

GENOTOXICOLOGY ANALYSIS OF AVEMAR AND AZOXYMETHANE IN THE RAT'S MARROW MICRO-NUCLEUS TEST

EXPERT'S OPINION

by

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Summary

The Department of Experimental Pathology and Mutagenic Research of the National Institute of Chemical Safety of the "Jozsef Fodor" Center of Public Health, Hungary (OKK-OKBI) has been involved in the experiment, carried out in the year 2000, by the First Institute of Pathology and Experimental Cancer Research of the School of General Medicine of the Semmelweis University, Budapest, entitled: "Effect of Avemar on the azoxymethane induced colonic carcinogenesis in rats". The animals were given per orally 3000 mg/kg body weight Avemar daily through stomach probe for 32 weeks long. Three subcutane treatments with azoxymethane on the 3rd, 4th and 5th week, in a dose of 15 mg/body weight, were performed. The rats were divided into four groups: 1. negative control (water). 2. Treated with Avemar on its own. 3. Treated with azoxymethane on its own. 4. Treated with Avemar + azoxymethane. Besides the study on the inhibition of the development of colonic tumors (which topic is not part of the present discussion), marrow smears from the femurs of the rats were prepared, and the frequencies of the micro-nucleided polychromasia erythrocytes were determined.

Within the given experimental circumstances, Avemar did not prove to be genotoxic in the rat's marrow micro-nucleus test.

Introduction

The micro-nucleus test serves for testing the chromosome or the damaging of the mitotic apparatus in the marrow's red blood-cells. The micro-nuclei are particles consisting of non-centric fragments, whole chromosomes occurring during the chromosome break-up caused by the clastogens – by damaging the mitotic apparatus.

The target cells of the analysis are the immature polychromasia erythrocytes (PCE) of the marrow whose cores get thrust out from the cells after the last maturing fission. Therefore the micro-nuclei remained in the cytoplasm can be easily recognised. During the analysis we compare the occurring frequencies of the micro-nucleated polychromasia erythrocytes (MPCE) in the marrow of the treated animals with the parallel ones and with the negative control.

Objective

Determining whether the treated groups feature an increased number of the MPCE against the negative control, therefore assessing whether the treatment had any clastogen/aneugen effect.

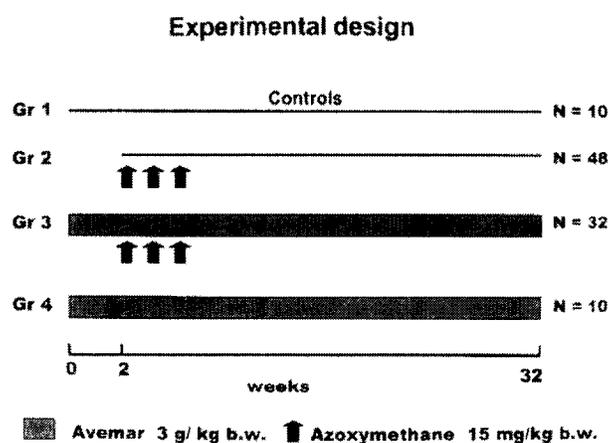
Materials and methods

Animals and diet

One hundred 4-week-old inbred male F-344 rats were used in this study. The animals were housed in air conditioned Animal Care Facility at our Institute under controlled humidity ($55\pm 5\%$) and temperature ($25\pm 2^\circ\text{C}$). They were caged in polyethylene boxes (5/cage) and all had free access to CRLT/N standard pelleted laboratory rodent chow (Charles River Ltd, Hungary) and tap water. This basal diet contained 86% dry material, 19% crude protein, 17% digestible protein, 4.5% crude fat, 6% crude fiber, 6% crude ash, 40% non-protein nitrogen material, 0.8% calcium, 11 000 IU/kg vitamin A, 600 IU/kg vitamin D3, plus amino acids as lysin, methionin and cystein.

Chemicals

Colon carcinogenesis has been induced by AOM (Azoxymethane, Sigma; A 9517). Avemar was produced and supplied by Biomedicina Co., Hungary. The product was a dried, standardized extract.



