EFFECTS OF MANGANESE ETHYLENE-BIS-DITHIOCARBAMATE (MANEB) ON RAT NASAL RESPIRATORY MUCOSA

DEVECI E[1], YORGANCILAR E[2], EKINCI C[1], KOPARAL[4] M, AKKUS Z[3], YAVUZ D[1]
[1]Department of Histology and Embryology, Faculty of Medicine, University of Dicle, Diyarbakir - [2]Department of Otorhinolaryngology, Head and Neck Surgery, Faculty of Medicine, University of Dicle, Diyarbakir - [3]Department of Biostatistics, Faculty of Medicine, University of Dicle, Diyarbakir - [4]Department of Maxillofacial surgery, Faculty of Dentistry, University of Dicle, Diyarbakir, Turkey

ABSTRACT

Maneb (Mn-ethylene(bis)dithiocarbamate is a dithiocarbamate pesticides. It is a fungicide pesticide used in the control of mildew disease on certain crops. In general, dithiocarbamates are considered chemicals of low toxicity for humans. However, the effects of maneb in humans are diverse. It has some effects to humans via absorption through skin and respiratory system. The nasal mucosa is the first part of the airways in contact with the environment and toxic agents. The aim of this study was todetermine the histopathological effects of maneb on nasal mucosa. In experimental group, the maneb was administered with inhalation to 10 male Wistar-Albino rats for five days a week. The control group (n=10) received distilled water with spray at the same time period. The experiment was terminated after three weeks. In each case, sections of the nosewere taken. In experimental group,microscopic examination of nasal respiratory mucosa revealed; Hypertrophy of epithelial cells and goblet cells, increasing of mucus secretion, decreasing in sizes of cilia and flattening of the cells near the basement membrane and apoptotic cells in the degenerated respiratory epithelial cells, detected a mild inflammatory reaction and a vascular dilatation in the connective tissue. All changes were statistically significant. In control group of 10 rats no significant histopathologic lesions were found. As a result, our study , maneb is potentially toxic agent to respiratory mucosa.

***Key words**: Maneb, nasal, mucosa, respiratory; rat.*

***Received** Septemper 24, 2013; **Accepted** Septemper 30, 2013*

Introduction

Maneb is a metal containing member of the ethylene-bis-dithio-carbamate group of fungicides. It is used in the control of early and late blights on potatoes and tomatoes and many other diseases of fruits, vegetables and field (Pastorelli et al, 1995). It is a non-systemic preventive fungicide and it has some moderately toxic effects to humans. On the other hand, occasional signs of local irritation or inflammation of the skin, eyes, or respiratory tract have been experienced upon contact with Maneb (Meister, 1992; Israeli et al, 1983). Acute inhalation of large amounts of Maneb dust or spray may cause irritation of the mucous membranes, resulting in a scratchy throat, sneezing, cough, and inflammation of upper respiratory tract (Extension Toxicology Network 1993; Israeli et al, 1983).The nose not only serves as the principal organ for the sense of smell, but it also functions to efficiently filter, warm, and humidify the inhaled air. Maneb inhalation may be irritating to the nasal mucosa.

Materials and methods

This study was carried out on 20 Wistar-Albino rats. These rats, weighing 180-210g, maintained in standard laboratory conditions at the Department of Medical Science and Research Centre of Dicle University (DUSAM). The animals were group-housed (10 per cage) under standard conditions (21 ± 2 °C) in the Animal Health and Research Center of Dicle University (DUSAM). The animals had free access to standard laboratory rat pellet and water. They were divided into two groups. The experimental group (n=10) received

