The Effect of a Hypoproteic Diet and Ethanol Consumption on the Yield of Chromosomal Damage Detected in Bone Marrow Cells of Mice

M. C. TERREROS, J. C. De LUCA, and F. N. DULOUT

CIGEBA (Centro de Investigaciones en Genética Básica y Aplicada) Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, 60 y 118 - CC 296-1900, La Plata, Argentina

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ABSTRACT. The relationship between protein malnutrition and ethanol consumption as modulating factors of the genetic response to xenobiotics was studied. BALB/c mice of both sexes were fed for three weeks after weaning either with a normal diet containing 25% protein or a hypoproteic diet containing 5% protein. Half of the animals received 20% ethanol in drinking water. Cytogenetic analysis was performed in bone marrow cells. Slides were stained for C-banding in order to assure the accurate scoring of dicentric chromosomes. Results obtained showed an increased frequency of dicentric chromosomes in mice fed with the hypoproteic diet (5.45 dicentrics per 100 cells) in contrast to mice fed with the normal diet (0.61 dicentrics per 100 cells). Ethanol consumption increased the frequency of chromosomal damage, but no differences in the effect of ethanol between mice fed with the normal diet and mice fed with the hypoproteic diet (16.33 and 16.80 dicentrics per 100 cells respectively). The enhanced frequency of dicentric chromosomes in animals fed with the hypoproteic diet might have been originated from the increase or the improper repair of chromosome breaks. The similarity in the response to ethanol consumption in animals fed either with the normal or the hypoproteic diet might have been provoked by a decrease of alcohol dehydrogenase (ADH) level in undernourished mice. The chromosomal damage due to ethanol may be lower in undernourished mice than in mice fed with the normal diet due to the reduced amount of circulating acetaldehyde able to induce chromosomal damage. The results obtained are an evidence of the role played by the diet in the modulation of the genetic response to xenobiotics.—KEY WORDS: chromosome, diet, ethanol.

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The relation between protein-caloric malnutrition (PCM) and genetic damage has been studied in human beings and laboratory animals obtaining contradictory evidences. It has been found that children aged 1-60 months with severe PCM exhibited an increase of chromosomal aberrations (dicentrics, gaps, isogaps and breaks) in peripheral lymphocytes and bone marrow cell cultures. These abnormalities persisted even after the children had attained normal height and weight [1]. Lymphocyte and bone marrow cultures of female mice fed with a diet containing 9% proteins showed an increase of chromosome breaks and deletions as compared with controls fed with a diet containing 23% protein [16]. Increased frequencies of chromosome breaks and deletions in bone marrow cells of rats fed with low-protein diets has been found [19]. Kindig *et al.* [10] analysed the effect of malnutrition on mouse oocytes. Those animals subjected to a diet with 50% less metabolizable energy than the control diet exhibited a significant increase of chromosomal abnormalities. This did not occur with those animals fed with diets with 12.5 and 25% less metabolizable energy than the control diet. In a second experiment, animals fed with diets containing 4 and 8% protein showed an increase of chromosomal damage in relation with animals fed with a diet containing 16% protein. Opposite results were obtained by other authors who did not find any difference between normal and malnourished children [9, 18]. In addition, the results obtained in mice did not show any influence of protein-deficient diets on the frequency of chromosomal aberrations [14]. These discrepancies between results obtained in human beings and laboratory animals have been accounted for the different methodologies employed. In human beings it is difficult to establish the degree of malnutrition in the individuals studied, the effect of malnutrition per se as well as the incidence of other factors such as infection, chemical exposures, etc. In laboratory animals the discrepancies can be accounted for the different experimental designs employed. For instance, in the experiment of Vijayalaxmi [19] the animals were fed with hypoproteic diet for 8 weeks. In the experiment of Rabello-Gay *et al.* [14] the malnutrition period was 3 weeks. Whereas in some experiments the frequencies of each kind of chromosomal aberrations were scored [1, 19], in the experiment of Rabello-Gay *et al.* [14] only the frequency of abnormal cells (i.e. cells with at least one chromosomal aberration) was scored.

Ethanol is a well known mutagenic, carcinogenic and teratogenic agent for human beings. Significant increases of chromosomal aberrations were found in peripheral lymphocytes of alcoholics [11]. The induction of non-disjunction in the second meiotic division of mouse germ cells by ethanol has been described [8]. It has been demonstrated that acetaldehyde, the main metabolite of ethanol, is the inducer of chromosomal damage [12, 13]. Moreover, it has been shown that acetaldehyde, but not ethanol is the main inducer of chromosomal aberrations and aneuploidy in mammalian cells in culture [5].

In order to evaluate the relationship between protein malnutrition and alcohol consumption as modulating factors of the genetic response to xenobiotics, we carried out an experiment in which the effect of a hypoproteic diet and alcohol drinking was analysed.

