THE CYTOGENETIC EFFECTS OF REPEATED EXPOSURE TO ULTRASONIC SOUND WAVES IN MICE AND THEIR ALTERATIONS BY A HOMOEOPATHIC DRUG, ARNICA MONTANA

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Abstract

Separate sets of healthy mice were directly exposed to sonication (with the aid of ultrasonic cell disrupter at a frequency of 23 kHz at energy output level of 70) for 2 min. 1 min at a time with an interval of 1 min. This dose of sonication was repeated at an interval of 20 days, so that mice sacrificed at 30,60, 75 and 90 days after the initial dose actually received 2, 3, 4 and 5 such doses of sonication, respectively. The genotoxic effects in sonicated mice were assessed through the study of chromosome aberrations (CA), sperm head anomaly (SHA), and micronucleated erythrocytes (MNE) as against suitable unsonicated controls. Further, a group of sonicated mice were orally administered with Arnica Montana-30, a homoeopathic drug commonly used against shock and injury and the results were compared with another set of succussed alcohol fed controls (the "vehicle" of the drug being ethyl alcohol). In the sonicated mice, elevated frequencies of CA (comprising mainly of physiological types), MNE and SHA were noted as compared to that of unsonicated controls. Correspondingly, the cytogenetical effects in sonicated and drug-fed (combined) series appeared to be relatively less as compared to succussed alcohol fed sonicated control, thereby indicating that the homoeopathic drug had positive modifying effect on genotoxicity produced by ultrasonication. The implications of the results have been discussed.

Introduction

The ultrasonic sound waves are being increasingly used in biology, medicine for diagnostic purposes, in physiotherapy, in hyperthermia for cancer therapy and in non-invasive thermometry of internal tumors [6,11,21,23]. Pregnant women are also often exposed to periodic ultrasonographic tests. Although some amount of work has been done for understanding the biological effects of ultrasonication in mammal[2,14,16], the effect on mammalian genetic system in vitro appears to be inadequately studied and the results have been inconclusive; some authors suggested no significant effects in in vitro system[7,9,22] while others claimed some positive genotoxic changes in in vivo[15,18,20] system. In the present investigation an attempt was made to assess the extent of genotoxic effects of ultrasonication, if any, in the mammalian model *Mus musculus, in vivo*. The other objective of the study was to examine if the potentized homoeopathic drug Arnica Montana-30, which showed antigenotoxic action against X irradiation[19], could also favourably modify the harmful effects of sonication, if any.

Materials and Methods

Healthy Swiss albino mice (*Mus musculus*) weighing between 25-30 grams of both sexes served as materials for the present study.

Experimental design

Batches of 5 mice each were subjected to whole-body ultrasonic sound waves with the help of an ultrasonic cell disrupter machine (LSL, SECFROID, Switzerland) operating at a frequency wave of 23KHz, and at an energy output level of 70 for a period of 2 min (twice for 1 min each with an interval of 1 min in between). This dose of sonication was repeated at an interval of 20 days, so that mice sacrificed at 30, 60, 75 and 90 days after initial dose actually received 2,3,4 and 5 such doses of sonication, respectively. One batch of sonicated mice was orally administered with dilute potentized homoeopathic drug, Arnica Montana-30 (procured from HAPCO, Calcutta), normally used against external and internal shock and injury. One drop (0.06 ml) of the drug in 90% alcohol medium was diluted with 10 ml of double distilled water

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for making the "stock solution" of the drug, from which experimental mice were fed 0.06 ml twice at an interval of 12h.

Another batch of sonicated mice which served as controls was fed with dilute "succussed alcohol-30" (alcohol 30 prepared as per homoeopathic potentization procedure of giving 10 succussions to the 90% alcohol "vehicle" of the drug and diluting 1 ml each time with 99 ml of fresh alcohol to increase one potency); further dilution of alcohol-30 was followed in the same manner as that of Arnica Montana-30.

Chromosome aberration study

Mice at all fixation intervals were injected intraperitoneally with 0.03% colchicine solution @ 1 ml/100g body weight one and a half hours prior to sacrifice. The conventional citrate-flame-drying-Giemsa technique was followed for the bone marrow chromosome preparation.

Micronuclei testine

A part of suspension of bone marrow cells in 1% sodium citrate solution was smeared on clean, grease free slides. The slides were briefly fixed in methanol and subsequently stained with May-Grunwald solution followed by Giemsa staining[26].

Sperm head anomaly

Epididymis of each side of the control and treated male mice was dissected and taken out separately into 10 ml of 0.87% normal saline. It was made free of fats, vas deferens and other tissues. The inner contents were taken out and it was thoroughly shaken to make the sperm suspend in saline solution. This suspension was filtered through a silken cloth to remove debris and was dropped on clean grease free slides uniformly. The slides were allowed to air-dry and then stained in dilute Giemsa as per the routine procedure[27].

