LIVER DISEASE

Liver dysfunction among workers handling 5-nitro-*o*-toluidine

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Background: 5-Nitro-*o*-toluidine is an aromatic nitro amino compound. While other aromatic compounds are known to damage the human liver and are registered as toxic substances, toxicity information concerning 5-nitro-*o*-toluidine is lacking.

Aims: To investigate the hepatotoxicity of 5-nitro-*o*-toluidine.

Patients and methods: Of 15 workers in the same factory who handled 5-nitro-*o*-toluidine, three were hospitalised with symptoms of acute liver dysfunction. Suspecting a link between liver dysfunction and working conditions, we correlated workplace factors with clinical findings in all 15 workers.

Results: Blood biochemistry tests indicated liver damage in seven of 15 study subjects. Workers who handled 5-nitro-*o*-toluidine and nitrosyl sulphuric acid often loosened their respiratory protective equipment shortly after 5-nitro-*o*-toluidine powder had been dispersed into the air of the room. No potential hepatotoxins were present except for 5-nitro-*o*-toluidine. Six of the affected workers had handled 5-nitro-*o*-toluidine 12 to 20 times; the seventh worker had handled the powder three times; and the other eight workers without liver dysfunction had handled the material once or twice. No other significant differences in background were evident between the affected and unaffected workers, such as age, sex, or protective measures. Histological findings during recovery from liver damage were similar to those of acute viral hepatitis. None of the 15 subjects has demonstrated liver damage since the factory was closed.

Conclusions: A link between liver dysfunction and 5-nitro-*o*-toluidine exposure is suggested by greater severity of liver dysfunction associated with more episodes of handling.

Certain aromatic compounds are known to damage the human liver and are registered as toxic substances. Information concerning such chemicals is available in toxicity manuals and more recently also via Internet sites of various industrial organisations and institutions.^{1,2} Only a few new entries designating substances harmful for humans have been added in the past decade.

5-Nitro-*o*-toluidine (CH₃C₆H₃-NH₂-NO₂; Chemical Abstracts Service (CAS) No 99-55-8) is an aromatic nitroamino compound that exists as a yellow powder with a melting point of 104°C at 1 Torr. This compound is used worldwide as a raw material for azo dyes used to colour cellulose fibres red. However, information concerning the toxicity of 5-nitro-*o*-toluidine is scant.

Three workers from the same workplace handling 5-nitro-*o*-toluidine as a raw material for the production of a hair dye were admitted to our hospital with liver dysfunction. As we suspected a link between symptoms and findings and patients' working conditions, we investigated the workplace and carried out a health surveillance of all workers in the factory. We studied the relationship between exposure to 5-nitro-*o*-toluidine and liver dysfunction and also characterised the clinical manifestations of 5-nitro-*o*-toluidine poisoning.

METHODS

Patients

Between 24 June and 7 July 1998, three workers from the same factory were admitted to our hospital because of liver dysfunction. The three patients worked in the same section where a total of 15 men had begun to handle 5-nitro-*o*-toluidine (Fast Scarlet G base) as a raw material for hair dyes. After the third patient was admitted, the prevalence of subjective symptoms was surveyed through interview, set up with

the assistance of the company. Blood and urine were sampled sequentially from all workers. Serum biochemistry tests, complete blood counts, and urinalyses were performed immediately after specimen collection in all subjects. Serum biochemistry tests included aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (TBil), albumin (ALB), and total cholesterol (TC). We also determined prothrombin time (PT) on admission in the hospitalised patients. With respect to infectious agents, we tested for hepatitis B antigen and hepatitis A and C antibodies in all subjects, and also for cytomegalovirus and Epstein-Barr virus antibodies in hospitalised patients. Liver morphology was monitored by ultrasonography in subjects with abnormal liver function, and needle biopsy of the liver was performed in the hospitalised patients one month after symptom onset.

Hygienic investigation

A hygienic investigation included: determination of frequency of handling of the suspected toxin in the 15 workers; review of Material Safety Data Sheets (MSDS), including the previous chemical safety record at the factory; review of risk management practices concerning exposure to chemicals; and backgrounds of the workers, including age, medical history, alcohol use, and prior liver function test results.

Abbreviations: CAS, Chemical Abstracts Service; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; TBil, total bilirubin; ALB, albumin; TC, total cholesterol; PT, prothrombin time; MSDS, Material Safety Data Sheets; CI, colour index.

Statistical analysis

The software package StatView 4.0 was used. Data for subject group are reported as mean (SEM). Unpaired Student's *t* tests for independent samples were used to compare data between individuals with high and low numbers of exposures. A value of $p < 0.05$ was considered to indicate statistical significance.