MATERIALS AND METHODS

A total of 55 weaning BALB/c mice of both sexes, with an initial body weight around 18-20 g were used. The animals were divided into four groups and subjected to the following treatments from weaning (30 days old) to the end of the experiment (51 days old): 1) control group: fed *ad libitum* with a semisynthetic diet containing 25% protein (S 25)(Table 1); 2) undernourished group: fed *ad libitum* with a semisynthetic diet containing 5% protein (S 5) (Table 1); 3) control plus ethanol: fed as the first group and receiving 20% ethanol in drinking water; 4) undernourished plus ethanol: fed as the second group and receiving 20% ethanol in drinking water. Two hours before sacrifice by cervical dislocation, mice were intraperitoneally injected with 1 mg/kg of colchicine.

The diets were prepared weekly in the laboratory and kept at 4°C until use. Food intake was measured daily and the animals were weighed weekly.

Cytogenetic preparations from bone marrow cells were made according to routine protocols [15] and fixed according to Islam and Levan [7]. Chromosome spreads were stained for C banding according to Sumner [17].

Cytogenetic analysis was performed in coded slides by scoring the frequencies of dicentric chromosomes. In this way the analysis could be performed with objectivity since double C-banded chromosomes were easily detected (Fig. 1).

Statistical analysis was performed by means of ANOVA [2].

Table 1. Composition of the control diet (25% protein) (S 25) and the hypoproteic diet (5% protein) (S 5) in g/kg

Component	Control (S 25)	Hypoproteic (S 5)
Casein	338.30	69.27
d-1-Methionine	3.40	0.73
Corn starch	343.30	615.00
Glucose	180.00	180.00
Cellulose	40.00	40.00
Corn oil	50.00	50.00
Salt mixture[a)]	40.00	40.00
Vitamin mixture[b)]	5.00	5.00
Total	1,000.00	1,000.00

a) Salt mixture (1,000 g)		b) Vitamin mixture	
K_2HPO_4	386.6 g	Vitamin A	6,000 UI
$CaCO_3$	340.0 g	Vitamin B1	2.0 g
NaCl	190.0 g	Nicotinamide	15.0 g
$CaHPO_4$	68.2 g	Vitamin B2	2.5 g
$FeSO_4$	28.0 g	Vitamin B6	1.0 g
$MnSO_4$	2.6 g	Vitamin B12	5.0 g
$ZnCO_3$	0.9 g	Vitamin D	3,000,000 UI
$CuSO_4$	0.3 g	Vitamin K	2.0 g
$MgSO_4$	0.2 g	Calcium pantothenate	10.0 g
		Choline chlorhydrate	10.0 g
		Ascorbic acid	20.0 g
		Inositol	20.0 g
		Folic acid	0.4 g
		Biotin	0.2 g
		Excipient to	1,000.0 g

RESULTS

Table 2 summarizes the average daily food intake as well as the initial and final average weekly body weights of the animals of different groups. A decrease of food intake as well as of final body weights in mice fed with the hypoproteic diet (S5) in relation with mice fed with normal diet (S25) was observed ($F=17.2$, $p<0.01$ for body weights). Food intake and final body weights were also lower in animals fed either with normal or hypoproteic diet and receiving ethanol in drinking water ($F=7.3$, $p<0.05$ and $F=16.00$,$p<0.01$ for body weights respectively) (Table 2).

Table 3 shows the frequencies of dicentric chromosomes in the different experimental groups. As no statistical differences between sexes were found, the data has been analyzed altogether. Mice fed with S5 showed an increased frequency of dicentric chromosomes in relation with the animals fed with S25 (5.45 and 0.61 dicentrics per 100 cells respectively) ($F=12.3$, $p<0.01$). Ethanol consumption induced an increase of chromosomal damage both in animals fed either with S25 or S5. A frequency of

Table 2. Average daily food intake and average initial and final body weights in the different experimental groups

Interval treatment	Treatment	Number of mice studied	Average daily food intake (g) ± SEM	Average body weights (g) ± SEM Initial	Final
	S25	13	4.95±0.37	20.20±0.07	22.40±0.32
First	S25+OL	12	3.55±0.35	19.85±0.19	21.70±0.26
week	S5	15	3.10±0.39	20.00±0.29	18.90±0.53
	S5+OL	15	2.90±0.39	20.25±0.19	19.25±0.11
	S25	13	4.70±0.37	22.40±0.32	24.00±0.24
Second	S25+OL	12	3.10±0.35	21.70±0.26	22.45±0.36
week	S5	15	3.55±0.39	18.90±0.53	18.70±0.46
	S5+OL	15	3.25±0.39	19.25±0.11	18.65±0.04
	S25	13	4.80±0.39	24.00±0.24	25.45±0.32
Third	S25+OL	12	4.30±0.35	22.45±0.36	22.00±0.34
week	S5	15	3.40±0.39	18.70±0.46	19.70±0.75
	S5+OL	15	3.70±0.39	18.65±0.04	18.15±0.05

SEM: Standard Error of Mean. OL: 20% ethanol in drinking water.

