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INDUCTION OF SINGLE-STRAND BREAKS IN DNA OF MICE AFTER INHALATION OF VINYL CHLORIDE

S.A. SOLVEIG WALLES and BO HOLMBERG

Unit of Occupational Toxicology, Research Department, National Board of Occupational Safety and Health, S-171 84 Solna (Sweden)

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SUMMARY

Female mice were exposed to 500 ppm vinyl chloride (VC) for 6 h/day 5 days/week for 1–8 weeks. Groups of mice were killed at different times during this period. DNA damage, expressed as single-strand breaks (SSB), was studied in liver, kidneys, lungs, spleen and brain. The level of SSB increased in liver, kidneys, spleen and lungs with time of exposure and reached a plateau for kidneys and lungs after 80 and 120 h of exposure. In spleen there was only a slight increase in the SSB, and in brain no detectable increase was found.

INTRODUCTION

VC is mutagenic to several organisms and carcinogenic to humans, mice, rats and hamsters [1]. In mice it produces tumors in several organs, e.g. the liver, lungs, kidneys and mammary glands [1–3].

VC is rapidly metabolized by a monooxygenase enzyme to chloroethylene oxide [4]. Chloroethylene oxide can spontaneously transform to chloroacetaldehyde. Both these compounds are electrophilic agents and react with proteins and nucleic acids in vivo [5].

In this study the induction of SSB have been studied in the DNA of female mice after inhalation of 500 ppm VC. The results show that VC induces SSB in DNA in the liver, kidneys, spleen and lungs.

MATERIALS AND METHODS

Chemicals

VC (>99.9%) from AGA, Sweden, was used. Hydroxylapatite (Bio-Gel HTP and DNA-grade Bio-Gel HTP Bio Rad Laboratories, Richmond, CA,

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TABLE 1

EXPOSURE SCHEME

	Exposure time (h)	No. of animals
Mice killed 2 h after exposure	39	4
	60	4
	117	4
	234	4
Mice killed 18 h after exposure	36	4
	114	4
	231	5

Control animals, exposed to air only, were killed after 36 h (n = 3) and 231 h (n = 4).

U.S.A.) and 4,6-diamidino-2-phenylindole · 2HCl (Serva-Feinbiochemica, Heidelberg, F.R.G.) were purchased.

Animals and exposure

Female mice (strain NMRI, 5 weeks old) were used. The mice were exposed for 6 h/day 5 days/week in 10-ℓ desiccators with 15 animals in each. The temperature in the desiccators was 24°C, the relative humidity 60—70% and the airflow 4 ℓ/min. The VC gas was diluted with air to a concentration of 500 ppm. The VC concentration was checked continuously with a Miran-1 A infrared spectrophotometer with a recorder. Control animals were exposed to air only. Animals were killed by cervical dislocation 2 or 18 h after the exposure period, and immediately dissected for SSB determination. Table 1 shows the exposure scheme.

Determination of DNA-strand breaks

Cell nuclei were prepared from liver, kidney, lung, spleen and brain according to the method described by Walles and Orsén [6]. SSB were determined by the DNA-unwinding technique of Ahnström and Erixon [7] as modified by Walles and Erixon [8]. The fraction (F_{DS}) of double-stranded DNA (DS) was calculated [9] as the ratio of the amount of DS and the sum of DS and single-stranded DNA (SS):

$$F_{DS} = \frac{DS}{DS + SS}$$

The negative logarithm of F_{DS} is a linear function of the number of SSB.