maneb with spray (Tech.87% purity obtained from Hoechst-Shering, Germany) at a concentration of 250 ppm (ppm/mg toxicant per 1 kg body weight) in 5 mL distilled water five days a week (total treatment time was three weeks). For application of maneb, rats of experimental group were placed into a glass vase. Maneb was achieved by squeezing of maneb spray inside it. Animals were kept in the glass vase for 1 hour. At non-exposure times, rats were kept inlaboratory animal house, which was far from the place of exposure with no maneb detection. The control group (n=10) received distilled water with spray. At the end of the study, the animals were sacrificed by decapitation under ether anesthesia. The skins were removed as well as all the soft tissues surrounding the nasal cavity. Then, the bony - framework of the nasal cavity including nasal septum were nibbled out by bone-nibbler. The samples were fixed with 10% formalin solution and decalcified with 5% EDTA (Ethylene-diamine-tetraacetic acid). After preservation, specimens were directly dehydrated in a graded series of ethanol and embedded into paraffin wax. Thin sections, 5-6 micrometres, were cut using a microtome (Rotatory Microtome, Leica, RM 2265, Germany) and stained with Periodic Acid Schiff (PAS), Mallory-Azan and then observed under Olympus BH2 light microscopy with multiplemagnifications (40x, 100x, 400x) to determine histopathological changes. These changes were evaluated by a semi-quantitative method. All morphological changes including epithelial hyperplasia, goblet cell hypertrophy, cell infiltration, basal membrane flattening and vascular dilatation were noted. The intensity of these change graded from 0 to 3 (0: no change, 1: low, 2: moderate, 3: intense). The apoptotic changes were also noted as positive or negative.

Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (version 15.0, SPSS Inc.,Chicago, IL, USA). The Kolmogorov-Smirnov and Fischer's Exact Chi-square test (for apoptosis) tests were used for the statistics as indicated, test and results were expressed as mean ±SD. P values below 0.05 were considered as statistical significant.

Results

As a result of histopathologic examination, in control group, sections of the respiratory mucosa no any pathological changes were observed (Fig.1). In experimental group, some sections were showed some histopathologic changes (Table 1). The hypertrophy of epithelial cells and goblet cells, increasing of mucus secretion, decreasing in sizes of cilia andflattening of the cells near the basement membrane were observed (Fig. 2). In some sections of experimental group animals, different numbers of apoptotic cells in the degenerated respiratory epithelial cells were also observed. There were also detected mild inflammatory reaction and vascular dilatation in the connective tissue (Fig. 3).There was however, no finding of dysplasia or carcinoma. According to the scoring, the all type of histopathologic changes were significantly overrepresented in the experimental group compared to the control group ($p < 0.05$) (Table I)

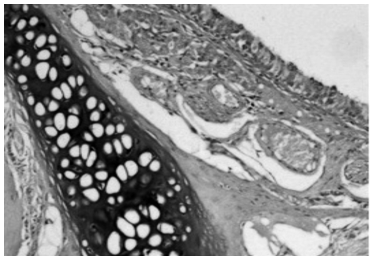

Figure 1: The nasal respiratory mucosa epithelium tissue (Bar x50) (control group) (PAS).

Histopathological Changes	Experimental Group (mean±SD)	Control Group (mean±SD)	P Value
Epithelial Hyperplasia	2±0,66	0	P<0,001*
Goblet Cell Hypertrophy	2,01±0,31	0,1±0,31	P<0,001*
Inflammatory Cell Infiltration	2,4±0,51	0,5±0,52	P<0,001*
Basal Membrane Flattening	2,3±0,48	0	P<0,001*
Vascular Dilatation	2,1±0,73	0,3±0,48	P<0,001*
Apoptosis (+/-)	6/10	0/10	P<0,05*†

Table 1: The nasal respiratory mucosa epithelium tissue (Bar x50) (control group) (PAS).

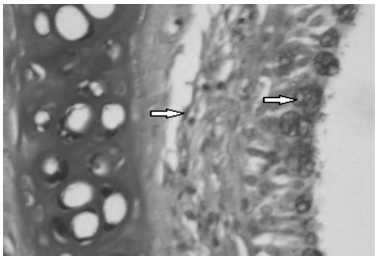

Figure 2: Hypertrophy of goblet cells, increased mucus-secretion,thinning of the basement membrane (white arrow)(Bar x100) (Experimental group) (PAS) .