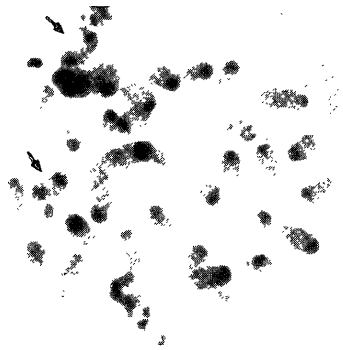

Fig. 1. Bone marrow metaphase showing two dicentric chromosomes (arrows).

Table 3. Frequencies of dicentric chromosomes in the different treatments

Treatment	Number of mice studied			Number of cells scored			Average dicentric chromosomes per 100 cells±SEM		
	Males	Females	Total	Males	Females	Total	Males	Females	Total
S25	7	6	13	350	300	650	0.57±0.28	0.66±0.36	0.61±0.09
S25+OL	6	6	12	300	300	600	18.00±0.68	14.66±0.66	16.33±0.49
S5	7	8	15	700	400	1,100	4.85±0.85	6.50±0.53	5.45±0.35
S5+OL	8	7	15	400	350	750	14.50±0.65	19.42±1.34	16.80±0.76

SEM: Standard Error of Mean.
OL: 20% Ethanol in drinking water.

16, 33 dicentrics per 100 cells was detected in mice fed with S25 ($F=458.6$, $p<0.001$ in comparison with mice which did not receive ethanol in drinking water). In undernourished mice the effect of ethanol consumption was similar to the effect observed in mice fed with S25 (16.80 dicentrics per 100 cells)($F=0.01$, not significant in the comparison with the animals fed with S25 which received ethanol in drinking water).

DISCUSSION

The fact that mice fed with the hypoproteic diet showed a frequency of dicentric chromosomes almost ninefold higher than those fed with normal diet is a finding coincident with those obtained by Armendares *et al.* [1] and Vijayalaxmi [19]. These authors performed cytogenetic studies in children with severe protein-caloric malnutrition and in rats fed with low protein diets respectively. The increase of chromosomal aberrations as a consequence of severe protein malnutrition can be explained by a failure of enzymatic mechanisms of DNA replication and repair. According to Kindig *et al.* [10], malnutrition can affect the synthesis of enzymes required for DNA repair. Moreover, both caloric or protein malnutrition can decrease energy or essential aminoacid availability respectively. Animals fed with low energy diets are energy deficient and probably use degraded protein as a source of energy and severe protein malnutrition can decrease the supply of essential aminoacids. Since the synthesis of enzymes necessary for DNA replication and repair requires both energy and aminoacids, their decrease may result in an increment of DNA chromosomal damage. However, since DNA replication and repair are very complex mechanisms, a simultaneous depletion of the large number of enzymes involved cannot be invoked to explain the dramatical increase of dicentric chromosomes in malnourished mice. Notwithstanding, it is possible to assume that dicentric chromosomes are produced after the improper repair of the large number of breaks induced.

In mice receiving 20% ethanol in drinking water during three weeks after weaning, the frequency of dicentric chromosomes was similar either in animals fed with the normal diet or in animals fed with the hypoproteic diet. It is well known that chronical ethanol consumption increases the frequency of chromosome aberrations, mainly dicentric chromosomes, in human peripheral blood lymphocytes [11]. It has been also demonstrated that acetaldehyde, the first metabolic derivative of ethanol, is the main inducer of chromosomal damage [5, 12, 13]. Thus, the metabolism of ethanol could be considered to explain the results obtained. Alcohol dehydrogenase (ADH) is the main enzyme acting on ethanol degradation, being the rate of ethanol oxidation limited by the level of ADH [4]. On the other hand, the ADH level can be influenced by the quality of the diet, that can decrease the metabolic rate of ethanol. It has been shown that low-protein diets decreased the ADH level in rat liver [3, 6] reducing the metabolic rate of ethanol both in human beings and rats. Accordingly, mice fed with the hypoproteic diet could have had a decrease of ADH level and therefore, a slow metabolic rate of ethanol to acetaldehyde. Consequently, the chromosomal damage might have been lower in these mice than in mice fed with the normal diet due to the reduced amount of circulating acetaldehyde able to induce chromosomal damage.

The results obtained are an evidence of the role played by the diet in the modulation of the genetic response to a xenobiotic like ethanol. Further studies with other genotoxic agents could give more information about the relation between diet and ethanol consumption and the induction of chromosomal damage.

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