Experimental protocol

Following randomization the animals were divided into 4 groups as shown in the Figure. Ten rats served as untreated controls (Negative control [water], group 1). For the treatment of the animals in group 2 AOM was dissolved in physiologic saline and the animals were given three

subcutaneous injections 1 week apart, 15 mg/kg body weight (b/w) each (n=48). In two additional groups the effect of the Avemar was investigated. The fermented wheat germ was freshly dissolved in tap water and was given by gavage at a dose of 3 g/kg b/w once a day, between 09:00 and 10:00. In group 3 (n=32) animals started to receive Avemar 2 weeks prior to the first injection of AOM daily and continuously thereafter (including weekends and holidays) until killed 32 weeks later. In group 4 basal diet plus Avemar were administered only (n=10), but no carcinogen was applied. At the end of the experiment all the rats were killed by exsanguination. We removed the marrow from a femur of the killed animals (F344 male rats) then we made 2 smears per animal. After drying we painted one smear according to the May-Grünwald/Giemsa method while the other one was painted with acridin orange fluorescent paint. The microscopic analysis was performed with a 100-immersion objective, with normal and fluorescent microscopes. The number of the micro-nucleated cells was determined by counting 2000 PCE per animal. In order to evaluate the eventual marrow-damaging effect we counted 200 eritrocites to determine the ratio between the immature policromasia and mature normo-cromasia eritrocites (PCE/NCE). For the statistical analysis we applied the Chi square at a significance level of 5%.

Results and discussion

When evaluating the results of the microscopic analysis (see Tables) the value of 2.0 per mill is significantly higher according to the statistical calculation compared to the control group (0.8 per mill) regarding the MPCE frequency. The groups treated with Avemar only and the interaction (Avemar + azoxymethane) featured an increased MPCE occurrence frequency (1,2 per mill, 1.0 per mill) compared to the water control, but the difference was not significant statistically ($p > 0.05$).

The number of the immature eritrocites (PCE) was less than in the treated groups (33%, 33%, 335) compared to the water control group (38%), but the difference between the treated and the control groups can't be considered significant from biological perspective.

It can be concluded that, within the given experimental circumstances, Avemar did not prove to be genotoxic in the rat's marrow micro-nucleus test.

Budapest, 2 June 2000.

Interactive examination of Avemar and azoxymethane in bone-marrow micronucleus test of rat (summing table)

Groups	Number of animals	Number of evaluated cells	MPCE ‰	PCE %
Negative control (water)	10	20,000	0.8	39
Avemar	9	18,000	1.2	33
Azoxymethane	10	20,000	2.0*	33
Avemar + azoxymethane	10	20,000	0.95	33

*p<0.005

Interactive examination of Avemar and azoxymethane in bone-marrow micronucleus test of rat (raw data)

Group: Negative control (water)

Group: Avemar

Number of animals	MPCE ‰ on the basis of 2000 PCE	PCE/NCE ratio	PCE %	Number of animals	MPCE ‰ on the basis of 2000 PCE	PCE/NCE ratio	PCE %
4101	0.0	0.51	34	4111	0.0	0.32	24
4102	1.0	1.26	56	4112	0.0	0.49	33
4103	0.0	0.36	26	4113	2.5	0.52	34
4104	1.5	0.78	44	4114	0.5	0.39	28
4105	0.5	0.79	44	4115	1.0	0.85	46
4106	1.5	0.42	29	4116	1.0	0.40	29
4107	2.0	0.66	40	4117	3.0	0.60	38
4108	0.0	0.51	34	4118	1.5	0.54	35
4109	0.5	0.94	49	4119	1.5	0.51	34
4110	1.0	0.57	36	4120	-		
Average	0.8	0.66	39	Average	1.2	0.51	33
± SD	0.71	0.27	9.2	± SD	1.03	0.15	6.3

Group: Azoxymethane

Group: Avemar + azoxymethane

Number of animals	MPCE ‰ on the basis of 2000 PCE	PCE/NCE ratio	PCE %	Number of animals	MPCE ‰ on the basis of 2000 PCE	PCE/NCE ratio	PCE %
4121	1.0	0.46	32	4131	1.0	0.46	32
4122	3.5	0.99	50	4132	1.0	0.43	30
4123	0.0	0.37	27	4133	0.0	0.60	38
4124	5.5	0.77	44	4134	0.0	0.41	29
4125	1.0	0.54	35	4135	0.5	0.45	31
4126	0.5	0.22	18	4136	2.0	0.35	26
4127	2.0	0.39	28	4137	1.5	0.33	25
4128	2.0	0.47	32	4138	1.0	0.88	47
4129	2.5	0.36	27	4139	1.0	0.68	41
4130	2.5	0.65	39	4140	1.5	0.44	31
Average	2.0*	0.52	33	Average	0.95	0.50	33
± SD	1.49	0.22	9.3	± SD	0.64	0.17	6.9

*p<0.005