Genetics and Molecular Biology, 23, 1, 105-107 (2000)

Short Communication

Effect of stannous chloride combined with caffeine on fecundity of *Drosophila prosaltans*

Mary Massumi Itoyama and Hermione Elly Melara de Campos Bicudo

Abstract

The present study analyzed the number of progeny of stannous chloride- and/or caffeine-treated *Drosophila prosaltans*. A significant decreasing effect was observed in every case when compared to the control, except for the smallest stannous chloride dose used. Combinations of both substances using two different stannous chloride doses did not differ significantly from each other but number of progeny decreased 15% with the higher concentration combination when compared with caffeine-treated flies. The present results and data in the literature indicate that the effects of continuous ingestion of stannous chloride should be studied.

INTRODUCTION

Several studies have considered the sum effect of caffeine and other agents affecting DNA, such as X-rays (Mendelson and Sobels, 1974), gama-rays (Targa, 1983), UV radiation (Cremer *et al.*, 1983), or substances such as the alkylating agent Thiotepa (Kilhman *et al.*, 1982), tumorigenic drugs (Granberg-Ohman *et al.*, 1980) and benzamide (Das, 1987). In some cases caffeine has shown a potentiating effect on mutagenic agents.

In this study we analyzed fecundity of *Drosophila prosaltans* treated with caffeine combined with stannous chloride ($SnCl_2$). In a previous study, in the same species, caffeine alone induced a dose-dependent fecundity decrease (Itoyama and Bicudo, 1992).

Mutagenic effects of caffeine were studied in prokaryotic as well as eukaryotic organisms with variable results, but in several cases it was considered a DNA repair inhibitor (Mendelson and Sobels, 1974; MacPhee and Leyden, 1985; Sasaki *et al.*, 1989). Contradictory results were obtained for stannous chloride, in some cases showing genotoxic activity, causing changes in *Escherichia coli* DNA (Cunha *et al.*, 1991) and decreasing SOS response in the same organism (Olivier and Marzin, 1987). In *Drosophila melanogaster* (Tripathy *et al.*, 1990) and *Saccharomyces cerevisiae* (Singh, 1983), toxicity was observed but no mutagenic effect seemed to occur. Other studies on *E. coli* indicated induction of SOS response by treatment with $SnCl_2$ (Bernardo-Filho *et al.*, 1994) and involvement of exonuclease III in repairing lesions caused by $SnCl_2$ (Cabral *et al.*, 1998). Increasing doses of $SnCl_2$ were also observed to strongly reduce surviving of *E. coli* cells (Cabral *et al.*, 1998). Thus, if $SnCl_2$ affects DNA and elicits DNA repair inhibited by caffeine, greater impairment might be expected from their ingestion together than separately.

Caffeine is largely consumed in coffee, teas and medicines (Benowitz, 1990) while stannous chloride, a powerful reducing agent, is used in radiopharmacy to synthesize scintigraphic tracers employed in nuclear medicine. The food industry uses it as a preservative (e.g., in soft drinks) and in some fluoride tooth pastes. Possible joint consumption of these drugs, therefore, makes relevant information on their effect, isolated or in interaction, even with other organisms used as indicators.

MATERIAL AND METHODS

Drosophila prosaltans (saltans group, saltans subgroup) is maintained in our laboratory at $20^{o} \pm 1^{o}C$, in banana-agar culture medium. The strain used is from Sangre Grande, Trinidad. Six-day-old virgin males and females were mass crossed (10 couples per vial) in bottles containing banana-agar culture medium (control experiments) or containing this culture medium with caffeine added (2,000 µg/ml; experiment named t_4) or with stannous chloride (500 µg/ml or 2,000 µg/ml; experiments named Sn_1 and Sn_2, respectively) or with both substances ($t_4 + Sn_1$ and $t_4 + Sn_2$). Six bottles were prepared for each type of medium. Flies were transferred once to new food of the same type, after five days. Parents were discarded five days later. Progeny were computed separately by sex.

Caffeine used was Cafeina Anidra, P.A. (1,3,7-trimethylxanthine), Reagen, Quimibrás, Indústrias Químicas S.A., Brazil. Stannous chloride ($SnCl_2.H_2O$) was Baker analyzed Reagent, Química Moura Brasil, S.A., Brazil.