Observations

The frequency distribution of various chromosome aberrations at different fixation intervals encountered in unsonicated mice, sonicated mice, sonicated mice fed with alcohol 30 (positive control) and in sonicated mice fed with the homoeopathic drug Arnica Montana 30 have been summarized in Table-1 and representative photomicrographs provided (PM 1 to 7). An analysis of the data would reveal that there was an increase in total aberration frequencies from 30 days through 90 days in the sonicated series as compared to unsonicated control, and in general the homoeopathic drug fed mice showed the lowest frequency of aberrations as compared to that in sonicated mice and in alcohol-30 fed sonicated mice, in respect of both the "major" and "other" types of aberrations (Table 1). Further, in the alcohol-30 fed sonicated mice there was an abrupt increase of total aberrations at 90 days. The data on induction of micronucleated erythrocytes, both NCE and PCE, have been summarized in table 2 and representative photomicrograph provided (PM 11); an analysis of the data would also reveal that the effects of sonication increased with the lapse of time and that in the drug fed series the incidence of total number of micronuclei was the lowest as compared to the other two sonicated series. The same trend of apparently "cumulative effect" of sonication was also reflected in the data of the sperm head anomaly (Table 3, PM 9, 10).

Discussion

Khuda-Bukhsh and Chakrabarti (1998) reported that even a single dose of ultra-sound irradiation could produce genotixic effects in mice as compared to normal unirradiated controls. In the present investigation, in addition to confirmation of the earlier findings, it would be revealed that repeated exposure to ultrasonic sound waves yielded a fairly appreciable number of chromosome aberrations, enhanced the induction of micronculeated erythrocytes and number of sperm with anomalous head shapes; and that there was a "cumulative action" for repeated exposure to ultrasonic sound waves. However, several workers[1,3,5,22,24] did not get elevated frequencies of SCE in cultured lymphocytes of human being exposed to ultrasonic sound waves. One the other hand, several other workers[12,13,15] reported positive effects of ultrasonic sound waves in lymphocytes of human beings and in egg lecithin.

The biophysical effects of ultrasound in aqueous solutions can be categorized as thermal effects, cavitation, and direct effects[14]. The mechanism of action of ultrasound is quite complex, in aqueous media the non-thermal effects of ultrasound is mainly due to cavitation. The degradation of the cavitation bubbles produces free radicals[10] and induces temporary local shock waves, on the other hand the "to and fro" motion of the cavitation bubbles produce hydrodynamic shearing stress[2,14]. This results in degradation of DNA in aqueous solution and even destruction of cells[8,24]. Chatterjee and his coworkers[16,17] also documented positive changes in enzymes related to lipid peroxidation and strongly held the view that ultrasonic irradiation caused cytotoxicity. In the present investigation the administration of the homoeopathic drug Arnica Montana-30 reduced the genotoxic effects

Table-1

Frequency distribution of chromosome aberrations in 500 bone marrow cells examined (100 cells from each of 5 individual) at different fixation intervals

Fixation Time intervals (Days)	Series	Major aberration types												% of other aberration types		Total aberration	Protection	
		GS	BS	CF	TR	F	PUL	RS	TA	PP	AP	No.	%	No.	%	%±SE	Unsonicated Control vs Sonication +Drug	Sonication vs Sonication +Drug
	Unsonicated control	0	0	0	0	0	0	0	0	0	0	0	0.0	0.04		0.4±0.245		
30	Sonication	1	3	0	0	4	9	3	0	6	0	26	5.2	154	30.7	35.9±0.231		
	Sonication +Drug	0	2	6	0	1	6	4	5	0	0	24	4.8	76	15.2	20.0±0.864	19.6**	15.9^{n}
	Sonication +Alcohol	0	4	4	0	1	6	4	5	5	1	30	6.0	78	15.6	21.6±0.833		
60	Sonication	1	2	4	0	0	5	6	3	5	0	26	5.2	89	17.8	23.0±0.766		
	Sonication +Drug	0	3	2	0	0	6	5	4	5	0	25	5.0	74	14.8	19.8±0.683	19.4***	3.2^{n}
	Sonication +Alcohol	1	3	10	1	0	9	6	3	5	0	38	7.6	96	19.2	26.8±1.774		
75	Sonication	0	2	4	0	0	6	5	5	4	1	27	5.4	101	20.2	25.6±0.503		
	Sonication +Drug	0	1	3	1	0	1	2	4	1	0	13	2.6	29	5.8	8.4±0.503	8.00**	17.2**
	Sonication +Alcohol	1	5	7	1	0	7	8	5	5	0	39	7.8	60	12.0	19.8±1.200		
90	Sonication	0	5	8	0	0	7	6	5	6	0	37	7.4	111	22.2	29.4±0.503		
	Sonication +Drug	0	4	7	1	0	7	5	6	4	0	34	6.8	88	17.6	24.4±0.400	24.0***	5.0^{n}
	Sonication +Alcohol	7	5	13	3	1	12	4	15	3	0	63	12.6	149	29.8	42.4±3.290		

"Major" types: GS= Gap; BS= Break; CF= Centric fusion; TR= Translocation; F=Fragment; PUL= Pulverisation; RS= Ring; TA= Terminal association; PP= Polyploidy; AP= aneuploidy.

