggs, although this number in C had been more than double greater than that in HU 0.1 and more than triple that in HU 0.25. In C, the oviposition until the fifth day accounted for approximately 80% of the total number of eggs while in the same period the eggs in HU 0.1 accounted for 67.5% and in HU 0.25 for 64%, suggesting some delay in the egg production or maturation in the treated groups. Kruskal Wallis followed by pairwise comparisons performed using Mann-Whitney test of the three groups showed significant differences in the second, third and fourth oviposition days. In every case, C was greater than HU 0.25 (2nd day: (W=487.5; P=0.029), 3rd day: (W=515.0; P=0.004), and 4th day: W-515.5; P=0.003), but C *versus* HU 0.1 and HU 0.1 *versus* HU 0.25 did not differ.

3.3. Emergence Time Duration, Mortality During Development and Mean Developmental Time from Egg to Adult

Data on these three characteristics are in Table **3**. As already mentioned, the emergence time duration was analyzed putting 200 eggs per experimental group to develop and computing the number of adults produced, from the first to the last fly emerged. Adults from the experimental groups C, HU 0.1 and HU 0.25 started the emergence period at the same day. However, while in C and HU 0.1 experiments, the emergence time was concluded in three days, in HU 0.25 it lasted five days. In the light of the numbers, the emergence in C and HU 0.1 groups was higher in the second emergence day (136 and 130 flies, respectively) and in HU 0.25 the emergence was greater in the third day (79 flies).

The total numbers of adults obtained in C, HU 0.1 and HU 0.25 experiments were 200, 195 and 176, respectively and may be used for evaluation of the mortality during development from egg to adult stages. In comparison with C, in which the number of adults produced was the same number of eggs used to start the experiment, the total numbers of adults produced in HU 0.1 and HU 0.25 showed 2.5% and 12.0% mortality during development, respectively.

Mean development time from egg to adult, in hours, was also computed. In the light of the numbers, this time was greater in HU 0.25, for both females and males than in the other groups. This was confirmed by statistical analysis, performed separately for males and females. The comparison of the mean development time of males in the three mediums, by ANOVA, indicated the existence of significant differences (F= 57.70; P<0.001). Pairwise comparisons (Tukey 95% confidence intervals) showed, for C X HU 0.1 (-3,69; 9,14), for C X HU 0.25 (17,41; 30,15) and for HU 0.1 X HU 0.25 (14,60; 27,51) confirming significant differences for C X HU 0.25 and HU 0.1 X HU 0.25. Significant differences were also detected for females, in the same comparisons.

Table 1. Productivity in the three experimental groups (control and hydroxyurea treated HU 0.1 and HU 0.25 mg/mL), per generation (F1 to F6), computing separately female (F) and male (M) offspring and their sum (T). N = total number of progeny; (χ) and (sd) = mean and standard deviation of progeny per parental female.

Generation	Group	F			M			T		
		N	χ	sd	N	χ	sd	N	χ	sd
	Control	671	14.9	4.3	635	14.1	4.2	1306	29.0	8.4
F1	HU 0.1	396	8.8	3.2	367	8.2	3.9	754	16.8	6.6
	HU 0.25	494	11.0	2.0	482	10.7	3.5	976	21.7	5.4
	Control	1736	38.6	11.0	1796	39.9	9.1	3532	78.5	20.1
F2	HU 0.1	963	21.4	15.2	1059	23.5	15.5	2022	44.9	30.8
	HU 0.25	434	9.6	1.2	399	8.9	1.3	833	18.5	2.1
	Control	1618	35.9	2.9	1575	35.0	5.4	3193	70.9	8.3
F3	HU 0.1	1416	31.5	6.5	1429	31.8	8.1	2845	63.2	14.6
	HU 0.25	1021	22.7	4.8	967	21.5	4.4	1988	44.2	9.0
	Control	1759	39.0	3.7	1766	39.2	3.8	3525	78.3	7.4
F4	HU 0.1	1560	34.7	0.5	1594	35.4	2.6	3154	70.0	2.3
	HU 0.25	1250	27.8	6.8	1294	28.8	5.0	2544	56.5	11.8
	Control	1451	32.2	4.2	1388	30.8	3.8	2839	63.1	8.0
F5	HU 0.1	647	14.4	5.3	587	13.0	5.0	1234	27.4	10.3
	HU 0.25	581	12.9	2.1	630	14.0	1.9	1211	26.9	3.1
	Control	1613	35.8	6.8	1480	32.9	5.6	3093	68.7	12.4
F6	HU 0.1	1525	33.9	4.9	1463	32.5	4.8	2988	66.4	9.6
	HU 0.25	1148	25.5	7.4	1170	26.0	7.6	2318	51.5	14.7

Table 2. Number of eggs daily lead by 20 *Drosophila melanogaster* females in nine days of oviposition, total laid in the entire period, daily mean number, median (md) and standard deviation (sd) for the experiments control (C) and hydroxyurea treated (HU 0.1 and HU 0.25 mg/mL).