RESULTS

Discovery of liver dysfunction in workers

The first patient (case No 1) consulted a community physician because of high fever and low back pain. As serum biochemistry tests indicated severe liver dysfunction (AST 328 IU/l; ALT 549 IU/l), he was referred to our hospital on the following day. The second patient (case No 2) developed fatigue and appetite loss about the same time. When he first sought medical attention, even more severe liver dysfunction was evident (AST 2610 IU/l; ALT 2700 IU/l; GGT 284 IU/l; ALP 1090 IU/l; LDH 3610 IU/l; and TBil 1.2 mg/dl). He was admitted to our hospital on the following day. The third patient (case No 3) noted dark urine and upper abdominal discomfort and consulted his primary care physician. When serum biochemistry tests indicated liver dysfunction (AST 1107 IU/l; ALT 1996 IU/l; GGT 246 IU/l; ALP 743 IU/l; LDH 554 IU/l; and TBil 3.6 mg/dl), he was sent to our hospital immediately. A fourth worker at the factory (case No 4) presenting with fatigue was diagnosed with liver dysfunction by another doctor, but was not admitted to the hospital.

Serum biochemistry tests were performed for the remaining 11 asymptomatic workers, 17 days after the first patient presented with symptoms. Of these 11 workers, three (case Nos 5, 6, and 7) had liver damage (table 1), and two (cases 5 and 6) were admitted to our hospital on the following day. The company that owned the affected factory closed the work site 34 days after the first handling of 5-nitro-*o*-toluidine.

Relationship between liver dysfunction and 5-nitro-*o*-toluidine

Six workers, case Nos 1–6, represented the individuals most engaged in processes involving 5-nitro-*o*-toluidine; the remaining nine workers sometimes substituted for the others in performing these tasks. The workers handled 5-nitro-*o*-toluidine for 4–5 hours at a time. The total amount of 5-nitro-*o*-toluidine that they processed before closure of the work site was 29 tons. The frequency of handling 5-nitro-*o*-toluidine in

the principally involved workers and the backup workers was 12–20 times and 1–3 times, respectively. Serum biochemistry tests indicated liver injury in the six frequently exposed workers (table 1). No significant difference in age was evident between workers with high and low frequency of exposure (54.3 (4.3) *v* 51.3 (4.8) years). No study subject took any medication or was a habitual drinker. Various serum markers for viral infection, including hepatitis viruses, were negative in all workers. Screening examinations of liver function, performed one month prior to initial handling of 5-nitro-*o*-toluidine in 15 of these men, showed no liver dysfunction (range: AST 12–34 IU/l; ALT 13–35 IU/l; GGT 21–40 IU/l; ALP 200–240 IU/l; and TBil 0.3–0.6 mg/dl).

A link between liver dysfunction and 5-nitro-*o*-toluidine exposure was supported by the finding that severity of liver dysfunction increased in relation to frequency of handling 5-nitro-*o*-toluidine ($p < 0.01$) (table 1). In addition, the latent period from initial handling of 5-nitro-*o*-toluidine to onset of symptoms was similar in each clinically evident case.

Working conditions

No information concerning the toxicity of 5-nitro-*o*-toluidine in humans was available in the MSDS. The company owning the affected factory had used approximately 100 tons of 5-nitro-*o*-toluidine per year for more than 15 years at another factory where handling was fully automated. Maintenance workers there were kept completely shielded from chemicals, and had never shown liver dysfunction.

Because of increasing demand for the product, the company assigned additional production using 5-nitro-*o*-toluidine to the affected factory, beginning 18 days before the first patient developed symptoms. The workers manually scooped 5-nitro-*o*-toluidine from barrels and tossed it into airtight mixing machine where it reacted with sulphuric acid (H_2SO_4 ; CAS No 7664-93-9) and nitrosylsulphuric acid (HOSO_2NO ; CAS No 7782-78-7) (fig 1). Under the conditions of scooping, raising of a cloud of 5-nitro-*o*-toluidine powder was unavoidable. The work area was surrounded by sheeting to prevent the powder from spreading, and the workers inside wore protective equipment including aprons, coveralls, boots, rubber gloves, goggles, and respirators. The respirators, gloves, and coveralls were exchanged twice a day, and aprons and goggles were washed every day. No other protective apparatus was present, such as air filters to remove aerosolised powder from the work

Table 1 Liver function in workers according to the number of exposures to 5-nitro-*o*-toluidine