"Other" types include : Precocious centromeric separation, centromeric stretching, Stickiness, C-mitotic effect.
n= non-significant, *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$.

Table-2

Frequency distribution of micronucleated erythrocytes in approximately 5000 bone marrow cells (1000 cells from each of 5 individuals) examined at different fixation intervals

Fixation Time intervals (Days)	Series	NCE			PCE			P/N	MNE in NCE and PCE		Protection	
		Total cells	No	%	Total cells	No	%		Total cells	%±SE	Unsonicated Control vs Sonication +Drug	Sonication vs Sonication +Drug
	Unsonicated control	3457	2	0.058	1543	1	0.065	0.446	3	0.06±0.025		
30	Sonication	2371	6	0.253	2695	14	0.519	1.136	20	0.40±0.051		
	Sonication +Drug	2770	7	0.252	2230	6	0.260	0.805	13	0.26±0.051	0.200*	0.135^{n}
	Sonication +Alcohol	3303	13	0.393	1776	9	0.506	0.537	22	0.43±0.042		
60	Sonication	3115	7	0.224	1895	14	0.739	0.608	21	0.42±0.099		
	Sonication +Drug	2950	4	0.135	2050	4	0.195	0.694	8	0.16±0.016	0.100*	0.259*
	Sonication +Alcohol	2593	29	1.110	892	17	1.900	0.344	46	1.32±0.254		
75	Sonication	3055	12	0.392	1840	12	0.652	0.602	24	0.49±0.105		
	Sonication +Drug	3165	10	0.315	1895	9	0.474	0.598	19	0.38±0.045	0.315***	0.115^{n}
	Sonication +Alcohol	3768	18	0.477	1259	49	3.800	0.334	67	1.33±0.662		
90	Sonication	3652	20	0.548	1348	10	0.742	0.369	30	0.60±0.093		
	Sonication +Drug	3085	7	0.226	1915	5	0.261	0.621	12	0.24±0.040	0.180**	0.360**
	Sonication +Alcohol	2730	44	1.610	2270	21	0.925	0.832	65	1.30±0.139		

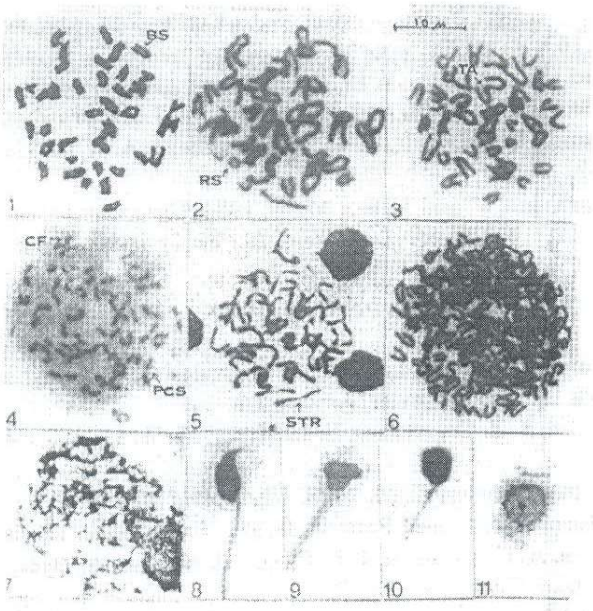
n= non-significant, *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$.

Table-3

Fix time intervals (days)	Series	Sperm observed	Sperm with abnormal heads		Protection	
			No.	%±SE	Unsonicated control vs Sonication + Drug	Sonication vs Sonication + Drug
	Unsonicated control	5000	9	0.18±0.037		
30	Sonication	5000	28	0.56±0.112		
	Sonication +Drug	5000	19	0.38±0.058	0.20	0.18
	Sonication +Alcohol	5000	30	0.60±0.110		
60	Sonication	5000	44	0.88±0.097		
	Sonication +Drug	5000	21	0.42±0.074	0.24*	0.46***
	Sonication +Alcohol	5000	83	1.66±0.181		
75	Sonication	5000	49	0.98±0.222		
	Sonication +Drug	5000	30	0.61±0.071	0.42***	0.38***
	Sonication +Alcohol	5000	130	2.60±0.192		
90	Sonication	5000	74	1.48±0.0107		
	Sonication +Drug	5000	32	0.64±0.117	0.46**	0.84**
	Sonication +Alcohol	5000	115	2.3±0.3271		

*= $p<0.05$, **= $p<0.01$, ***= $p<0.001$.

to a considerable extent, for which its use may be recommended in patients who have to undergo repeated ultrasonographic tests, either for diagnostic purpose or as a therapy.



Legends for Photomicrographs (PM):

Photomicrographs of some chromosome aberrations: PM 1-break (BS), PM 2-ring (RS), PM 3-terminal association (TA). PM 4- precocious centromeric separation (PCS) and centric fusion (CF). PM 5-stretching (STR), PM 6-polyploidy (PP), PM 7-stickiness; PM 8-normal sperm and PM 9 to 10- sperm with abnormal head shape; PM 11- erythrocyte with micronucleus Magnification (Bar represents 10 µm).

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