RESULTS

The results are presented in Fig. 1. When the animals were killed 2 h after

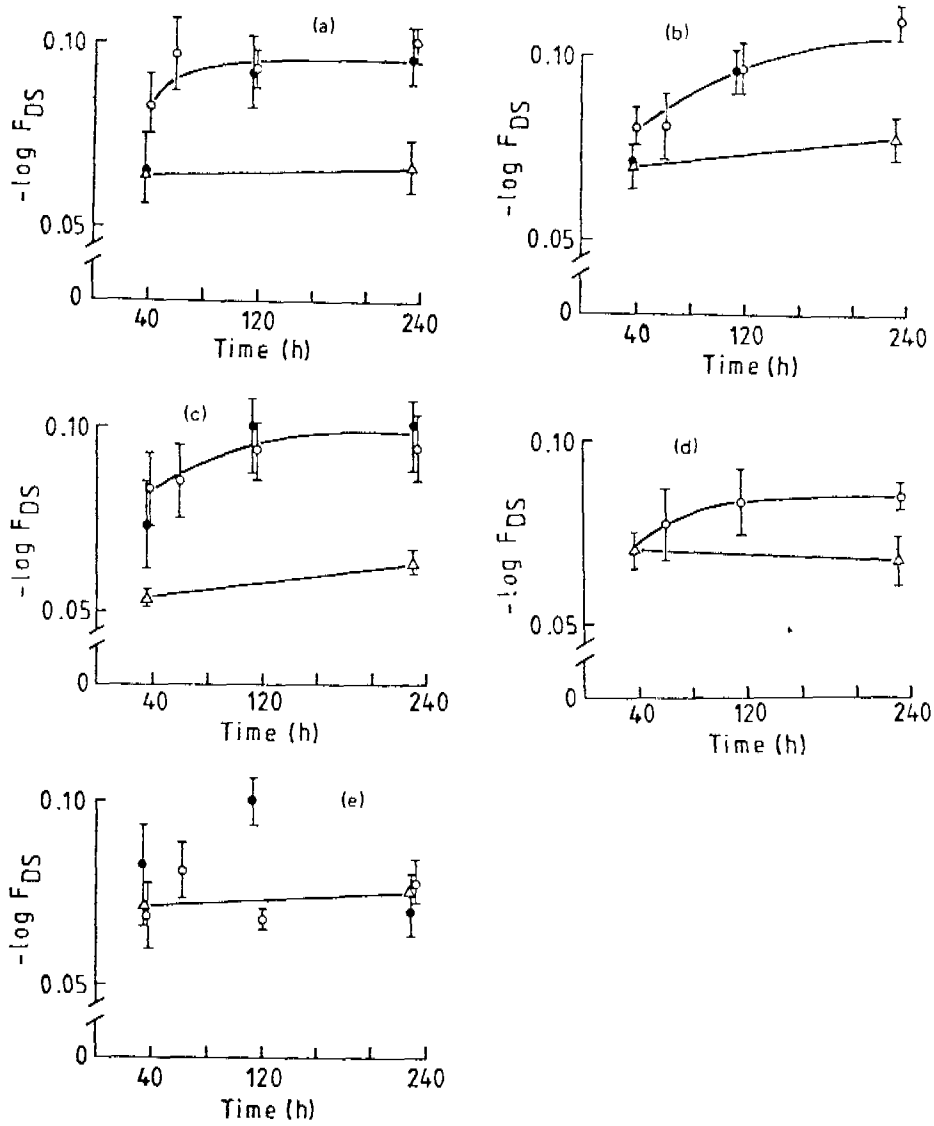

Fig. 1. Level of SSB expressed as $-\log F_{DS}$ for DNA of (a) kidney, (b) liver; (c) lung; (d) spleen; (e) brain after different times of exposure to VC. SSB were determined after 2 h (○) and 18 h (●) after exposure; control animals (△) mean ± S E. Statistical analyses were performed with the Student's *t*-test. The lowest statistical significance of the difference between mean values of controls and of exposed animals are given: kidneys (39 h) $*P < 0.05$; liver (117 h) $*P < 0.05$; lungs (39 h) $**P < 0.01$; spleen (234 h) $**P = 0.01$.

an exposure period of 117 h, increased levels of SSB were found in lungs, liver, kidneys and possibly spleen, but not in brain. For kidneys and lungs a plateau was reached after 80 h and 120 h exposure, respectively. The level of SSB in liver, however, seemed to increase slightly throughout the

exposure period. A statistically significant increase in SSB occurred in spleen only at 234 h. The level of SSB after 234 h of exposure was about the same for kidneys, lungs, and liver when the mice were killed 2 h after exposure.

