MUTAGENIC EFFECTS OF THE FOOD COLOUR ERYTHROSINE IN RATS

Hamdy A. MEKKAWY[1], A. A. MASSOUD[2], A. M. EL-ZAWAHRY[3]

[1]*The National Center of Social and Criminological Research, Cairo, Egypt*

[2]*Faculty of Sciences, Tanta University, Tanta, Egypt*

[3]*NODCAR, Cairo, Egypt*

ABSTRACT: Male rats were treated of with a diet supplemented daily by the synthetic food colour erythrosine (0.08 and 0.4 g/kg diet) for 30 days. Changes in mutagenic activities, as an index for evaluating of possible toxic effects, were monitored by measuring chromosomal aberrations of rat bone marrow, nucleic acids and total protein concentrations of rat liver and brain. The present study found that erythrosine induced chromosomal aberrations. Chromosomal aberrations of bone marrow cells were centromeric attenuation, centric fusion, deletions, ring shape, stickiness, end-to-end association and polyploidy. The mitotic index was statistically increased with a small dose of erythrosine but was inhibited with the higher dose. Biochemical assays revealed that the nucleic acids and the total protein increased markedly during the various periods of treatment. Results indicated that the two doses of erythrosine were found to be mutagenic agents, the high dose of erythrosine having more effect than the lower one.

KEY WORDS: Erytrosine; Mutagenics; Chromosomal aberration.

Problems of Forensic Sciences, vol. XLIII, 2000, 184–191
Received 9 September 1999; accepted 16 May 2000

INTRODUCTION

Erythrosine (Cl Food Red FD and C Red number 3) (Iodoeosin; 2,4,5,7-tetraiod fluorescenic disodium salt; $C_{20}H_6I_4Na_2O_5$ mol. wt. 879.87. Erythrosine has been listed as a permitted food colouring agent by the FAO/WHO Expert Group [19]. However, the Group did recommend that this substance be properly evaluated for its mutagenic potential [37]. Although data on acute toxicity of this compound is not available, short-term studies conducted on rats fed for 28 days on a diet containing 65 mg erythrosine reveal adverse effects compared with controls [8, 21]. Similarly long-term studies carried out on rats and does fed for 2 years on a diet containing erythrosine reveal reduced growth and spleen weight [24, 25].

The aim of this work is to evaluate the effect of erythrosine at two doses on the chromosomes of bone marrow cells of the laboratory rats. Biochemical assays were

done to determine the effect of erythrosine on the amount of DNA, RNA and total proteins in brain and liver homogenates of rats.

MATERIALS AND METHODS

Adult male rats (*Rattus norvegicus*) weighing 100–120 g each were used. They were randomly selected from an experimental breeding station in Helwan, Egypt. They were offered a balanced standard maintenance diet with free access of water [10, 26].

The experiments were carried out on 60 male rats which were divided into three groups including control and treated groups. The first subgroup served as a control and the second subgroup was daily given a high oral dose (0.4 g/kg diet) of erythrosine [23], and the third subgroup was daily given small oral dose (0.08 g/kg diet) – 1/10 dose of Ibrahim et al. [27].

Ten of the control and treated animals were used in each of the biochemical and chromosomal studies and were sacrificed after 6 hours at the end of the experimental period. The method used for chromosomal preparation from the bone marrow cells was bassed on the techniques devised by Yosida and Amano [38].

The liver and brain were removed immediately and homogenized in 0.9% NaCl solution for the determination of nucleic acid and total protein. Nucleic acids were extracted from liver and brain homogenates [35], deoxyribonucleic acid [14], ribonucleic acid [33], and total protein [12] were determined.

The results obtained were statistically analysed by Kurtz's method [30].

RESULTS

Chromosome analysis

In the present work, the effect of both doses 0.4 and 0.08 g/kg diet supplement of erythrosine on the chromosomes of bone marrow cells of male rats (*Rattus norvegicus*) after 30 days was studied.

In 30 days of treatment, the mitotic index (number of divided cells per 1000 of the rat bone marrow cells) in the group treated with the small dose of erythrosine increased statistically but in the group was inhibited with the higher dose (Table I). Chromosomal aberrations were centromeric attenuation, centric fusion, deletions, ring shaped, stickiness, end-to-end association, polyploidy, gaps and break (Table II).

TABLE I. EFFECT OF ERYTHROSINE ON THE MITOTIC INDEX IN RATS (*RATTUS NORVEGICUS*)

Doses [g/kg diet]	Treatment [days]	No. of dividing cells/1000 cells/rat	
		Statistical analysis	
		Mean ± S.E.	Changes of control [%]
Low dose (0.08 g/kg diet)	Control	40.0 ± 1.23	–
	erythrosine (30 days)	48.0 150 ± 1.02**	20.0
Highdose (0.40 g/kg diet)	Control	41.0 ± 1.27	–
	Erythrosine (30 days)	25.2 ± 2.30**	38.5

Results are expressed of mean ± S.E. of five rats.
S.E.= Standard error.
** High significant.

