
Histamine release by Western red cedar (*Thuja plicata*) from lung tissue *in vitro*

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Evans, Elizabeth and Nicholls, P. J. (1974). *British Journal of Industrial Medicine*, 31, 28-30. **Histamine release by Western red cedar (*Thuja plicata*) from lung tissue *in vitro*.** Various respiratory symptoms have previously been observed in workers exposed to dust from Western red cedar (*Thuja plicata*). Although an allergic basis for these effects has been proposed, the possibility that the dust may contain a pharmacologically active agent was investigated. Aqueous extracts of two samples of red cedar released significant amounts of histamine from pig and human lung *in vitro*. For one of these samples, using pig lung, a dose-response relation was found over a narrow range of concentrations. These dusts possessed the same order of histamine-releasing activity as a sample of cotton dust. Potassium cyanide reduced the release of histamine caused by low concentrations of Western red cedar. Similar effects of cyanide on the histamine-releasing activity of cotton dust and compound 48/80 were observed. It is possible that release of histamine in the lungs and upper respiratory tract occurs on inhalation of dust from Western red cedar and this may be a contributory factor to the development of respiratory symptoms in workers exposed to the dust of this wood.

Respiratory symptoms following exposure to dust of Western red cedar (*Thuja plicata*) were briefly described by Doig (1949), who reported two minor episodes of asthma among men working with this wood. The respiratory syndrome resulting from exposure to Western red cedar has been reported in detail more recently by Gandevia and Milne (1970) and Chan-Yeung, Barton, McLean, and Grzybowski (1971). These authors describe the gradual development of dry irritating cough and chest tightness throughout the working day accompanied by nocturnal attacks of cough and wheeze. In a small number of cases there was an immediate asthmatic reaction on inhalation of dust or dust extracts, and some individuals experienced rhinitis of varying severity. Partial or complete recovery was observed during weekends or holidays.

Positive bronchial reactions, measured as a small but significant decrease in the forced expiratory volume at one second ($FEV_{1.0}$), were observed in men working exclusively with this timber (Gandevia, 1970) and were also seen in response to provocative in-

halation of extracts of cedar dust (Gandevia and Milne, 1970). While it is thought that the basis of the asthmatic responses is allergic in nature (Chan-Yeung *et al.*, 1971), Gandevia (1970) has suggested that the most likely interpretation of the changes in ventilatory capacity found in the Western red cedar workers is that they reflect a non-specific bronchial reaction to inhaled particulate matter.

It is possible that other mechanisms may also be involved in the production of these respiratory symptoms. Since histamine-releasing activity has been detected in certain wood dusts such as the tropical woods, ukola and okumen, which cause rhinitis, wheezing, and eye irritation (Subiza, Alizo, and Diaz, 1970), it was of interest to examine whether Western red cedar possessed pharmacological activity of this type.

Materials and methods

Two samples of Western red cedar were examined. Sample A was from a carpenter's workshop in Australia

and was provided by Dr. B. Gandevia. Sample B was obtained from the workshop of the Welsh School of Pharmacy. These samples consisted of sweepings from benches and floors. Both samples were ground mechanically to a moderately coarse powder (22/60; British Pharmacopoeia, 1968). Airborne cotton dust was collected from a Lancashire mill spinning a coarse grade of cotton. The synthetic histamine releaser, compound 48/80, was purchased from the Wellcome Research Laboratories, Beckenham, Kent.

Aqueous extracts of the dusts were prepared by grinding the material (3g) with Tyrode solution (18 ml) in a mortar for 15 minutes. After filtration through cotton gauze, the extracts were centrifuged at 1000 g for 15 minutes. The supernatant was adjusted to pH 7.4 and the volume was made up to 18 ml with Tyrode solution. Compound 48/80 was dissolved in Tyrode solution.

Pig lung was obtained from the local abattoir and macroscopically normal human lung was obtained from surgical lung resections. In all cases, the lung was immediately placed in Tyrode solution at 4°C until it was prepared for the incubation experiments two to three hours later.

The histamine-releasing activity of the extracts was determined according to the procedure of Nicholls, Nicholls, and Bouhuys (1967). Chopped lung (100 mg) was incubated with various amounts of either the dust extracts, compound 48/80 or Tyrode solution at 37°C for 25 minutes. The final volume was 3 ml. At the end of incubation histamine released into the medium was determined spectrophotofluorimetrically (Shore, Burkhalter, and Cohn, 1959). For each lung specimen employed, the histamine content was also determined by this method.

Glucose-free Tyrode solution was employed for experiments in which potassium cyanide (KCN, 10^{-3} M) was used. The KCN in Tyrode solution was incubated with the lung for 15 minutes before addition of either the dust extracts or compound 48/80.

Results

Extracts of both samples of Western red cedar released histamine from human and pig lung *in vitro* (Table). In both species, the activity of these extracts was of the same order as an extract of cotton dust prepared from an equivalent amount of material. The effect of compound 48/80 is included for comparison, and it is evident that this substance is a potent histamine releaser in human and pig lungs.