When the animals were killed 18 h after VC exposure, the SSB levels in kidneys, lungs and liver had returned to normal values for animals exposed for 36 h. However, after 114 h and 231 h of exposure the SSB levels in kidneys, lungs and liver remained elevated even 18 h after termination of exposure. In brain the SSB level 18 h after VC exposure of 114 h, was the only sample elevated. The SSB levels in brain at other exposure periods did not show a consistent pattern.

DISCUSSION

VC is rapidly metabolized to chloroethylene oxide [5], which can be conjugated to glutathione or be transformed to chloroacetaldehyde, which can be further metabolized [10]. The short-lived [5] epoxide is the most reactive metabolite [11] and binds to nucleophilic sites in macromolecules [5]. The main product of the alkylation of DNA is *N*-7-(2-oxoethyl)guanine [5]. The VC metabolites also alkylate proteins [12] in the liver, small intestine, kidneys, lungs and spleen, but not in the brain. The tissue protein binding was roughly correlated to the monooxygenase activity. The binding to protein is 5 times higher in liver than in kidneys, lungs, or spleen [12,13].

A $-\log F_{DS}$ value of 0.11, which was reached for liver after 234 h of exposure in animals killed 2 h after termination of exposure, corresponds to about 0.5 SSB/10^9 daltons (calculated from Refs. 8 and 9). Thus, after 4—8 weeks of exposure, one SSB seems to be induced per every 2 DNA molecules in liver, lungs and kidneys.

For kidneys and lungs, the SSB levels reached a plateau. There are several possible explanations for the formation of the plateau, e.g. only a limited amount of SSB can be formed during the base excision repair process. Chloroethylene oxide may also act as a cross-linking agent, and thus restricts a further increase of SSB. A third possibility could be that chloroethylene oxide might, after a sufficiently long exposure time, induce cell death, leading to a degradation of DNA. Finally, induced and repaired SSB may attain a steady state. This latter hypothesis could be further illuminated by combining VC exposure with administration of substances inhibiting excision repair.

The observation that the SSB levels are normalized 18 h after the first 36 h of exposure, but not after longer exposures, indicates that the induced SSB can no longer be repaired after a sufficiently long induction time, which lies somewhere between 36 h and 114 h of exposure. The curves shown for the different organs in Fig. 1a—c suggest that this induction time may be different in various tissues.

SSB are formed by base excision repair of alkylated bases, e.g. *N*-7 and

N-3-alkylguanine [14,15]. The alkylation of O^6 in guanine, which is related to mutagenic and carcinogenic effects of chemicals [16], is not repaired by excision repair [17] and does thus not lead to SSB. However, as alkylating agents react to a higher degree with *N*-7 than with O^6 of guanine, an increase in the SSB levels might at least tentatively be used for definition of critical organs. However, much work has to be done to further illuminate this possibility.

It has been shown that DNA fragmentation caused by a carcinogenic substance in a particular organ is related to the carcinogenic effect. This has for example been shown for nitrosoamines and alkyl alkansulfonate [22,23]. In the present study the highest levels of SSB were reached in liver but DNA damage appeared earliest in lungs and kidneys. The spleen seems to be less sensitive than liver, lung and kidney, as judged from the lower SSB value in the spleen at the end of the experiment. The lung is the most sensitive organ for VC carcinogenicity in mouse [3,20]. However, although liver hemangiosarcomas are induced also in mouse [2,3], they are less common than hemangiosarcomas in other organs and they represent only a minor portion of all tumor types formed. Reticulum cell sarcoma of the spleen was observed in mice in one study [3]. Kidney tumors have not been detected in NMRI mice, although VC induced tumors occurred in perinephral fat tissue [3]. However, kidney tumors occurred in Swiss mice in Maltoni's study [2]. The absence of a difference in SSB between brain tissue of VC-exposed mice and control animals is in accordance with the observations [13] that no radioactivity bound to brain DNA could be detected in VC-exposed mice and that no brain tumors have been observed in experimental animals. Brain tumors have been found among occupationally exposed men [21—24].

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