Group	Number of Eggs and (sd) per day									Total	χ/day	md	sd
	1	2	3	4	5	6	7	8	9				
C	91	165	147	273	108	89	65	47	6	991	110	91	77.9
	(7.8)	(10.3)	(7.5)	-12	(6.1)	(8.1)	(4.8)	(3.9)	(0.6)				
HU 0.1	137	95	97	128	115	68	103	99	9	851	94.6	99	37.8
	(6.7)	(4.5)	(6.2)	(8.8)	(6.0)	(4.8)	(7.4)	(5.5)	(0.9)				
HU 0.25	41	67	56	80	41	33	69	53	6	446	49.6	53	22.3
	(4.7)	(6.3)	(4.4)	(7.4)	(3.0)	(2.4)	(4.9)	(4.9)	(0.8)				

3.4. Frequency of Mating and Premating and Mating Time Duration

As mentioned before**,** these observations were made for C and HU 0.25 treated groups, in four combinations of C and T flies. The observation lasted one hour in the morning and in the afternoon. Data in Table **4** for comparison of the four combinations showed, in the light of the numbers, that the frequency of mating was higher for combinations involving males and females C, in the two periods of observations.

The mean duration of the premating time in C couples was greater than in the other combinations, but the difference was apparently meaningful only in the comparison of C X C

Table 3. Duration of the emergence period (in days), daily distribution and total (T) of emerged *Drosophila melanogaster* flies, computing separately adult males (M) and females (F), and mean duration of the development time from egg to adult in hours [χ (h)]. Two hundred eggs started each experiment (control (C) and treated with hydroxyurea (HU 0.1 and HU 0.25mg/mL).

Group	Emerged Flies per Day										Total			χ (h)	
	1		2		3		4		5						
	M	F	M	F	M	F	M	F	M	F	M	F	T	M	F
C	5	28	49	87	18	13	-	-	-	-	72	128	200	316	309
HU 0,1	3	19	42	88	23	20	-	-	-	-	68	127	195	319	312
HU 0,25	1	9	13	29	34	45	17	16	5	7	70	106	176	340	332

Table 4. Mating frequency (in %) and premating and mating time duration (in min) observed twice a day in *Drosophila melanogaster*, using couples formed by males (M) and females (F) from experiments control (C), treated with hydroxyurea (HU 0.25/mL) (T) and combinations of C and T in both directions. Ten virgin couples, five days old, were used to start every experiment, in each period.

Couples			Period	Mating Number and %	Premating Time			Mating Time		
F		M			Minim	Max	χ	Minim	Max	χ
C	X	C	Morning	5	9	60	33	19	28	22
			Afternoon	8	7	42	19	3	22	16
			Total	13 (65%)						
T	X	T	Morning	2	21	35	28	21	23	22
			Afternoon	5	6	29	16	13	18	16
			Total	7 (35%)						
C	X	T	Morning	4	17	47	31.5	20	27	23
			Afternoon	1			13			16
			Total	5 (22%)						
T	X	C	Morning	2	6	7	6.5	20	26	23
			Afternoon	6	10	32	17	15	22	18
			Total	8 (40%)						

with the mean duration obtained in the morning for T X C combination, exactly in the period in which the premating time was greater for the remaining combinations. The duration of mating time was only slightly greater in the combinations involving males and females from different groups. The sample size for analysis of the premating and mating times duration need to be increased in order to reinforce the present observations

4. DISCUSSION

We tested, in *D. melanogaster*, the effect of two HU concentrations (HU 0.1 and HU 0.25) on the biological traits productivity, oviposition rate, emergence period duration, developmental time from egg to adult, mortality during development, frequency of mating, and premating and mating times duration. HU affected all these traits.

The number of progeny that was evaluated in six successive generations of treatment was reduced in every of them, in the two used concentrations, although statistically significant differences in relation to C were found in a single generation, The experiments treated with HU 0.25 had lower productivity than the experiments treated with HU 0.1 in all generations, except the first. Compared with C, the sum of female and male progeny in the six generations was reduced 26% in HU 0.1 and 44% in HU 0.25 treatments. HU did not affect differentially male and female progeny numbers.

We studied the effect of HU on productivity in six successive generations in order to detect eventual increase of the toxic effects through generations. The results do not support this hypothesis since the profiles from control and treatments presented the same pattern of variation along generations.

The dose dependent effect observed in the decrease of productivity was also observed in the other characteristics treated with the two HU concentrations.

