Aplastic anaemia associated with organochlorine pesticide: case reports and review of evidence

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Abstract
Three patients with aplastic anaemia had a history of substantial previous exposure to organochlorine pesticides. The temporal association between chemical exposure and the onset of first symptoms of anaemia was strongly supportive. Organochlorines have the property of lipid affinity and accumulation in adipose tissue. Objective evidence of clinically important concentrations of tissue pesticide residues may be a useful confirmation of previous exposure. In the patients studied the presence of Lindane (γ hexachlorocyclohexane) was shown using gas chromatography/mass spectrometry with selective ion monitoring of fragments obtained from one heavily exposed patient, with concentrations about five times greater than a matched control. The presence of clinically important tissue concentrations of pentachlorophenol was also confirmed in a second patient exposed to this agent.

The long term safety of organochlorine pesticides remains doubtful as they were introduced before adequate toxicological screening tests had been developed. The central registration of possible haematological adverse reactions, however, forms an important epidemiological method in the study of environmental chemical hazards and should be complied with whenever possible.

Case reports
CASE 1
A 12 year old boy presented with a four month history of pallor, easy bruising, exertional dyspnoea and headache. No antibiotics or other medication had been given. On admission he had noticeable pallor of skin and mucous membranes, with peripheral bruising. Height and weight were normal for age. Radial or thumb abnormalities, cutaneous hyperpigmentation, or neurological abnormalities were not present. There was no lymphadenopathy or hepatosplenomegaly.

The full blood picture was as follows: haemoglobin concentration 7.4 g/l; white cell count 2.1 x 10^9/l—neutrophils 1.3 x 10^9/l, lymphocytes 1.3 x 10^9/l, monocytes 0.2 x 10^9/l, eosinophils 0.1 x 10^9/l; and his platelet count was 19 x 10^9/l. Bone marrow aspirate showed fragments of variable cellularity with some dyserythropoiesis and a trephine biopsy specimen confirmed aplastic anaemia (<20% cellularity). Repeated full viral serology, liver enzyme tests, and Ham’s test were non-diagnostic. His HLA type was A2 B7 Bw6 DR2 DR14.

Three months after presentation he remained transfusion dependent with a deteriorating marrow cellularity of less than 10%; neutrophils were 0.3 x 10^9/l and platelets 18 x 10^9/l—that is, severe aplastic anaemia. The patient’s mother subsequently volunteered the information that the home had been chemically treated for woodworm about two to three months before the first appearance of symptoms. Moreover, the chemical had been used on woodwork in the child’s bedroom which had been hermetically sealed by polythene insulating sheets covering all the windows. The patient had reoccupied this room 48 hours after pesticide treatment. The agent was subsequently confirmed by chemical analysis from woodwork as Lindane (γ hexachlorocyclohexane).

CASE 2
A 28 year old male computer operator presented with a one month history of headaches, exertional dyspnoea, and easy bruising. There was no relevant medical history, recent medication, or viral infection. For several months before presentation, however, he had been actively involved in renovating an old building and had applied a proprietary solution of pentachlorophenol to timber work.

The full blood count on admission was as follows: haemoglobin concentration 5.3 g/l; white cell count was 1.5 x 10^9/l—neutrophils 0.5 x 10^9/l, lymphocytes 0.7 x 10^9/l, monocytes 0.3 x 10^9/l; and platelet count 13 x 10^9/l. Bone marrow aspirate and trephine biopsy specimen confirmed severe hypo-cellularity (<10%) consistent with severe aplastic anaemia. Marrow cytogenetics were normal. A separate 36 mg marrow biopsy specimen was frozen at −70°C and subsequent analysis showed pentachlorophenol at 200 μg/ kg (control, 100 μg/kg). Ham’s test, full virology including hepatitis B antigen, and hepatitis A IgM, yielded negative results. Biochemical profile and B₁₂/folate concentrations were normal. HLA tissue type was A1 B8 Bw6 DR3 DR6.

CASE 3
A 26 year old labourer presented with a six month history of increasing fatigue and right middle lobe pneumonia. Haemoglobin concentration was 6.0 g/l; white cell count was 1.4 x 10^9/l, with neutrophils 0.5 x 10^9/l, lympho-
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cytcs $0.7 \times 10^9/l$, and monocytes $0.2 \times 10^9/l$; platelet count was $10 \times 10^9/l$. Autoantibody

screen, full viral serology, and Ham's test

yielded negative results and the biochemical

profile was normal. Bone marrow aspirate and

biopsy specimen confirmed severe aplasia

(< $10^5\%$, cellularity). HLA tissue type was A3

A11 B5 B7 Bw4/6 DR2 DR4.

There was no relevant medical history or

preceeding medication. Some months before he

had been occupationally exposed to Lindane

while cleaning out the blocked drains from a

pesticide plant at his work place.

Pathology

CASE 1

Lindane (like DDT) is a persistent residue in

human adipose tissue. A $10.3\, g$ specimen was

biopsied from subcutaneous abdominal adipose

tissue under general anaesthesia. Fat was

extracted in Hexane and the resulting solution

analysed for Lindane using gas chromato-

graphy/mass spectrometry with selective ion

monitoring.

