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CYTOGENETIC EFFECTS OF INHALATION OF A MOSQUITO REPELLENT VAPOUR ON TARGET AND NON-TARGET CELLS OF RATS

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ABSTRACT

Genotoxic potential of inhalation of "All Out" (AO), a liquid mosquito repellent, vapour (AOV) was evaluated in target (pulmonary alveolar macrophages or PAMs) and non-target (bone marrow or BM) cells of rats. Evaluation was based on analysis of chromosome aberrations (CAs) in PAMs and BM cells, and micronuclei (MN) in PAMs. The rats were exposed to static vapour intermittently in a closed inhalation chamber (15 min/h, 8 h/day) for short-term (1 day) exposure and (15 min/h, 8 h/day, 7 day/week) for long-term (7 days) exposure. Animals were killed 24 h and 32 h after the final exposure for CA and MN studies respectively. Concentration-response analysis done by exposing the animals in AOV collected for different periods (1, 5 and 10 min) for both CAs and MN showed a good positive correlation; significantly higher frequencies, compared to the respective controls, of CAs and MN were obtained for the highest concentration tested. In no case the incidence of CAs in BM cells differed significantly from the control value. Insecticide present in AO is supposed to be responsible for causing chromosome damage. Differential response of PAMs and BM cells has been discussed from the point of target and non-target cells. Lack of any remarkable difference in effect for 1 and 7 day exposures seems to be due to the proliferative nature of the cells.

RESUMEN

Se evaluó el potencial genotóxico de la inhalación de "All Out" (AO), forma de vapor (AOV) del repelente líquido contra mosquitos, en células blanco (macrófagos de los alveolos pulmonares ó PAMs) y no blanco (médula osea ó BM) de ratas. El estudio estuvo basado en el análisis de las aberraciones cromosómicas (CAs) en células PAMs y BM y en micronúcleos (MN) en PAMs. Las ratas fueron expuestas intermitentemente a vapores estáticos en una cámara de inhalación cerrada en tratamientos cortos de 1 día (15 min/h, 8 h/día) y largos de 7 días (15 min/h, 8 h/día, 7 días/semana). Los animales se sacrificaron 24 h y 32 h después de la exposición final para los estudios de CA y MN, respectivamente. Los análisis de concentración-respuesta hechos al exponer a los animales al AOV colectados en diversos períodos (1, 5 y 10 min) tanto para CAs como para MN, mostraron buena correlación positiva; en las concentraciones más altas probadas se obtuvieron frecuencias significativamente mayores de CAs y MN, comparadas con sus respectivos testigos. En ningún caso la incidencia de CAs en células BM difirió significativamente del valor testigo. Se supone que el insecticida presente en el AO sea el responsable del daño causado a los cromosomas. Se discutió la respuesta diferencial de las células PAMs y BM desde el punto de vista de sus características como células blanco y no blanco. La carencia de una diferencia marcada del efecto en las exposiciones de 1 y 7 días parece debida a la naturaleza de proliferación de las células.

INTRODUCTION

Varieties of mosquito repellent (coil, mat and liquid type) are now being used to combat mosquito menace in tropical and subtropical countries. They are either burnt or evaporated in closed or semi-closed rooms to keep the mosquitoes at bay and users are exposed for hours to the smoke/vapour. "All Out" (AO) is one such repellent which is available in liquid form in a 'spirit lamp'-like container provided with a fibre wick. The liquid contains a pyrethroid insecticide having a

good knocking down effect on mosquitoes. During use the AO lamp is covered with an electrically operated cap which maintains a constant temperature and facilitates slow evaporation of the liquid.

Very recently mosquito coil smoke (MCS) and mosquito mat vapour (MMV) have been demonstrated to be clastogenic in pulmonary alveolar macrophages (PAMs) of rats by us (Das *et al.* 1994, Sahu and Das 1995). Liu and his co-workers (Liu and Wong 1987, Liu and Sun 1988, Liu *et al.* 1989) have earlier reported morphological and biochemical changes of the cells of the respiratory tract of rats exposed to MCS. So far as the authors are aware, AO or any other liquid type mosquito repellent has not been tested for its potential genotoxic effect.

In view of its large scale use, indoor air pollution caused by its evaporation and human exposure we have evaluated the possible clastogenic efficiency of AO vapour (AOV) in pulmonary alveolar macrophages (PAMs) of rats by using chromosome aberration (CA) analysis and micronucleus (MN) test. The incidence of CAs in the target cells (PAMs) has also been compared with that in non-target bone marrow (BM) cells to substantiate the importance of studying the target cells.

