Death associated with volatile substance inhalation—Histologic, scanning electron microscopic and energy dispersive X-ray spectral analyses of lung tissue

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Abstract

The investigation of deaths due to the inhalation of volatile substances may be complicated by a lack of scene and autopsy findings. Mechanisms of death may not be determinable at autopsy, and there may be very few markers of inhalant abuse. A 21-year-old man is reported who died from the combined effects of methadone toxicity and toluene inhalation. Histological examination of the lungs revealed congestion and edema, as well as particles of blue, pigmented material within the interstitium and in macrophages. Scanning electron microscopy was undertaken, revealing that the particles contained granules that measured 0.15–0.2 μm in diameter, within the range of mean particle sizes for inorganic paint pigments. Energy dispersive X-ray spectral analysis of the granules demonstrated a significant percentage of titanium (12%) confirming their origin from paint. Ancillary investigations such as electron microscopy and X-ray spectral analysis in cases of possible lethal volatile inhalation may prove useful adjuncts in determining the type of substance inhaled and in providing evidence of previous non-lethal episodes.

Keywords: Volatile inhalation; Paint sniffing; Scanning electron microscopy; Energy dispersive X-ray spectral analysis

1. Introduction

Inhalation of volatile substances is a form of substance abuse that tends to occur among young people living in poor, isolated communities [1,2]. A variety of different substances are used that contain volatile chemicals [3] the effects of which include intoxication, with a rapid onset of drowsiness or a feeling of well being. More severe manifestations include visual hallucinations, loss of consciousness or convulsions [4].

The autopsy diagnosis of death due to the effects of volatile inhalation relies on toxicological evaluation of fluids, tissues or headspace air; such investigations often being initiated by the findings at the death scene. The physical manifestations at autopsy may, however, be relatively non-specific [5]. To assist in the evaluation of such cases a survey was conducted of the literature, and the findings in a specific case are reported, to summarise possible features at the scene and autopsy that may either point to, or corroborate, the possibility of an inhalant death. The usefulness of scanning electron microscopy and energy dispersive X-ray spectral analysis of lung tissue in suspected cases is also demonstrated.

2. Case report

A 21-year-old male was found dead in his bed. He had a known history of paint sniffing and when last seen alive had been in an intoxicated state, smelling strongly of paint. There was no history of occupational exposure to paint.

At autopsy traces of paint were noted on the abdomen, left hand and right thigh (Fig. 1). A reddened scaly rash was present around the nostrils and upper lip, in keeping with the effects of chronic irritation by paint solvents (Fig. 2). The only other findings were of pulmonary congestion and edema, and mild cervical lymphadenopathy. There was no evidence of trauma.
and there were no underlying organic diseases present that
could have caused or contributed to death.
Toxicological analysis of blood revealed a low ethanol
concentration of 0.02% and a high, potentially lethal, level of
methadone of 0.63 mg/L. No other common drugs were
detected. Toluene was detected in analysis of the headspace
over blood and lung tissue specimens, consistent with recent
toluene inhalation. Death was attributed to mixed hydrocarbon
and methadone toxicity.

3. Materials and methods

Routine tissue sections including lung were taken and stained with hema-
toxylin and eosin for light microscopy.
Following identification of pigmented foreign material within the lungs,
small sections of lung were air dried, mounted on aluminum pin stubs, and
coated with a 15 nm layer of carbon in an evaporative vacuum coater. The
samples were then examined in a Philips XL30 Field Emission scanning
electron microscope equipped with an EDAX DX4i energy dispersive X-ray
spectrometer. Images were recorded using a secondary electron detector to
demonstrate the morphology of the specimen, and with a back-scatter electron
detector to highlight material in the lung with a higher mean atomic number
than background lung tissue constituents.

4. Results

Sections of lung revealed congestion and edema with early
putrefactive changes. Also present were aggregates of blue
granular pigment within the interstitium and within alveolar
and interstitial macrophages, which also contained typical
darker anthracotic pigment. The blue granules were present on
both hematoxylin and eosin stained (Fig. 3) and on unstained
slides.
Scanning electron microscopy revealed collapsed lung
parenchyma and red blood cells with scattered particles of
rounded material measuring approximately 2–5 μm in dia-
meter (Fig. 4A). These corresponded to the aggregates of blue
pigment noted on routine staining. Use of the backscatter
electron detector highlighted granular material within the
particles measuring approximately 0.15–0.2 μm in diameter.
The brighter signal indicated that the components of the
granules had higher mean atomic numbers than components of

Fig. 1. Traces of silver-colored paint on the abdomen of a 21-year-old paint
sniffer.

Fig. 2. Reddened scaly rash around the nostrils and upper lip indicating chronic
irritation from paint/solvent inhalation in the same case.