TABLE II. MEANS OF CHROMOSOMAL ABERRATIONS INDUCED BY ERYTHROSINE PER 50 METAPHASE NUCLEI OF RATS AFTER 30 DAYS

Type of aberration	No. of cells with aberration/50 metaphase		
	Control	Erythrosine [g/kg diet]	
		Low dose (0.08 g/kg diet)	High dose (0.40 g/kg diet)
Diploidy	0.21	4.5**	12.4**
Centric fusion	0.00	3.6**	6.1**
Break	0.00	1.1**	2.7**
Gap	0.00	3.1**	4.1**
Centrometric attenuation	0.01	4.2**	6.5**
Deletion	0.00	3.8**	5.1**
Ring shaped	0.00	1.4**	1.8**
Stickness	0.01	2.2**	4.3**
End-to-end	0.00	1.2**	3.8**

Results are expressed of mean ± S.E. of five rats.
S.E.= Standard error.
** High significant.

Biochemical analysis

The results gained presented in table III indicate that administration of both doses of 0–08 and 4 g/kg diet of erythrosine to rats over 30 days caused a significant increase of nucleic acids (DNA and RNA) and total protein in brain and liver of rats (Table III).

TABLE III. EFFECT OF ERYTHROSINE [G/KG DIET] ON NUCLEIC ACIDS [MG/G TISSUE] OF LIVER AND BRAIN OF MALE RATS AFTER 30 DAYS

Tissues	Parameter	Low dose (0.08 g/kg diet)		High dose (0.40 g/kg diet)	
		C	T	C	T
Liver	DNA	3.71 ± 0.21	4.67 ± 0.06**	2.93 ± 0.04	3.46 ± 0.08**
	RNA	6.81 ± 0.37	8.63 ± 0.12**	7.11 ± 0.06	8.22 ± 0.05**
	Total protein	276.52 ± 4.15	361.41 ± 3.21**	256.41 ± 4.02	300.81 ± 5.21**
Brain	DNA	1.91 ± 0.05	2.22 ± 0.06**	1.74 ± 0.07	2.16 ± 0.05**
	RNA	1.82 ± 0.04	2.31 ± 0.05**	1.81 ± 0.01	2.21 ± 0.04**
	Total protein	280.1 ± 5.05	300.2 ± 4.02**	198.5 ± 2.34	300.51 ± 2.35**

Results are expressed of mean ± S.E. of five rats.
S.E. = Standard error.
** High significant.

DISCUSSION

There has been increasing concern in recent years about the mutagenic potential of a variety of food additives and food colours and it is believed, that such substances may present a possible hazard to man by causing gene mutations and/or chromosomal aberrations [37].

The effect of erythrosine administration on the mitotic index (number of dividing cells/1000) was recorded.

The low dose (0.08 g/kg diet) of erythrosine provoked a significant increase at 30 days but the higher dose (0.4 g/kg diet of erythrosine) caused a significant decrease after 30 days. Our results are consistent with the results of Maugh and DeHondt et al. [13, 32] using cannabis; and Baset et al. [13] using cypennethrin). It has long been known that the inhibition of nuclear DNA synthesis by certain chemicals invariably blocks mitosis [4, 11].

Chromosomal aberrations were observed following administration erythrosine at both doses, consisting mainly of: diploidy (Figure 1a) and centric fusion (Figure 1b). These are presented in Table II.

Damage to chromosomes after the GI stage of the cell cycle causes chromatid lesions and results in chromatid breaks [36]. This finding was observed in the present study and is consistents with that of Zhurkov [39]. Therefore, it may be possible to conclude that erythrosine exerts its mutagenic effect after the GI stage of the cell cycle.

Mutagenic substances are known of causing hereditary malformation by then effect on germ cells or non hereditary malformation by their effect on somatic cells [2, 29].

From the present results, it is evident that erythrosine, at both doses of 0.4 and 0,08 g/kg diet has the effect of significantly increasing liver and brain DNA and RNA content after 30 days may have resulted from induction of RNA synthesis [20].

Brachet [6] has demonstrated that cellular RNA synthesis is a DNA-dependent process, thus the increasing RNA content after treatment with erythrosine was probably

Fig. 1a. Metaphase spread from bone marrow of treated rat with erythrosine showing deploidy and endomitosis (Giemsa stain X 1250).