MATERIALS AND METHODS

Inbred Charles-Foster rats of both sexes having a body weight of 80-100 g and with an age of 8-12 weeks were used. The repellent (AO) was purchased from the local market (Batch No. GCA, 7/94). The animals were exposed to AOV in a closed glass inhalation chamber (for details see Das *et al.*1994). In brief, AOV was first collected by placing the AO-lamp, with its switch on, inside the inhalation chamber for different periods (1, 5 or 10 min) to obtain different gas concentrations. The lamp was then taken out quickly through a small door and rats were released into the chamber. After 15 min they were taken out. Thus the rats were exposed to the static vapour intermittently @ 15 min/h and 8 h/day for short-term (1 day) exposure, and @ 15 min/h, 8 h/day and 7 day/week for long-term (1 week) exposure. After use the inhalation chamber was always made gas-free for next use.

For CA and MN assays separate sets of animals were employed. The animals for the former received colchicine (4 mg/kg) pretreatment, but not the animals for the latter. Further, the same animals provided both PAMs and BM cells for CA assay. MN study was done only in PAMs. The animals were killed 24 h and 32 h after the final exposure for CA and MN assays, respectively. Control animals were reared and maintained side by side but without exposure to AOV. Collection of pulmonary lavage, and preparation and staining of slides for MN assay were done following the technique mentioned elsewhere (Das *et al.* 1994, Sahu and Das 1995). For CA analysis both lavage fluid and BM cells were processed and slides were prepared according to the conventional hypotonic KCl-flame-drying-Giemsa schedule.

Student's t test was conducted to know the level of significance between the treated and control data. To find out if the data showed any correlation with the concentration of the vapour a correlation coefficient analysis (r-test) was done. Mean data for CAs and MN obtained at various concentration levels were compared following analysis of variance (F-test).

RESULTS

For CA analysis in both PAMs and BM cells only break type aberrations were taken into consideration; gap type aberrations, though encountered, were not considered here because of their doubtful significance. Chromatid breaks, fragments of untraceable origin, rings (resulting from sister chromatid union) and exchanges comprised the break type aberrations; the first two types being the most common. Data on types and frequencies of structural CAs in both the cell types are presented in **table I**. The frequencies of CAs in BM cells for different AOV concentration levels did not differ significantly from each other (F-test, $p > 0.05$) and also from the control value. The data for long-term and short-term exposures were the same and close to the control value. On the other hand, in PAMs the frequencies of CAs (**Table I**) as well as MN (**Table II**) exhibited a distinct concentration-dependent increase (r = +0.929 and +0.995 respectively); however, the increase was statistically significant only in animals exposed to AOV collected for 10 min. The data for 1 day and 7 day exposures for both CA and MN in PAMs were close to each other (t-test, $p > 0.05$) and significantly high, compared to the respective control value (**Tables I** and **II**).

Elevated incidences of mitotic index (MI) were also noted in PAMs of exposed rats particularly at higher concentrations (**Table II**).

DISCUSSION

Significantly high incidences of CAs and MN in PAMs, at least at higher concentration, clearly demonstrate genotoxic ability of inhalation of AOV. Thus AOV, like MCS and MMV, is also genetically harmful. This liquid mosquito repellent contains (as indicated on the packet) d-allethrin (3.6 % w/w), dibutyl hydroxy toluene (0.31 % w/w), perfume (0.15 % w/w) and deodorised kerosine (95.94 % w/w). The active principle is d-allethrin, a pyrethroid insecticide. Although a number of synthetic pyrethroids have been reported to be non-mutagenic in *Salmonella* and V79 Chinese hamster cells (Bartsch *et al.* 1980) some of them have already been shown to be clastogenic in mammalian *in vivo* systems (Amer and Aboul Ela 1985, Bhunya and Pati 1988, 1990, Pati and Bhunya 1989). Agents showing negative effect in *Salmonella* assay but positive in mammalian *in vivo* systems are not uncommon (Das and Roy 1990, Roy and Das 1990). It is quite likely that the pyrethroid insecticide present in AO is responsible for

TABLE I. INCIDENCE OF CHROMOSOME ABERRATIONS IN TARGET (PAMs) AND NON-TARGET (BM) CELLS OF RATS EXPOSED TO "ALL OUT" VAPOUR

Cell type	Collec. of AOV (min)	Exposure schedule (day)	Metaphases/animals	"Break type" aberrations				
				Chromatid breaks	Fragments	Rings	Exchanges	Total (mean% ± SE)
PAM	0	-	200/4	2	-	1	-	2.00 ± 0.47
	1	1	200/4	2	1	-	-	1.50 ± 0.43
	5	1	200/4	2	1	-	1	2.50 ± 0.83
	10	1	200/4	11	-	-	1	6.50 ± 0.43**
	10	7	200/4	6	3	1	-	5.50 ± 1.29*
BM	0	-	400/4	2	2	-	-	1.00 ± 0.35
	1	1	400/4	1	2	-	-	0.75 ± 0.21
	5	1	400/4	1	1	1	-	1.00 ± 0.35
	10	1	400/4	4	2	-	-	1.50 ± 0.25
	10	7	400/4	2	4	-	-	1.50 ± 0.25