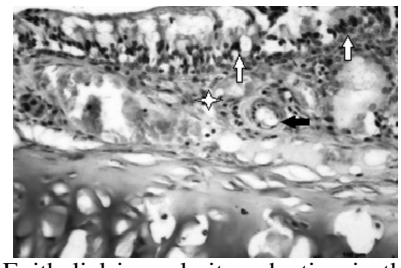

Figure 3: Epithelial irregularity,reduction in the size of cilia and a increase in the number of apoptotic cells (whitearrow), polymorph nucleated cell infiltration (asterix) and vascular dilatation (black arrow). (Bar x50) (Experimental group)(Mallory-Azan).

Discussion

The results of this study indicate that exposure to maneb may result in pathological changes in the nasal mucosa when compared with non-exposed.

Maneb is a toxic agent to humans.It enter the body mainly through the respiratory tract (aerosol, dust) skin and mucous membranes. Local allergic reactions (dermatitis, conjunctivitis, rhinitis and bronchitis) (Israeli et al, 1983), CNS effects (de Tollenaeret al, 2006; Mecoet al. 1994) and accumulation in the liver (Deveci et al, 1999) testicular damage (Deveci, 2006) andnephrotoxicity (Guvenet al, 1998) have been reported in literature. The exposure to maneb has been linked to an increase in the risk of Parkinson-like disease (Meco et al. 1994). In a study, exposure to a combination of the fungicide maneb andthe herbicide paraquat in mice leads to increasea substantia nigra neuronal pathology (Costello et al, 2009). Srivastava et al. (2012) also determined that mancozeb (it is a combination of Zinc and maneb) exposure can induce genotoxicity and apoptosis in cultured human lymphocytes.

In the literature, there are only two reports of dermal effects of maneb. Kimuraet al. (1998) reported that maneb induced delayed type dermatitis in the dorsal skin of Mexican hairless dogs. Kuroki et al. (1998) also examined the dermatotoxic effects of maneb on the skin of rats. In this study they demonstrated that, maneb caused type degeneration of the epithelial root sheath of hair follicles (in catagen phase) due to apoptosis.

The inhalation of toxic gases and particles, ingested chemicals can also affect the respiratory mucosa. Then, the respiratory mucosa is an important site of injury for toxic agents. The rat nasal mucosa was selected for this experimental toxic agent inhalation study as afirst potentially affected part of respiratory system. The histopathologic mucosal changes secondary to toxic agents are include edema, inflammation, fibrosis, mucosal ulceration, necrosis, hyperplasia, squamous metaplasia, and neoplasia (Renne& Gideon, 2006).The dithiocarbamates have been known to cause some upper and lower airway symptoms in humans, ie, rhinitis, pharyngitis, and bronchitis (Israeli et al, 1983). However, there is report of histopathologic findings in nasal mucosa induced by maneb.Warheitet al. (1989) demonstrated that a Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (it is a kind of dithiocarbamade fungicide) inhalation may cause olfactory epithelium degeneration in rats. Further histopathological studies are needed to evaluate the effects of maneb respiratory mucosa and olfactory epithelium. In our study we observed that, the hypertrophy of epithelial cells and goblet cells, increasing of mucus secretion, decreasing in sizes of cilia and flattening of the cells near the basement membrane and apoptotic cells in the degenerated respiratory epithelial cells. There were also detected mild inflammatory reaction and vascular dilatation in the connective tissue.Our study was consistent with Israeli etals' report. In our study we observed a few apoptotic cells in the degenerated respiratory epithelial cells in nasal mucosa. However, we didn't observe any neoplastic changes probably due to limited exposure duration time.

In this study we prefer the Mallory-Azan and PAS for staining. We focused on the early histopathologic changes after maneb application. We pointed out that, further studies are needed to clear evaluate the apoptosis and other degenerative and neoplastic changes in late period of application.