Oviposition rate was also reduced in the treated experiments, although the period of oviposition has had the same duration (nine days), in the three groups. Oviposition days with significant egg number differences among groups exhibited values higher in C than in HU 0.25 and smaller in HU 0.25 than in HU 0.1. Considering the totality of eggs laid in the nine days, HU 0.1 and HU 0.25 treatments showed 14% and 55% decrease in relation to C, respectively,

The decreased numbers of eggs and progeny caused by HU indicate a relationship of cause and effect: smaller number of eggs, fewer adults. However, the mortality during development from egg to adult also analyzed in this study, which was responsible for 2.5% and 12% death in HU 0.1 and HU 0.25 treatments, respectively, has also to be considered. HU toxicity that affected embryo life in a lethal way produced statistically significant differences in the comparisons C *versus* HU 0.25 and HU 0.1 *versus* HU 0.25. Because the mortality of males did not differ among the three experimental groups, contrarily to females, it seems that HU susceptibility of females is greater than that of males, relatively to this trait.

HU produced delay in the development of the flies from egg to adult stages, statistically greater in HU 0.25 treated flies than in C and HU 0.1 experiments. The time increased did not differ between males and females analyzed separately, indicating similar effect of HU on both sexes. The developmental delay seems to be responsible for the increase of two days in the duration of the emergence period observed in flies from HU 0.25 treatment when compared to C and HU 0.1. While the emergence time of adults lasted five days in HU 0.25 treatment, in C and HU 0.1 it lasted three days.

Molecular and cytological information available in the literature may be used in hypotheses to explain the observed effects on the analyzed traits. As already mentioned, HU inhibits ribonucleotide reductase, an enzyme necessary for production of the building blocks for DNA synthesis. In this case, cell division does not proceed and if the HU treatment is maintained, the arrest in cell division can cause death of the dividing cells [7].

Another damage of HU is that it may impair the chromosome stability, producing chromosome aberrations. Previous studies in *D. melanogaster* showed that HU affects several genes and products involved in the control of cell division. When the DNA that codes for these regulatory proteins is damaged, the process of mitosis may proceed, even in the presence of HU, producing anomalous cells. For example, kinases Chk1 and Chk2 are proteins that evaluate the genomic normality in the second checkpoint of cell division, active at G2/M [35]. In the presence of HU, these kinases fail in their task, causing entry of the cell in division even in the presence of impaired DNA integrity, delaying and altering mitosis and meiosis. These effects may cause larval lethality, which was attributed to an abnormal enhancement of chromosome aberrations reducing the stability of the mitotic chromosomes.

Such impaired effects of HU treatment on mitotic and meiotic cells may be at the basis of some deleterious effects we detected in the present study. They may affect oogenesis, thus explaining the decrease of the egg numbers; and may be causing the increase in development and emergence times. The increase in the mortality rate during development may be dependent on the physiological importance of cells lead to death, parallel to the degree of larval HU susceptibility. All these aspects deserve further analyses, which at least in part are relatively easy to be done in *Drosophila*.

As it was also mentioned, the literature shows that HU affects sperm in different ways [22, 23]. The present study in *Drosophila* indicates that the female gametes are also affected by HU treatment. It will be important to study the cytological and molecular processes underlying the HU harmful effects on oogenesis.

In the present data, *Drosophila* reproduction was also HU affected in relation to the frequency of couples that mate. The frequency of mating was higher for C X C than for T X T and "hybrid" combinations and, among these last, the frequency was greater in the couples formed by T females and C males than in the opposite direction, which was the more affected among combinations.

The mean premating time (in the morning, which was the period of longer premating times) for couples formed by T females and C males was about five times smaller than that in the opposite combination; the mating time in turn was slightly greater in the "hybrid" than in C X C and T X T combinations. Although we recognize the need to increase the sample size in this experiment, these observations already suggest that some changes due to HU may occur.

In *Drosophila*, the occurrence of mating is dependent on the male courtship behavior whose steps must be recognized by females. Thus, hypothetically, changes of the courtship components in HU treated males may preclude their recognition by females. The male courtship in *Drosophila* is a complex process that involves cellular components of neural circuits [36, 37]. Chemical ablation of neural cells due to HU treatment has been used for studying nervous system development and function in *Drosophila* [38, 39]. Among many of these ablation results exhibited in the literature, it is important for the present discussion to mention those revealing that one of the main structures of the brain, which is named central complex, coordinates behavior programs in *D. melanogaster*, including the program of the sound produced by males, which is an important component of the courtship [40, 41]. It is known that this sound is species-specific and its changes difficult or even block the male acceptance by female, thus acting as a mechanism of sexual isolation [42]. These findings incite the idea of association between brain damage due to HU action and the performance in mating. This is another possibility for further studies. .

From data in the literature, to which we may add the present results, it is clear that the knowledge of the mechanisms that generate both benefits and harmful side-effects of HU are still dependent on many studies. In the present study, *Drosophila* showed to be quite susceptible to the effects of HU opening possibilities to improve knowledge on the effects of this substance through cytological and molecular approaches.

In conclusion, the present observations reinforce the concern about the effects of HU on reproduction and development. Considering that at now there is no appropriate substitute for HU in the SCD treatment, and considering that its harmful effects are dose dependent, it is important to focus on the need of a special care to find the minimal therapeutic dose and the minimal effective treatment time for keeping the treated patients less vulnerable to them.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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