TREMORGENIC EFFECTS OF MOULD EXTRACTS ISOLATED IN WOOD INDUSTRY TESTED IN RATS

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Among workers in the Swedish wood industry acute alveolitis, known as "woodtrimmer's disease", has been recognized for some time. One of the signs in the acute phase of the disease is transitory tremor. The etiology of the disease is still not clearly understood, but it is assumed to be associated with wood dust containing fungal spores. One of the dominant fungal strains isolated from wood at the saw-mills was found to be Aspergillus fumigatus. As it is known that Aspergillus fumigatus has the ability to produce tremorgenic mycotoxins, the moulds from the working environment were isolated, cultivated and tested for the ability to produce mycotoxins, to estimate a possible relation between fungal spores and tremorgenic effects. Extracts of moulds were prepared and tested on rats after oral administration.

The ability to induce tremors in rats was found in six out of nine extracts tested. A very good agreement was found between the results of the chemical analysis of extracts and those of the study in vivo. The results imply that woodtrimmer's disease may at least partly, be mycotoxicosis.

Exposure to certain organic dusts may cause various pulmonary diseases among exposed workers. The inhalation of air with a high concentration of airborne fungal spores is particularly deleterious producing a damage known as allergic alveolitis, hypersensitive pneumonitis or pulmonary mycotoxicosis. Depending upon the origin of exposure it is also commonly called farmer's lung or maltworker's lung (1).

The acute form of the disease is characterized by febrile illness, cough, shivering, dyspnoea and malaise beginning a few hours after exposure. Repeated exposure can lead to the chronic conditions with progressive dyspnoea and lung fibrosis (2, 3). Most reports on lung disease connected with the working environment are described in farmers handling mouldy hay (4). Among workers in the Swedish wood industry the working environment in the saw-mills is responsible for developing acute alveolitis known as woodtrimmer's disease affecting particularly the woodtrimmers and sorters (5). The acute
symptoms (sometimes called febrile attacks) in woodtrimmer's disease can also be seen among the workers handling wood chips (6). One of the signs in the acute phase of the disease is transitory tremor. In general, the acute form seems to be much more common than the manifest one. According to Belin (7) 10–20 per cent of the exposed workers develop an acute form of the disease. The etiology of the disease is still not clearly understood, but it is assumed that it is associated with wood dust containing spores of various moulds.

The most common species of moulds isolated from the drying chambers and kilns in Scandinavian countries are Aspergillus fumigatus, Paecilomyces variotii and Rhizopus rhizopodiformis (8). In 1988 the National Board of Occupational Safety and Health in Sweden issued an Ordinance (Wood Mould, AFS 1988:6) containing provisions on wood mould and General Recommendations for the implementations of the provisions. The purpose of the rules was to prevent the exposure to wood moulds.

In order to better understand toxicological characteristics of such an exposure, a special study in experimental animals was performed jointly by the Department of Forest Products, The Swedish University of Agricultural Sciences, Uppsala, the Department of Biochemistry and Biotechnology, The Royal Institute of Technology, Stockholm and the Department of Toxicology, The Institute for Medical Research and Occupational Health, University of Zagreb, Zagreb. The moulds isolated from different kilns and saw-mills in Sweden were tested as to their ability to induce tremors in experimental animals. To estimate a possible relation between fungal spores and tremorgenic effects, the moulds from the working environment were isolated and cultivated under laboratory conditions. As the moulds from Aspergillus spp. are known to produce tremorgenic mycotoxins (9) only Aspergillus fumigatus isolated from different workplaces was used. The cultivation and extraction of moulds were performed in a liquid medium by means of the modified method of Yamazaki and co-workers (10). One strain was also cultured on gamma-sterilized sapwood blocks of Scotch pine.

Nine unpurified, coded mould extracts were submitted for chemical analysis to the Department of Biochemistry and Biotechnology of the Royal Institute of Technology in Stockholm and an aliquot for toxicological testing in vivo to the Department of Toxicology of the Institute for Medical Research and Occupational Health, University of Zagreb in Zagreb.

The results of chemical analysis showed that six out of nine extracts contained tremorgenic mycotoxins. In two extracts verruculogen was detected, three samples contained fumitremorgen C, whereas in one extract both verruculogen and fumitremorgen C were found (11).

The unpurified extracts, dissolved in peanut oil, were administered by gavage to female rats (three in each group). Each animal received 1.5 ml of the tested solution and control rats received the solvent only. The animals were kept under observation and the size of appearance and intensity of tremor were recorded. The tremorgenic effect was observed in six out of nine tested groups, the intensity varying from very mild to very pronounced. Three extracts and four control solutions did not show any tremorgenic effect. After comparing the results obtained by chemical analysis and tremorgenic reactions in animals good agreement was found in all but in two cases. In the first case the
extract obtained after cultivation of moulds produced a very strong tremor, but analyti-
cally, none of the tremorgenic mycotoxins was detected. Later it was discovered that
the amount of extract in this case was not large enough for both analytical purposes and
studies in vivo. Therefore a second cultivation of the same mould isolate was performed
and the extract was used for chemical analysis. For the testing in vivo an extract from
the first cultivation was used. It is possible that this isolate of Aspergillus fumigatus lost
its ability to produce mycotoxins after it had been cultivated for the second time. In the
second case although analytically the extract contained a detectable amount of verru-
culogen, no reaction in animals was noticed. The level of verruculogen in that extract is li-
kely to have been insufficient to produce tremor. The results indicate that the tremor-
genic effect in experimental animals might have been due to the gastrointestinal absorp-
tion of some mould extracts isolated from the working environment.

The common occurrence of Aspergillus fumigatus at saw-mills and the ability of so-
me isolates to produce tremorgenic mycotoxins might pose a serious health problem to
the workers working in highly 'polluted' indoor environments. The problem seems to
be even more pronounced in Scandinavian countries because of the conditions inside
the kilns, the high relative humidity and the relatively low temperatures (35 and 65 °C)
compared to drying schedules used for types of wood in non-Scandinavian countries.

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427
Sazetak

TREMOROGENI UČINCI EKSTRAKATA PLIJESNI IZOLIRANIH IZ PRAŠINE DRVETA U ŠIATOKA

U Švedskoj, u radnika zapuslenih u drvnoj industriji, javlja se akutni alveolitis poznat i pod nazivom Woodtrimmer's disease, popraćen prolaznim tremorom. Pojava je nepoznate etologije ali se povezuje s izloženošću drvenoj prašini u kojoj se nalaze spore različitih plijesni. Aspergillus fumigatus je plijesan koji se najčešće izolira u sušarama drveta. Iz radne sredine izolirane su plijesni Aspergillus flavus, te je nakon kultiviranje testirana njihova sposobnost stvaranja tremorogenih mikotoksina analitičkim metodama i u pokusima na štakorima. Ekstrakti plijesni apsirirani su pokusu nih životinjama oralno.

Šest od devet ekstrakata izazvalo je pojavu tremora u testiranih životinjima. Nađena je vrlo dobra korelacija rezultata kemijskih analiza i rezultata dobivenih u pokusima in vivo. Podaci upućuju na moguću vezu između Woodtrimmer's disease i izloženosti radnika tremorogenim mikotoksinima.

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428