Significantly higher than the respective control value (*t*-test) at * $p < 0.05$, ** $p < 0.001$
For each point 2 males and 2 females were used
r value for different concentrations for 1 day exposure in PAMs = +0.929, $p < 0.05$

chromosome damages in PAMs. In view of some positive reports available in the literature regarding the efficiency of toluene in inducing CA (Kreijl and Slooff 1985, Mavournin *et al.* 1990) and of organic compounds emitted from kerosine space heater in inducing mutation in *Salmonella* Ames test (Mumford *et al.* 1992) their role in causing chromosome damage cannot be ruled out. The amount of perfume present in AO is extremely small and its possible involvement in chromosome damage seems to be remote. Testing of different components of "AO" may throw some light regarding the causative factor(s) for chromosome damage.

Very recently lung has drawn the attention of the geneticists for evaluation of genotoxic potential of gaseous or volatile environmental agents (Conner *et al.* 1979, 1980, Au *et al.* 1988, Rithidech *et al.* 1989, 1990, Chorvatovicova and Kovacikova 1992, Das *et al.* 1994). Earlier for the lack of suitable technique in handling pulmonary cells following inhalation non-target cells like BM cells, gonadal cells, lymphocytes, etc. were conventionally used for the purpose (Kligerman *et al.* 1987, Kar *et al.* 1989, Moorthy and Murthy 1994). But the non-target cells as they are less exposed or not exposed at all to the agent may provide false negative results. Differential response of PAMs and BM cells in the present study (**Table I**) is explained from the point of target and non-target cells and clearly demonstrates the importance of using the target cells for such study. Our results thus support the findings of Conner and Chang (1983), Scott *et al.* (1983), Lewtas *et al.* (1993) and Binkova *et al.* (1994).

In the present study "concentration" of AOV was expressed in terms of period of collection of vapour inside the chamber. It is no doubt a crude method. However, as evaporation in this device takes place uniformly at a constant temperature the amount of vapour in the closed chamber is expected to be nearly proportional to the period of evaporation. It clearly gives an idea about positive correlation between amount of the vapour and chromosome damage.

Both spindle poisons and clastogens can induce MN. From our data one cannot be sure if MN resulted from clastogenic or spindle poisoning effect or both. However, clastogenic efficiency of AOV demonstrated in CA study and non-availability of polyploid or aneuploid cells in metaphase preparation and of abnormal mitotic figures in MN preparation suggest that MN

TABLE II. INCIDENCE OF MICRONUCLEATED PAMs IN RATS EXPOSED TO "ALL OUT" VAPOUR

Collection of vapour (min)	Exposure schedule (day)	No. of PAMs scored/animals	PAMs with MN (mean % ± SE)	Mitotic index (mean % ± SE)
Control	-	2000/4	0.15 ± 0.04	0.78 ± 0.09
1	1	2000/4	0.15 ± 0.08	1.15 ± 0.15
5	1	2000/4	0.25 ± 0.04	1.18 ± 0.09*
10	1	2000/4	0.35 ± 0.04* (r = +0.995 $p < 0.001$)	1.75 ± 0.13*** (r = +0.935 $p < 0.05$)
10	7	2000/4	0.55 ± 0.08**	1.55 ± 0.10**

Significantly higher than the control value (*t*-test) at * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$
For each point 2 males and 2 females were used

were most likely due to clastogenic effect. Identical trends for concentration-response data for both CAs and MN also support the assumption. 32 h post-exposure gap was reported to be highly suitable for MN assay in PAMs (Sahu and Das 1995).

The data for 1 day and 7 day exposures for both CA and MN fail to exhibit any remarkable difference, and that is explained from the point of proliferative nature of PAMs. This aspect has been discussed earlier by us in detail (Das *et al.* 1994).

Toxic exposure not only induces mitosis in the macrophages but causes influx of them in the bronchoalveolar air space also (Evans *et al.* 1973, White and Garg 1981). Concentration-dependent increase of MI (**Table II**) thus indicates toxic nature of the vapour. If there is such an influx, absolute values for CAs, MN and MI are expected to be more, because the influx would lead to dilution of the affected cells with the lately influxed unexposed cells.

Our finding reveals that AOV like MCS and MMV has chromosome damaging ability, at least at higher concentration. A comparative study of relative genotoxic efficiency of these three mosquito repellents, which is in progress, would be important to identify the less toxic with still good insecticide capacity.

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