Gas chromatography was performed on an

OV351 capillary column with a linear tem-

perature gradient of $120^\circ C$ to $200^\circ C$ over eight

minutes: under these conditions the $\gamma$-isomer of

Lindane eluted at seven minutes 54 seconds.

Benzil (molecular weight 210), used as an

internal standard, eluted at eight minutes 25

seconds.

Fragments corresponding to Lindane-HCl

(masses 252, 254, 256) and Lindane-2 HCl

(masses 217, 219, and 221) were clearly observed. The relative intensities of these

signals and their retention times in the gas

chromatography column were also characteris-
tic of Lindane (fig 2).

The concentrations of the pure $\gamma$-isomer were about five times higher than the age

matched urban control with no evidence of

occupational or high exposure. The absolute

value reported (25–30 parts per billion) is of

uncertain importance as we could find no

comparable studies using this method of

analysis to establish the range of isomer con-

centrations in the “normal” population.

This result has, however, been subsequently con-

firmed by independent analysis.

Progress

As there was no available HLA matched sibling

bone marrow donor, treatment was started

with intravenous equine anti-thymocyte

globulin $15\, mg/k$ for eight days. Progress was

complicated by the associated symptoms of

serum sickness controlled by a reducing dose

of prednisolone. Treatment was supplemented

with oral oxymethalone at $2\, mg/k$ daily. Three

months later a dose of anti-thymocyte

globulin was given. There was a subsequent

partial response with a reticulocytosis at five

weeks and an eventual increase in haemo-
geglobulin to $11.1\, g/l$. After 18 months without

requiring transfusions he remained neutro-

penic and thrombocytopenic with further

blood transfusions required over subsequent

months.

CASE 2

Progress

Following a period of observation with no

change in peripheral blood counts a central

venous line was inserted and treatment started

with anti-thymocyte globulin at $20\, mg/k/day$

intravenously for eight days with oral

oxy-
methalone $100\, mg$ twice a day. On completion

of anti-thymocyte globulin treatment he was

given (on a reducing dose) prednisolone to treat

the associated serum sickness. Subsequent

progress was complicated by several septi-

caeic episodes, which responded to intra-

venous antibiotics, and persistent retinal

haemorrhage requiring platelet support. There

was no evidence of response to anti-thymocyte

globulin at 10 weeks. Although histologically

incompatible in one HLA type with a sibling,

the mixed lymphocyte culture was non-re-

active and arrangements were made for

allogeneic bone marrow transplantation.

Unfortunately, he developed severe pneu-

monia, which did not respond to antibiotics,

with sputum evidence of aspergillus. In spite

of intravenous amphotericin he deteriorated

with progressive pulmonary failure that required

assisted ventilation. Pulmonary aspergillosis

was confirmed at necropsy.

CASE 3

Progress

Initial treatment with antibiotics was started,

followed by high dose intravenous methyl

dexamethasone at $20\, mg/k/day$ for three days,

reducing to $10\, mg$, $5\, mg$, $2\, mg$, and $1\, mg$

each for three days. Four weeks later peripheral

blood count showed a response with haemo-
geglobulin at $11.5\, g/l$, $4.7 \times 10^9/l$, neutrophils $2.3 \times 10^9/l$, lymphocytes $2.0 \times 10^9/l$, and mono-
cytes $0.4 \times 10^9/l$, and platelet count at $20 \times 10^9/l$. He remained severely thrombocytopenic

and progress was complicated by recurrent

melaena that required blood and platelet trans-
fusions. Continuous oral prednisolone and

oxymethalone $200\, mg$ a day was given, but no

improvement in peripheral counts was noted.

Nine weeks later a course of anti-thymocyte

globulin at $20\, mg/k/day$ for eight days was

administered in addition to prednisolone

$30\, mg$ daily initially. After four weeks platelet

count had increased to $44 \times 10^9/l$, and over the

subsequent weeks he achieved normal peri-

cpheral counts and no longer required blood and

platelet transfusions. He remained well two

years after initial diagnosis.

Discussion

Lindane ($\gamma$-hexachlorocyclohexane) is an

organochlorine that was first used as an insect-
cide in 1942 (fig 1). Its vapour pressure is

low, but it is highly volatile, and anti-thymocyte

globulin was given. There was a subsequent

partial response with a reticulocytosis at five

weeks and an eventual increase in haemo-

globulin to $11.1\, g/l$. After 18 months without

requiring transfusions he remained neutro-

penic and thrombocytopenic with further

blood transfusions required over subsequent

months.

The predominant body storage form in adipose tissue is the $\beta$

isomer followed by the parent $\gamma$-isomer. Lindane is partially metabolised in the liver to

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Pentachlorophenol (fig 1) (used as a wood preservative fungicide) and other chlorinated phenols. Acute toxicity is very well recognised as stimulation of the central nervous system and convulsions, particularly in children. Chronic exposure induces liver microsomal enzyme activity, and liver damage, including tumour formation, has been confirmed in animal studies. The WHO report on Lindane concluded that there was sufficient laboratory evidence that the chemical was carcinogenic in animal experiments and that it is reasonable to regard such a chemical as posing a carcinogenic risk in man.