For statistical purposes, exploratory data analysis, variance analysis for transformed data (square root) and lin-

Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas de São José do Rio Preto, Universidade Estadual "Júlio de Mesquita Filho" - UNESP, 15054-000 São José do Rio Preto, SP, Brasil. Send correspondence to H.E.M.C.B.

ear model adjustment were used (Neter and Wasserman, 1974; Peres and Saldiva, 1983).

RESULTS AND DISCUSSION

Fly fecundity in the six experiments is in Table I. Because the numbers of males and females did not differ significantly from each other in the progeny of every experiment, only the total progeny numbers are presented. ANOVA (analysis of variance) applied to the transformed data showed significant values ($F = 58,36$; $P \leq 0.05$). Multiple comparisons of Tukey (Table II) showed significant differences in comparisons of control experiment with every other experiment except that in which 500 µg/ml of stannous chloride was used alone. Both mixtures of substances ($t_4 + Sn_1$ and $t_4 + Sn_2$) differed significantly from Sn_1 and Sn_2 but not from t_4. Combined treatments also did not differ. Besides, results using the two doses of stannous chloride did not differ significantly from each other.

Administered isolatedly, caffeine as well as stannous chloride in a 2,000 µg/ml concentration of culture medium decreased fly fecundity significantly. As mentioned, for caffeine this had already been shown by Itoyama and Bicudo (1992) and Itoyama *et al.* (1998). However, when the flies were fed stannous chloride and caffeine combined, fecundity decrease did not differ significantly from flies fed caffeine alone. Nor did results differ when, combined with caffeine, stannous chloride in the doses of 500 µg/ml and 2,000 µg/ml was used, indicating that its effects were not cumulative with those of caffeine in decreasing fecundity when jointly administered. However, considering progeny number, there was a decrease of about 15% between $t_4 + Sn_1$ or $t_4 + Sn_2$ and t_4 (caffeine alone), suggesting that some additional toxic effect of stannous chloride cannot be completely discarded. Between Sn_1 and Sn_2, there was a decrease of about 10%. Thus, the present results in *Drosophila prosaltans* and the other data in literature, previously mentioned, recommend for further study continuous ingestion of stannous chloride.

Table I - Total fecundity in the experiments. Drugs and doses per ml of banana-agar medium: t_4 = 2,000 µg/ml caffeine; Sn_1 = 500 µg/ml of stannous chloride; Sn_2 = 2,000 µg/ml of stannous chloride; $t_4 + Sn_1$ and $t_4 + Sn_2$ = combined treatments.

	Fecundity					
	C	t_4	Sn_1	Sn_2	$t_4 + Sn_1$	$t_4 + Sn_2$
Bottle 1	1177	747	883	895	578	586
Bottle 2	1159	692	1241	1033	656	634
Total	2336	1439	2124	1928	1234	1220

C, control.

Table II - Multiple comparisons of Tukey for fecundity in the control and treated flies.

Experiments	Control	t_4	Sn_1	Sn_2	$t_4 + Sn_1$
t_4	3.30*				
Sn_1	0.73	2.57*			
Sn_2	1.42*	1.88*	0.69		
$t_4 + Sn_1$	4.20*	0.90	3.47*	2.78*	
$t_4 + Sn_2$	4.27*	0.97	3.54*	2.85*	0.07

*$P < 0.05$.

ACKNOWLEDGMENTS

We thank Antonio José Manzato for assisting with the statistical analysis. M.M.I. is the recipient of fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasil) and Coordenadoria de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES). Publication supported by FAPESP.

RESUMO

A fecundidade de *Drosophila prosaltans* foi analisada em indivíduos tratados com íon estanoso, cafeína ou íon estanoso, em duas concentrações, combinado com cafeína. Em todos os casos, a fecundidade foi significativamente menor quando comparada com o controle, exceto na menor concentração de íon estanoso. Nas combinações das duas substâncias, a redução não foi significativamente maior que a causada pela cafeína sozinha, podendo indicar que o efeito das duas substâncias juntas corresponde ao próprio efeito da cafeína separada. Mas a redução da fecundidade foi 15% maior no tratamento que utilizou íon estanoso em maior concentração, sugerindo que seu efeito tóxico nesse parâmetro, mesmo em combinação com a cafeína, não deve ser desconsiderado.

REFERENCES

Benowitz, N.L. (1990). Clinical pharmacology of caffeine. *Annu. Ver. Med. 41*: 277-288.

