Effects of Isoflurane Exposure on Oncogene and Tumour Suppressor Gene Expressions in Vital Organs of CBA/CA Mice

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Abstract. Background: Isoflurane is a halogenated volatile anesthetic. It is generally believed that modern inhaled anesthetics are not themselves toxic but their intermediates might be. Whether isoflurane exposure results in any alteration in oncogene and tumour suppressor gene expression was investigated here. Materials and Methods: CBA/CA H-2k mice were anesthetized with isoflurane for 1 hour. The expression alterations of c-myc, Ha-ras and p53 genes were investigated in the vital organs 3, 24 and 48 hours later. Results: Expression patterns of the investigated genes differed both in magnitude and time course according to gender. Overexpressions were detected after 24 hours in both sexes, but in females, overexpression of all investigated genes remained high in all organs at 48 hours, except in the bone-marrow. Discussion: Considerable overexpression of two oncogenes and a tumour suppressor gene after isoflurane exposure were found.

Isoflurane is a halogenated methyl ethyl ether, (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether). The three fluorine atoms on the terminal ethyl carbon confer considerable molecular stability, making isoflurane resistant to chemical and physical reactions. Isoflurane, like enflurane, requires no preservative, does not react with metal and is stable in soda lime and ultraviolet light (1-3).

It is now generally believed modern inhaled anesthetics themselves are not hepatotoxic but their metabolites and intermediates might be (4). Toxicity of anesthetics may be related to anesthetic biotransformation. Biotransformation may produce injury by one or more mechanisms: lipid peroxidation depletion of antioxidants and covalent binding which permanently alters molecular structure (5-7).

Kharasch and Thummel identified cytochrome P450 2E1 as the predominant enzyme catalysing human liver microsomal defluorination of isoflurane (6). They suggested that metabolism also contributes significantly to the elimination pharmacokinetics of volatile agents. Kim et al. demonstrated DNA damage, lipid peroxidation and protein oxidation in rats exposed to isoflurane, observed in such vital organs of the animals as lymphocytes, bone marrow, spleen, brain, liver and lung (7). However, the biological effect of ionic and non-ionic fluoride has not yet been clarified (8).

There have been no reports on whether isoflurane exposure results in oncogene and/or tumour suppressor gene expression alterations as a biological effect of exposure. The aim of this study was to determine whether such changes occur in the vital organs of CBA/CA mice after isoflurane anesthesia.

Materials and Methods

Conventionally kept 5-week-old CBA/Ca (H-2k) inbred mice (sensitive to chemical carcinogen exposure) weighing 20 to 23.5 g were used. Six groups were maintained in both genders, containing six animals in each group. Three groups were exposed to isoflurane for 1 hour in a plastic chamber, with a volume of approximately 3 liters, connected to an anesthetic machine with a half opened system, which is generally used for anesthesia of small mammals. Anesthesia was administered with a forane vaporizer calibrated for isoflurane at concentration of 2% (1.5 minimum alveolar concentration) for 1 hour, employing 90% oxygen 3 l/min as the carrier gas. The first group was autopsied 3 hours, the second 24 hours and the third 48 hours after anesthesia. Fourth, fifth and sixth groups were control groups of both sexes. For the control group 90% oxygen at inhalation of 3 l/min was maintained for 1 hour. The fourth group was autopsied 3 hours, the fifth 24 hours and the sixth 48 hours after oxygen inhalation (in paralell with the groups

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anesthetized with isoflurane). Thymus, spleen, lung, liver, kidney, lymph nodes and bone marrow were removed during autopsy and 100 mg samples of each tissue were pooled separately according to groups (one pooled sample per group). After homogenization of the organs, total cellular RNA was isolated using TRIZOL reagent (Invitrogen, Paisley, Scotland, UK). After RNA concentration and quality check at 260/280 nm, 10 µg of RNA was dot blotted onto Hybond N+ nitrocellulose membranes (Amersham, Little Chalfont, UK) and hybridized with chemiluminescently labelled (ECL kit, Amersham, Little Chalfont, UK) c-myc, Ha-ras and p53 (Prof. J. Széberényi, University of Pécs, Hungary) gene-specific probes. RNA isolation, hybridization and detection were performed.