Fig. 3. Aggregates of blue, pigmented foreign material within alveolar macro-
phages (A) and within macrophages and the interstitium (B), in lung sections
from a 21-year-old paint sniffer (hematoxylin and eosin, 450×). (For inter-
pretation of the references to color in this figure legend, the reader is referred to
the web version of the article.)
surrounding lung tissue (Fig. 4B). X-ray spectrometry revealed carbon, oxygen, sodium, aluminum, phosphorus and titanium. Carbon and oxygen are expected components of lung tissue and may also be present in paint, and sodium and phosphorus may have derived from a sodium phosphate buffer used in formalin tissue preservative. The presence of titanium and traces of aluminum, however, indicated that typical components of paint were present (Fig. 5).

Semiquantitative analysis of the entire particle shown in Fig. 4 revealed the following components:

- carbon: 65%; oxygen: 30%; sodium 0.6%; aluminum: 0.3%; phosphorus: 0.4%; titanium: 3.7%.

Analysis of granular material within the particle revealed:

- carbon: 54.5%; oxygen: 31.6%; sodium 0.6%; aluminum: 0.8%; phosphorus: 0.5%; titanium: 12%.

5. Discussion

Paints are liquids that are used to coat surfaces of a variety of objects that when dry provide a tinted, durable and protective layer. Paints are made up of a number of components including pigments such as titanium dioxide, binders, fillers and solvents such as toluene. Titanium dioxide was introduced as a pigment in paint shortly after 1918 and is most useful in enamel and other high gloss paints due to its marked opacity [6]. Unfortunately solvents in paints predispose to their misuse, and the detection of toluene in headspace analyses in the reported case indicate that this had occurred.

The recreational inhalation of volatile substances is most often found among young people who utilise materials such as gasoline, glue, lighter fluid, shoe polish and paint. These substances are readily available, inexpensive and can be inhaled immediately without special preparation or equipment [2]. Deaths may result from accidents, inhalation of gastric contents, cardiac rhythm disturbance from direct cardiac toxicity, or suffocation [4,5]. Lethal outcomes are, however, relatively uncommon with inhalant deaths representing only 0.2% in one study of coronial autopsies in South Australia over the past two decades [5].

Different inhalants may be used in different communities, and in a South Australian series extending from 1983 to 2002 (prior to the death of the reported case) there were no cases of paint sniffing, with over 50% of the deaths being attributed to inhalation of gasoline, butane or propane [5]. While most deaths are accidental, occurring during recreational substance abuse, inhalants may also be used to augment sexual activity and in suicide attempts [5,7].
The diagnosis of possible deaths due to inhalants may be difficult if the victim has left, or been moved from, the area where sniffing occurred, or if there is a prolonged postmortem interval with resultant loss of volatiles from the blood and tissues. Indications at the scene may include containers of volatiles, with plastic bags or cans that have been used to inhale from [8]. The body may also smell of volatiles and there may be paint or correction fluid staining of the hands or face (as in the reported case) [9]. Prolonged sniffing may cause a rash around the nose and mouth, and gasoline sniffers may have marks on their face from the rim of sniffing cans [8]. The features on internal examination have been summarised in Table 1, including neuropathological findings of cerebral cortical and hippocampal neuronal loss, gliosis, Purkinje cell loss and chromatolysis of neurons in the cerebellum, brainstem and basal ganglia [10,11]. Toxicological evaluation must include blood levels for common prescription and illicit drugs, as well as headspace analyses for volatiles, as victims may combine a variety of substances, as in the reported case where death was due to the combined effects of methadone and toluene.

Although titanium in the lungs has been associated with the development of pneumoconiosis and alveolar proteinosis in rare individuals who have had occupational exposure [12,13], the finding of paint pigment in the lungs of inhalant abusers has not been emphasized previously [14] and there has been little written on possible investigative steps. In the present case the finding of blue, pigmented material in paint sections initiated both scanning electron microscopy and energy dispersive X-ray spectral analysis of lung tissues. The striking finding was of aggregated foreign material composed of numerous small granules containing titanium that measured 0.15–0.2 μm. This fell well within the range of mean particle sizes for inorganic pigments in paint of 0.1–1 μm. Titanium dioxide particles that are under 0.2 μm tend to scatter light of a shorter wavelength and thus may appear blue [6], in keeping with the initial light microscopic observation of blue granular material within lung tissues.

In conclusion, careful examination of lung sections in cases of possible volatile inhalation deaths may provide evidence of previous episodes of paint sniffing. Ancillary examination of lung tissue with scanning electron microscopy followed by energy dispersive X-ray spectral analysis may then be used to confirm that the granules within particles are of a comparable size to those found in paint pigments and are composed of typical material such as titanium. This, combined with headspace analysis and routine toxicology, may allow a more complete picture of the victim’s activities to be constructed from the autopsy findings than is usually possible.

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References