Bernardo-Filho, M., Gutfilen, B. and **Souza-Maciel, O.** (1994). Effect of different anticoagulants on the labeling of red blood cells and plasma protein with ^{99m}Tc. *Nucl. Med. Commun. 15*: 730-734.

Cabral, R.E.C., Leitão, A.C., Lage, C., Caldeira-de-Araújo, A., Bernardo-Filho, F.L.S., and **Cabral-Neto, J.B.** (1998). Mutational potentiality of stannous chloride: an important reducing agent in the Tc-99m radiopharmaceuticals. DNA repair. Elsevier Science, Amsterdã.

Cremer, C., **Cremer, T., Hens, L., Baumann, H., Cornelis, J.J.** and **Nakanishi, K.** (1983). UV micro-irradiation of the Chinese hamster cell nucleus and caffeine post-treatment. Immunocytochemical localization of DNA photolesions in cells with partial and generalized chromosome shattering. *Mutat. Res. 107*: 465-476.

Cunha, M.C., Oliveira, E.F., Guillobel, H.C.B., **Bernardo-Filho, M.** and **Alcântara-Gomes, R.** (1991). Íon estanoso: um agente genotóxico. *Livro de Resumos do I Simpósio Latino-Americano de Mutagênese Ambiental (ALAMCTA)*, Caxambú, MG, p. 29.

Das, S.K. (1987). Further evidence for a different mode of action of caffeine and benzamide on mammalian cells. *Mutat. Res. 192*: 69-74.

Granberg-Ohman, I., Johansson, S. and **Hjerpe, A.** (1980). Sister-chromatid exchanges and chromosomal aberrations in rats treated with phenacetin, phenazone and caffeine. *Mutat. Res. 79*: 13-18.

Itoyama, M.M. and **Bicudo, H.E.M.C.** (1992). Effects of caffeine on fecundity, egg laying capacity, development time and longevity in *Drosophila prosaltans*. *Rev. Bras. Genet. 15*: 303-321.

Itoyama, M.M., Bicudo, H.E.M.C. and **Manzato, A.J.** (1998). The development of resistance to caffeine in *Drosophila prosaltans*: productivity and longevity after ten generations of treatment. *Cytobios 96*: 81-93.

Kilhman, B.A., Hansson, K. and **Anderson, H.C.** (1982). The effects of post-treatments with caffeine during S and G_2 on the frequencies of chromosomal aberrations induced by Thiotepa in root of *Vicia faba* and in human lymphocytes *in vitro*. *Mutat. Res. 104*: 232-330.

MacPhee, D.G. and **Leyden, M.F.** (1985). Effects of caffeine on ultraviolet-induced base-repair substitution and frameshift mutagenesis in *Salmonella*. *Mutat. Res. 143*: 1-3.

Mendelson, D. and **Sobels, F.** (1974). The inhibiting effect of caffeine on the maternal repair of radiation-induced chromosome breaks in *Drosophila*. *Mutat. Res. 26*: 123-128.

Neter, J. and **Wasserman, W.** (1974). *Applied Linear Statistical Models*. Richard D. Irwin, Inc., Homewood, IL.

Olivier, P. and **Marzin, D.** (1987). Study of the genotoxic potential of 48 inorganic derivatives with the SOS chromotest. *Mutat. Res. 198*: 263-269.

Peres, C.A. and **Saldiva, C.D.** (1983). Planejamento de experimentos. *5º Simpósio Nacional de Probabilidade e Estatítica, 23 a 30 de julho de 1982*, IME, USP, São Paulo, p. 98.

Sasaki, Y.F., Imanishi, H., Ohta, T. and **Shirasu, Y.** (1989). Modifying effects of components of plant essence on the induction of sister-chromatid exchanges in cultured Chinese hamster ovary cells. *Mutat. Res. 226*: 103-110.

Singh, I. (1983). Induction of reverse mutation and mitotic gene conversion by some metal compounds in *Saccharomyces cerevisiae*. *Mutat. Res. 117*: 149-152.

Targa, H.J. (1983). Synergism between caffeine and γ-radiation in the induction of dominant lethal mutations in oocytes and spermatozoa of *Musca domestica*. *Mutat. Res. 110*: 311-325.

Tripathy, N.K., Wurgler, F.E. and **Frei, H.** (1990). Genetic toxicity of six carcinogens and six non-carcinogens in the *Drosophila* wing spot test. *Mutat. Res. 242*: 169-180.

(Received May 3, 1999)