The association between the development of aplastic anaemia and previous exposure to drugs or industrial chemicals has been recognised for many years. This is often based on epidemiological evidence of the temporal relation between exposure and the development of aplasia, but no obvious aetiological agent is suspected in about 70% of cases. Clearly the importance of an accurate and detailed history of chemical exposure cannot be over emphasised.

The first nine reports of bone marrow injury associated with Lindane, particularly vapourisers, were documented in the United States by 1953, and sharp restrictions in its domestic use were then urged. In spite of attempted pro-scription in 1971 residents in the Mid West continued to use vapourisers in their homes; by 1979 there were about 30 case reports of aplastic anaemia plus a further 10 cases associated with exposure to Lindane in combination with other compounds.

Lindane has been identified in the tissues of patients in very few case reports. The sensitive method of mass spectrometry has only recently become available for tissue analysis and we accept that the incidence of aplastic anaemia from confirmed pesticide exposure must be very low. Lindane (hexachlorocyclohexane), however, has well documented direct biological activity: hexachlorocyclohexane is a structural analogue of inositol and inhibits phosphatidyl-inositol synthesis as the 2° transmembrane messenger for growth factor stimulation. Hexachlorocyclohexane or its metabolites induce chromatid breaks in vitro in human lymphocytes indicating potential genotoxicity to haemic stem cells.

Both the above mechanisms seem unlikely to be the cause of aplastic anaemia, as you would expect to observe inevitable dose dependent pancytopenia which is reported more commonly with organochlorine pesticide exposure. Our case reports must therefore represent a rare, probably idiosyncratic, reaction.

Aplastic anaemia has been regarded as a pre-malignant disorder of haemopoesis or even a variant type of pre-leukaemia. A well documented and convincing case reports the simultaneous development of “paramyeloblastic” leukaemia in two 20 year old cousins after prolonged and simultaneous occupational exposure to hexachlorocyclohexane. Familial or genetic sensitivity is therefore strongly suggested in these cases.

It has been shown that marrow sensitivity to chloraphenicol seems to have a genetic element and that aplastic anaemia of any cause has been associated with certain histocompatibility antigens and to DR218 and DPw319 in particular. It may therefore be important that cases 1 and 3 both possessed DR2 antigens. Several diseases with an immunopathological component are known to be associated with the DR2 antigen, including Goodpasture’s syndrome and multiple sclerosis. A cell mediated immunological mechanism has long been proposed in the aetiology of aplastic anaemia. The success of specific immunosuppressive treatment (anti-thymocyte globulin or cyclosporine) in up to half the patients with aplastic anaemia in recent trials would also support an immunological mechanism in these cases.
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Moreover, the two cases reported here which responded to anti-thymocyte globulin both shared the DR2 antigen, suggesting that there were shared features of autoimmunity.

Certain biological features associated with organochlorines and Lindane, in particular, also suggest why this agent may produce idiopathic marrow damage, possibly via immunological mechanisms.

**Lipophilic accumulation** Fat is an important element in the stromal support of haemopoiesis, and therefore a propensity of hexachlorocyclohexane to persist in narrow adipose tissue may increase the risk of interaction with lympho-haemopoietic function. For example, hexachlorocyclohexane inhibits phythaemagglutinin stimulation of lymphocyte growth.21

We suggest that organochlorine pesticides or their metabolites may become antigenic by interacting with certain rare unidentified human leucocyte antigen molecules (possibly in linkage disequilibrium with known HLA groups) and thus induce an autoimmune reaction that is responsible for the continuing marrow damage.

Apart from immunological mechanisms, idiosyncratic reactions may be due to abnormal metabolism or excretion of a chemical or drug, leading to its accumulation in excessive concentrations. One elegant study showed impaired oxidation of phenylbutazone in patients with aplastic anaemia attributed to this drug.22

Alternatively, metabolic or immunological mechanisms may be superimposed on a genetically determined haemopoietic stem cell abnormality or previous stem cell damage, thus resulting in aplastic anaemia.

Clearly there is a need for a more open and stringent evaluation of the hazards posed by agricultural chemicals.23 Little is known of the long term effects or mechanisms of toxicity of many pesticides.24 The patterns of exposure and rarity of many disorders show the limitation of purely epidemiological research. Indeed, the incidence of aplastic anaemia in developed temperate countries is only of the order of 3–6 million population per annum.25

Investigation of the aetiology of aplasia is further obfuscated by the delay of up to six months between exposure to a toxin and the development of pancytopenia.11 Well documented case reports, however, may contribute information on the suspected toxicity of these substances and serve to corroborate the recent recommendation of a standard centralised record system.24

We are grateful to Drs J Martin and PA Stevenson for permission to report their patients, and Sir Richard Body for his assistance as Chairman of the House of Commons Agriculture Committee on the effects of pesticides on human health.

The address for reporting haematological disorders and occupational hazards is as follows: Mrs J Hopkins, Haematological Disorders and Occupational Hazards Database, Department of Haematology, University Hospital of Wales, Heath Park, Cardiff CF4 4XN.

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