Using pig lung, a dose-response relation was shown for cotton dust over the concentration range 5-80 mg/ml. Compound 48/80 also possessed an effect that was related to its concentration. Dose-related effects for cedar wood were clearly observed only in the narrow range of 5-10 mg/ml (Table).

The effect of the metabolic inhibitor, KCN, upon histamine release from pig lung was studied in order to indicate whether energy-dependent mechanisms in the lung were involved in the action of cedar and cotton dusts. It may be seen that the inclusion of KCN (10^{-3} M) in the incubation medium reduced the

TABLE
HISTAMINE RELEASE FROM LUNG TISSUE IN VITRO

Material	Concentration ¹ (mg/ml)	Histamine released (%) ²		
		Human lung ³	Pig lung ⁴	
			No KCN	KCN ⁵
Western red cedar Sample A Sample B	80	5.8	6.7	
	5		2.3	1.0
	10		5.9	3.7
	25	8.1	5.4	
	50		6.3	
	80		6.1	
100		6.4		
Cotton dust	5	9.1	1.4	0.9
	10		3.3	2.5
	25		5.0	
	80		7.4	
Compound 48/80	0.01	11.2	5.5	2.9
	0.03		7.0	
	0.5		31.0	

Chopped lung (100 mg) was incubated in Tyrode solution (3 ml) for 25 minutes at 37°C. Histamine liberated into the incubation medium was assayed fluorimetrically.

¹final concentration in incubation medium; this represents the soluble fraction from the weight of material listed;

²histamine released expressed as % of the total histamine in the lung sample (100 mg);

³results are the means of duplicate determinations from one lung sample;

⁴results are means from three experiments;

⁵KCN (10^{-3} M) present in incubation mixture.

histamine released by both dusts and compound 48/80 (Table).

Discussion

On a weight basis, the two samples of Western red cedar possess about the same degree of histamine-releasing activity as the cotton dust sample examined. There is evidence that inhalation of this cotton dust by normal human subjects causes the release of histamine (Edwards *et al.*, 1970). Therefore, it is possible that cedar wood dust may release histamine from the lungs following inhalation by workers exposed to the dust, and this could contribute to the development of respiratory symptoms. Because the decreases in ventilatory capacity observed in subjects exposed to cedar dust were relatively inconsistent in the most heavily exposed workers (Gandevia, 1970), the presence of a pharmacologically active bronchoconstrictor substance in the dust was thought to be unlikely. However, it should be noted that inter-subject variation in response to an inhaled histamine releaser is to be expected as it will depend upon (a) the histamine content of the lung, (b) the releasability

of histamine from its stores in the lung tissue, and (c) the sensitivity of the bronchial muscle to the released histamine, all of which are probably independent variables (Nicholls *et al.*, 1967).

Various metabolic inhibitors, including cyanide, reduce the release of histamine from mast cells by compound 48/80 (Mota and Ishii, 1960; Uvnäs and Thon, 1961) and this has been interpreted as demonstrating the involvement of an energy-dependent mechanism in the action of this histamine releaser. As it has been shown that histamine release from mast cells by compound 48/80 is difficult to block by metabolic inhibitors when the releaser is acting at high concentrations (Yamasaki and Endo, 1967), the effect of KCN upon the activity of the dusts was examined only at low concentrations of the latter. The reduction in the histamine released by cedar and cotton dust extracts in the presence of KCN indicated that cellular respiration or an energy-dependent process may be essential for the activity of these agents.

Further studies of the histamine-releasing activity of Western red cedar would be of interest. In particular, the isolation and purification of the active agent would enable definitive provocative tests to be performed which would establish what respiratory effects, if any, are due to the release of histamine when cedar dust is inhaled. The histamine-releasing activity present in Ukola wood (Subiza *et al.*, 1970) is associated with the saponin fraction. This is unlikely to be so for cedar wood because of the results with KCN. Saponins are surface-active agents and probably release histamine by a physicochemical mechanism which would not be inhibited by KCN (Mongar and Schild, 1957).

Other pharmacological properties of constituents in cedar wood may also be of importance in determining the respiratory effects seen after inhalation of the dust. It is of interest that tropolones, which form the major portion of the volatile fraction of Western red cedar (Chan-Yeung *et al.*, 1971), possess β -adrenoreceptor antagonist activity (Belleau and Burba, 1963). Such antagonists are potentially capable of altering the balance between vagal and sympathetic stimuli reaching airway smooth muscle and, therefore, of altering smooth muscle sensitivity. Indeed, it has been shown that small doses of propranolol, administered orally, potentiate the effects of a histamine aerosol as well as the response to inhalation of hemp dust in man (Bouhuys, 1971).

However, it should be noted that Canadian red cedar is almost unique in causing delayed attacks of breathlessness which may occur on several successive nights following one inhalation by some sensitized individuals (Gandevia, 1970; Chan-Yeung *et al.*, 1971). This cannot be directly due to histamine release and it is difficult to attribute it to β -receptor blocking activity until more is known about the

pharmacological activity and fate of the cedar wood tropolones following inhalation.

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