Fig. 1b. Metaphase spread from bone marrow of treated rat with erythrosine showing centric fusion (Giemsa stain X 1250).

caused by the increase in DNA which is in complete agreement with the results obtained by Maugh [32]. It is clear that there an increase in RNA or DNA base. In fact Allen and Abrahamson [3] reported similar results in rats fed mixture of PCB. In the same studies a number of hepatic cells exhibited an increase in the size of nuclei an observation which

coincides with those reported by Banhawy [5], El-Beih [18], Sanad [34] and El-Banhawy et al. [16].

Administration of erythrosine at 0.08 g/kg diet causes a significant increase in the total protein concentration in the rat brain and liver. Increasing the dose to 0.4 g/kg diet induced a significant increase in protein content of liver and brain. Similar increases in total proteins were found with mercuric in fishes [28] and with insecticide in rats [31] and with radiation sensitivity in human cells [15], with chloramphenicol in rats [9] and with chloramphenicol in rats [17].

The general picture of results illustrates that erythrosine influences the level of protein and nucleic acids, which probably control the genetic pattern of potential enzymes and metabolic pathways in the cell at the nuclear level. Also, erythrosine decreased mitosis, as well as causing chromosomal structural aberrations, which may be suggestive of its mutagenicity.

References:

1. Abdelbaset S. A., Ali M. O., Kamal A. M., Zaki N. G., The effect of cypermethrin insecticide on the chromosomal pattern and chemistry in rats, *Egyptian Journal of Anatomy* 1986, vol. 9.
2. Alexander G., Miles B., Gold G., Alexander R., LSD: injection early in pregnancy produce abnormalities in rats, *Science* 1967, vol. 157, pp. 459–460.
3. Allen J. R., Abrahamson, L. J., Responses of rats exposed to poly chlorinated diphenyls for fifty two weeks. II. Compositional and enzymatic changes in the liver, *Archives of Environmental Contamination and Toxicology* 1979, vol. 8, pp. 191–200.
4. Badr A., Hamoud M. A., Haroun S. A., Effect of the herbicide gespax on mitosis, mitotic chromosomes and nucleic acids in Vicia faba meristems, *Proc. Saudi Biol. Soc*. 1985, vol. 8, pp. 359–370.
5. Banhawy M. A., Effect of ionizing radiation on the deoxyribonucleic acid content of the liver cells, *Proc. Zool. Soc*. 1969, vol. 3, p. 323.
6. Brachet J., Handbuch der Histochemie, Fischerverlag, Stuttgart 1959, S. 111–112.
7. Brown W. J., Yoshida N., Organic mercurical encephalopathy an experimental electromicroscopic study, *Advances in Neurology Sciences* 1965, vol. 9, pp. 34–42.
8. Butterworth K. R., Gaunt I. F., Grasso P., Gangolli S. D., Acute and short-term toxicity studies in erythrosine BS in rodents, *Food and Cosmetics Toxicology* 1976, vol. 14, pp. 525–534.
9. Cadavid N. F., Bello E. M., Ramirez J. L., Differential long-term effects of d-chloramphenicol on the biogenesis of mitochondria in normal and regenerating rat liver, *Biochemical Journal* 1970, vol. 118, pp. 577–586.
10. Campell J. A., Methodology of protein evaluation RAG, Nutr. document R. 101 add. 37, June Meeting, New York 1961.
11. Chand S., Roy S. C., Effects of herbicide 2,4-dinitrophenol on mitosis, DNA, RNA and protein synthesis in Nigella Sativa L., *Biologia Plantarum* 1981, vol. 23, pp. 195–202.
12. Daughaday W. H., Lowry O. H., Rosedrough N. J., Fields W. S., Determination of cerebrospinal fluid protein with folin phenol reagent, *Journal of Laboratory and Clinical Medicine* 1952, vol. 39, pp. 663–665.