As a result, this is the first histopathological animal study to provide strong evidence that maneb is potentially toxic agent to respiratory mucosa. Therefore, one must take extreme caution when these toxic compounds are used.

References

1) Costello, S., Cockburn M., Bronstein J., Zhang X. & Ritz, B. *Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California*. Am J Epidemiol., 2009; 169(8): *919-26*.

2) Deveci, E. *Histopathological effects of organometallic maneb on testis in rats: a light and electron microscopic study*. ToxicolInd Health., 2006; 22(9): *395-8*.

3) Deveci E., Guven K., Bashan M., Onen A. & de Pomerai D. *The accumulation and histological effects of organometallic fungicides propineb and maneb in the livers of pregnant rats and their offspring*. J Toxicol Sci., 1999; 24(2): *79-85*.
4) *Extension Toxicology Network, Maneb pesticide information profile*, 9/1993 http://pmep.cce.cornell.edu/profiles/extoxnet/haloxyfop-methyl-parathion/maneb-ext.html
5) Guven K., Deveci E., Akba O., Onen A. & de Pomerai D. *The accumulation and histological effects of organometallic fungicides Propineb and Maneb in the kidneys of fetus and female rats during pregnancy*. Toxicol Lett., 1998; 99(2): *91-8*.
6) Israeli R., Sculsky M. & Tiberin P. *Acute intoxication due exposure to maneb and zineb. A case with behavorial and central nervous system changes*. Scand J Work Environ Health, 1983; 9: *47-51*.
7) Kimura T., Kuroki K. & Doi K. *Dermatotoxicity of agricultural chemicals in the dorsal skin of hairless dogs*.Toxicol Pathol., 1998; 26(3): *442-7*.
8) Kuroki K., Kimura T., Nakayama H. & Doi K. *Manganese ethylene bis (Maneb)-induced degeneration of hair follicle epithelia in hte dorsal skin of WBN/ILA-Ht rats*. J. Toxicol. Pathol. 1998; 11: *205-207*.
9) Meco G., Bonifati V., Vanacore N. & et al. *Parkinsonism after chronic exposure to the fungicidemaneb (manganese ethylene-bis-dithiocarbamate)*. Scand J Work Environ Health, 1994; 20: *301-305*.
10) Meister, R.T. (ed.). Farm Chemicals Handbook '92. Meister Publishing Company, Willoughby, OH., 1992.
11) Pastorelli R., Allevi R., Romagnano S., Meli G., Fanelli R. & Airoldi L. *Gaschromatography-massspectrometrydetermination of ethylenethioureahemoglobinadducts: a possible indicator of exposure to ethylene bis dithiocarbamate pesticides*. Arch Toxicol., 1995; 69(5): *306-11*.
12) Renne RA. & Gideon KM. *Types and patterns of response in the larynx following inhalation*. ToxicolPathol., 2006; 34: *281-5*.
13) Srivastava AK., Ali W., Singh R., Bhui K., Tyagi S., Al-Khedhairy AA., Srivastava PK., Musarrat J. & Shukla Y. *Mancozeb-induced genotoxicity and apoptosis in cultured human lymphocytes*. Life Sci., 2012; 90(21-22): *815-24*.
14) De Tollenaer SM., Buysse C., van den Anker JN., Touw DJ. & de Hoog M. *Lifethreateningcentralnervoussystem manifestations and hypothermia due to maneb intoxication in a child: a case report*. Ther Drug Monit., 2006; 28(6): *813-5*.
15) Warheit DB., Kelly DP., Carakostas MC. & Singer AW. *A 90-dayinhalationtoxicitystudy with benomyl in rats*. Fundam Appl Toxicol., 1989; 12(2): *333-4*.

Request reprints from:
Professor (PhD) ENGIN DEVECİ
Dicle University, Medical Faculty
Histology and Embryology Dept. 21280
(Turkey)