Carcinogenic Potential of Trans-2-hexenal is Based on Epigenetic Effect

EDIT NADASI¹, TIMEA VARJAS¹, LASZLO PAJOR² and ISTVAN EMBER¹

¹Department of Public Health and Preventive Medicine and
²Department of Pathology, Faculty of Medicine, University of Pécs, 7624 Pécs, Szigeti u. 12, Hungary

Abstract. Background: Trans-2-hexenal (2-hexenal) is an \(\alpha,\beta\)-unsaturated carbonyl compound protecting plants against harmful substances. Since humans have a permanent intake of 2-hexenal via vegetable products, this genotoxic and mutagenic compound is considered to play a role in human carcinogenicity. Materials and Methods: Ha-ras and p53 gene expression changes and tumor development were investigated in mice and rats after 2-hexenal administration. Results: 2-Hexenal exposure did not result in gene expression alterations 24, 48 or 72 hours after administration while 10 out of the 72 mice and rats included in the long-term study developed a malignancy by the end of the 18-month follow-up. Conclusion: Our results suggest that, although 2-hexenal showed no effect on the expression of the investigated onco- and suppressor genes, it has a marked carcinogenic potential, which may be explained only by an epigenetic effect of the compound.

Materials and Methods

Conventionally kept 6- to 8-week-old CBA/Ca(H-2\(^b\)), AKR/J(H-2\(^b\)) and C3He-mg(H-2\(^b\)) mice and Long-Evans, Fischer 344 and Wistar rats (6 females and 6 males of each strain) received 3x50 mg/kg body weight 2-hexenal (Sigma-Aldrich, Hungary) dissolved in corn oil i.p. in the "short-term" system. Controls received the same volume of corn oil. Animals were autopsied 24, 48 and 72 hours after drug administration. In the "long-term" study, mice and rats received 150 mg/kg body weight of 2-hexenal i.p. (50 mg/kg on the 1st, 8th and 15th day) and were autopsied after 18 months of survival. Developed tumors were removed and 5-\(\mu\)m formalin-fixed, paraffin-embedded sections were routinely stained by haematoxylin-eosin and evaluated by light microscopy, (50x and 100x).

One hundred mg tissue samples of lung, thymus, kidney, liver, spleen, paracoeal lymph nodes and bone marrow were removed from each animal and total cellular RNA was isolated by using TRIZOL Reagent (GIBCO, Grand Island, NY, USA). After RNA concentration and quality check at 260/280 nm, 5 \(\mu\)g of RNA was dot blotted onto Hybond N+ nitrocellulose membranes and hybridized with chemiluminescently-labelled (ECL Kit, Amersham, Little Chalfont, UK) Ha-ras (Prof. J. Szeberényi, University of Pécs, Hungary) and p53 (ATCC, Manassas, USA) DNA probes. Signals were detected on X-ray films and quantified by Quantiscan software (Biosoft, Cambridge, UK). Gene expressions were evaluated as percentages of \(\beta\)-actin. The arbitrary unit in the Figures is equivalent to 100% expression of \(\beta\)-actin.

Results

No significant difference between the treated and the control groups and no overexpression of Ha-ras and p53 genes could be observed in the "short-term" investigations (Figure 1).

In the "long-term" study, no tumors were found in the 12 CBA/Ca mice, while 1 of the 12 AKR mice developed leukaemia and 3 of the 12 C3He-mg mice exhibited malignant diseases (1 liver carcinoma and 2 kidney tumors); of the 12 LE rats, 1 had carcinoma of the parotideal gland and 1 had adenocarcinoma, 2 lung tumors were found among the 12 Fischer 344 rats and 2 other lung tumors among the 12 Wistar rats. Two tumors are shown in Figure 2.

Correspondence to: Edit Nadasí, MD, Department of Public Health and Preventive Medicine, University of Pécs, H-7624 Pécs, Szigeti u. 12, Hungary. Tel: 36/72/536-394, Fax: 36/72/536-395, e-mail: edit.nadasí@aok.pte.hu

Key Words: Trans-2-hexenal, carcinogenic effect, gene expression, "short-term" and "long-term" studies, epigenetic effect.
Figure 1. Expression of Ha-ras and p53 genes in lung, thymus, kidney, liver, spleen, lymph node and bone marrow of control and treated animals 24, 48 and 72 hours after 2-hexenal administration.
Discussion

2-Hexenal is a common component of plants (2, 11-13) and the main intake for humans is from the consumption of fresh vegetables, fruit and fruit juices and beverages like black tea (14). All data available suggest that, because of the permanent exposure and its mutagenic/genotoxic activities, 2-hexenal may play a role in human carcinogenesis.

2-Hexenal exposure did not result in gene expression alterations 24, 48 or 72 hours after administration in our "short-term" investigations. On the other hand, 10 (7.2%) out of the 72 animals included in the "long-term" study developed tumors by the end of the 18-month follow-up, while no tumors were observed in the control group of 18 animals.

Although our "short-term" and "long-term" animal model system could detect the effect of different carcinogenic compounds (15-20), 2-hexenal, which was shown to be genotoxic in other studies (6-9), induced no gene overexpression in our investigations. Considering that: i) 2-hexenal, unlike the well-known genotoxic 7,12-dimethylbenz(α)anthracene (DMBA; 18, 19), showed no effect on the expression of the investigated key genes (Ha-ras and p53) in the "short-term" experiments but exhibited carcinogenicity in the "long-term" study, and that ii) the coincidence between the genotoxic and carcinogenic effects of a certain substance is 90%, the carcinogenic effect of 2-hexenal may probably be explained by supposing an epigenetic effect of the compound.

Further "long-term" studies are needed to investigate the late effects of 2-hexenal on the expression of onco- and suppressor genes. Individually designed DNA-chips may also be useful to identify new biomarker genes underlying the supposed epigenetic effect of 2-hexenal.

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References


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