13. De Hondt H. A., Fahmy A. M., Abdel Baset S. A., Chromosomal and biochemical studies on laboratory rats exposed to cannabis extracts, *Egypt. J. Genet. Cyt.* 1979, vol. 9, pp. 217–231.
14. Dische Z., Some new characteristic colour tests for thyrmonucleic acid and a microchemical method for determining the same in animal organs by means of these tests, *Mikrochemie* 1930, vol. 8, pp. 4–32.
15. Djordjevic B., Szybalski W., Genetics of human cell lines. III. Incorporation of 5-bromo and 5-iododeoxyuridine into the DNA of human cells and its effect on radiation sensitivity, *Journal of Experimental Medicine* 1960, vol. 112, pp. 509–531.
16. El-Banhawy M. A., Sanad S. M., Al-Zahaby A .S., Al-Attar E. M., Effect of chloramphenicol treatment on the nucleic acids contents in the epithelial ileal mucosal and liver cells of the white rat, *Journal of Egyptian Society of Toxicologist* 1992, vol. 9, pp. 65–74.
17. El-Banhawy M. A., Sanad S. M., Al-Zahaby A. S., Al-Attar E. M., Effect of chloramphenicol treatment on the protein contents in the rat liver cells, *Journal of Egyptian Society of Toxicologist* 1992, vol. 9, pp. 87–93.
18. El-Beih Z. M., Effect of gamma-irradiation on the deoxyribonucleic acid content of the rat liver cells, *Egyptian Journal of Hist.* l980, vol. 3, pp. 41–44.
19. FAO/WHO Expert Committee on Food Additives 1974, pp. 19–29.
20. Gale G. R., Smith A. B., Atkins L. M., Walker F. M., Gadsen R. H., Pharmacology of captan: Macromolecular synthesis, *Toxicology and Applied Pharmacology* 1971, vol. 18, pp. 426–441.
21. Gaunt I. F., Grasso P., Ganglli S. D., Butterworth K. R., Acute and short-term toxicity studies on erythrosine BS in rodents, *Food and Cosmetisc Toxicology* 1976, vol. 14, pp. 525–532.
22. Goldwater L. J., The Birth of mercaptan, *Archives of Environmental Health* 1965, vol. II, p. 597.
23. Hammama A. A., Hewedi F. M., Ibrahim M. H., Abdel-Rahim F. A., Effect of synthetic dyes and natural pigments on some energetic metabolites in albino-rats, *Journal of Drug Research* 1991, vol. 20, pp. 117–123.
24. Hansen W. H., Davis K. J., Groham S. I., Perry C. H., Jacobson K. H., Long-term toxicity studies of erythrosine II. Effects on haematology and thyroxine and protein-bound iodine in rats, *Food and Cosmetics Toxicology* 1973, vol. 11, p. 535.
25. Hansen W. H., Zwiceky R. E., Brouwer J. B., Fitzhugh O. G., Long-term toxicity studies of erythrosine. I. Effects in rats and dogs, *Food and Cosmetics Toxicology* 1973, vol. 2, pp. 527–534.
26. Hegested D. M., Mils R. C., Eluehjem C. A., Hart E. B., Choline in il; nutrition of chicks, *Journal of Biological Chemistry* 1941, vol. 138, p. 349.
27. Ibrahim A. Y., Abdel Rahim E. A, Ramadan M. E., Abdel Rahim G. A., Effect of some natural and synthetic food colorants on protein, nucleic acids and nucleases in albino rats organs. Minia, *J. Agric. Res. and Dev*. 1988, vol. 10.
28. Ibrahim M. S., Abd-Allah G. A., Abdel-Baky T. E., Bahnsawy M. H. , Toxic effects of environmental pollution by herbicide gallant and mercury on liver function of the Nile Clarias lazera, *J. Egypt. Ger. Soc. Zool*. 1991, vol. 6 (A), pp. 189–199.
29. Kaher H., Chemical mutagens, Hollaender A. [ed.], Plenum Press 1971, vol. I, p. 57.
30. Kurtz N. R., Introduction to social statistics, Mc-Grow Hill Book Co., New York 1983, pp. 163.

31. Lin T., Dorough H. W., Influence of insecticide exposure on the in vivo and in vitro Metabolic activity of rats, *Archives of Environmental Contamination and Toxicology* 1974, vol. 2, pp. 364–377.
32. Maugh T. H. N., Marihuana: The grass may no longer be greener, *Science* 1974, vol. 185, p. 683.
33. Merchant D. J., Kalhn R. H., Murph W. H., Handbook of cell and organ culture, Burgess, Minneoplis 1969.
34. Sanad S. M., Histochemieal studies on the effect of the pyrethroid insecticide (Permethrin) on the rat liver cells, *Bulletin of Faculty of Science Zagazig University* 1989, vol. II, pp. 450–471.
35. Schneider W. C., Phosphorus compounds in animal tissues: I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid, *Journal of Biological Chemistry* 1945, vol. 161, p. 293.
36. Stevenson A. C., Bedford J., Hill A., Chromosomal studies in patients taking phenylhutazone, *Annales of the Rheumathic Diseases* 1971, vol. 30, pp. 487–500.
37. Vija Y., Genetic effects of turmeric and curcumin in mice and rats, *Mutation Research* 1980, vol. 79, pp. 125–132.
38. Yosida T. H., Amano K., Autosomal polymorphism in laboratory bred and wild Norway rats, Rattus norvegicus, found in Misima, *Chromosoma* 1965, vol. 16, pp. 658–667.
39. Zhurkov V. S., Investigation of the mutagenic activity of drug preparations and food additives on a culture of human lymphocytes, *Soviet Genetics* 1974, vol. II, pp. 528–530.