Figure 1. C-myc, Ha-ras and p53 gene expression in male mice after 1 hour isoflurane exposure during anesthesia or 1 hour 90% oxygen inhalation as control.
according to the manufacturer’s instructions. Signals were detected on X-ray films. Dots were evaluated by Quantiscan software (Biosoft, Cambridge, UK). β-Actin activity was used as an endogenous control. This gene shows permanent expression and its activity is considered as 1 unit or 100%. Gene expressions were evaluated as percentages of β-actin activity.

Results

Overexpression of c-myc and p53 was observed in the bone marrow in both sexes, and in the kidney in male mice 3 hours after isoflurane exposure (Figures 1 and 2). The early
gene expression declined in the first 24 hours. We supposed that this was due to the high turnover rate of the tissue resulting in higher sensitivity but quicker clearance after exposure to environmental agents. We detected considerable gene expression alterations (c-myc, Ha-ras and p53) in other organs (liver, spleen, lung, thymus and lymph nodes) 24 hours after exposure in both sexes.

Gene expression alterations showed different patterns between the two genders. In kidney tissue, there was a difference between females and males, as in male mice overexpression of c-myc and p53 was not detectable at 24 and 48 hours. In male 48 hours after exposure, we detected overexpressions only in the liver, spleen and lung. In female mice, overexpression of all three genes was detectable 24 and 48 hours after exposure in all organs except the bone marrow. The cause of the differences in expression kinetics between the sexes is possibly multifactorial; hormonal and body composition disparities might play a role. In liver, spleen and lung, we detected overexpression of all investigated genes in both sexes at 24 and 48 hours.

Discussion
General anesthetics are administered to patients and animals with the aim of minimizing the effect of external stimuli, such as surgical interventions. Cells respond to external stimuli as well as to exposure to environmental agents with a cascade of intracellular events. Although anesthetics can affect various steps in this series of molecular events, our knowledge of the mechanism of action of general anesthetics at the molecular level is fragmentary (9-11).

Immediate activation of transcription of a family of proto-oncogenes located in the nucleus has been found to precede changes in the expression of other stress-induced genes (12-16). Hamaya et al. reported immediate early expression alterations of two proto-oncogenes, c-fos and c-jun, in vital organs of male Sprague-Dawley rats after isoflurane anesthesia. Their results show time-related overexpression of both c-jun and c-fos, primarily appearing in the first 5 minutes, continuously increasing within the two-hour long investigation period in the brain, kidney and liver (16).

In our present study, we aimed to characterize the effect of isoflurane on the expression of oncogenes and a tumour suppressor gene, considered as biomarkers of the biological effect (17) of chemical exposure in vital organs of CBA/CA mice. We found that the investigated genes were overexpressed and the expression differed both in magnitude and time course according to gender. Overexpressions were detected after 24 hours parallel in both sexes, but in females, overexpression of all investigated genes remained high in all organs at 48 hours, except in the bone marrow. The cause of these differences needs further explanation but neurohormonal disparities, differences of sex hormone concentrations and the sensitivity of sex hormone receptors in the tissues might play a role. Sex differences involves genes participating in DNA repair, stress response, immunoregulation and cell signaling. Therefore, major differences of gene regulation exist between males and females (18, 19).

Bastian investigated the effects of isoflurane on RNA synthesis in isolated rat liver cell nuclei and found a significant increase in RNA transcription 18 hours after isoflurane anesthesia and found this effect disappear by the fourth day (20). We detected that the overexpression of the investigated oncogenes and tumour suppressor gene showed a peak at 24 hours; furthermore in females, expression alterations extended beyond 48 hours after exposure. Time-dependent DNA damages investigated by Kim et al. were found to be significantly higher in the same organs in which we detected overexpressions. Our result might be another indication that isoflurane can stimulate intracellular molecular events rather then suppress them.

References

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