Case No	Age (y)	No of exposures	AST (IU/l)	ALT (IU/l)	GGT (IU/l)	ALP (IU/l)	TBil (mg/dl)	ALB (g/dl)	TC (mg/dl)	PT (INR)
Principally exposed workers										
1	49	15	231	426	477	717	1.3	3.6	153	0.94
2	59	20	1224	2510	301	780	1.6	3.9	158	0.96
3	57	18	1107	1996	247	743	3.6	3.7	131	1.38
4	49	12	82	99	116	304	0.6	3.8	161	—
5	57	17	1344	1914	288	928	1.0	3.7	123	1.23
6	57	15	377	389	80	385	0.4	4.1	174	0.94
Mean (SEM)	54.3 (4.3)	16.2 (1.1)**	728 (228)**	1222 (421)**	252 (58)**	643 (100)**	1.4 (0.5)	3.8 (0.1)	153 (9)	1.09 (0.09)
Backup workers										
7	52	3	93	190	129	256	1.0	3.6	246	—
8	48	2	20	19	24	256	0.4	4.1	196	—
9	55	2	21	37	54	209	0.8	4.2	151	—
10	57	2	23	23	40	184	0.9	4.2	162	—
11	45	2	21	11	52	276	0.9	3.5	205	—
12	47	2	15	17	20	208	0.8	4.2	152	—
13	53	1	20	23	26	209	0.4	3.8	174	—
14	58	1	24	24	22	236	1.0	3.6	157	—
15	47	1	15	33	22	236	0.4	3.9	173	—
Mean (SEM)	51.3 (4.8)	1.8 (0.2)	28 (8)	42 (19)	43 (12)	227 (9)	0.7 (0.1)	3.9 (0.1)	180 (10)	—

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; TBil, total bilirubin; ALB, albumin; TC, total cholesterol; PT, prothrombin time.

** $p < 0.01$ compared with backup workers (unpaired Student's *t* tests).

Figure 1 Reaction sequence for producing hair dye. Workers manually loaded 5-nitro-*o*-toluidine, sulphuric acid (H_2SO_4), and nitrosylsulphuric (HOSO_3NO) into a closed mixing vessel where these compounds reacted with one another.

area, and airborne powder was visible as a cloud during handling. During short breaks taken within the sealed off space, the workers often slightly loosened the uncomfortable respirators. During the breaks, the airborne powder was no longer visible, although considerable concentrations presumably remained in the air.

Nitrosylsulphuric acid is a strong acid that can damage skin and the mucous membranes of the airway and gastrointestinal tract. No major chemical, physical, or biological hazards except for the acid and 5-nitro-*o*-toluidine were present in the work environment.

Clinical features

Symptoms appeared in the principally involved workers two weeks after initial handling of 5-nitro-*o*-toluidine. All noted fatigue, loss of appetite, and upper abdominal discomfort. In addition, case Nos 1 and 2 had high fever and joint pain. Case Nos 1, 2, and 3 noted dark urine.

Elevated serum values for AST, ALT, and ALP were consistent with mixed hepatocellular and cholestatic liver injury. The abnormal PT values in case Nos 3 and 5 implied hepatic synthetic dysfunction although other hepatic synthetic parameters such as albumin and total cholesterol showed no significant difference between workers with high and low frequency of exposure (table 1). Ultrasonographic findings included liver swelling and gall bladder wall thickening in case Nos 1, 2, and 3, as is commonly observed during the early phase of acute

viral hepatitis. Liver function in the five hospitalised patients gradually improved (fig 2A, B). Needle biopsy of the liver was performed in case Nos 1, 2, 3, and 6. Histological findings in case Nos 1, 2, and 6 included ceroid accumulation in Kupffer cells as well as focal necrosis such as that commonly seen during recovery from acute viral hepatitis. Unexpectedly, case No 3 showed fatty change and mild fibrosis surrounding central veins. No evidence of damage to the biliary ducts was present (fig 3A, B).

DISCUSSION

5-Nitro-*o*-toluidine is also designated 2-amino-4-nitrotoluene, 4-nitro-2-aminotoluene, 6-methyl-3-nitroaniline, 2-methyl-5-nitroaniline, 5-nitro-2-methylaniline, *p*-nitro-*o*-toluidine; colour index (CI) 37105; and azoic diazo component 12. The compound is not subjected to worldwide regulation unlike other toxic aromatic nitro and amino compounds. We sought toxicity information concerning 5-nitro-*o*-toluidine using chemical dictionaries, toxicity manuals, and Internet sources.^{1,2} We found a report of hepatocellular carcinogenicity in rats³ but no report of toxicity to the human liver. However, 4-nitro-2-aminotoluene (CI 37030; azoic diazo component 7),

Figure 2 Liver function tests over time in two hospitalised patients. All values for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were normal one month prior to exposure. Patient Nos 2 (A) and 3 (B) handled 5-nitro-*o*-toluidine 20 and 18 times, respectively, as indicated by the arrows. All values for AST, ALT, and ALP after handling of 5-nitro-*o*-toluidine initially were high, gradually decreased, and were finally normalised.

Figure 3 Histological findings in the cases shown in fig 2. (A) Case No 2 showed ceroid accumulation in Kupffer cells (arrowheads) (haematoxylin and eosin; original magnifications $\times 40$). (B) Case No 3 showed fatty change and mild fibrosis surrounding the central veins, with no apparent damage to the biliary ducts (haematoxylin and eosin; original magnifications $\times 20$).

used as an artificial sweetener, has shown liver toxicity.⁴ No information about toxicity in humans was found using the names 5-nitro-*o*-toluidine (CAS No 99-55-8) although searching for a synonym, *p*-nitro-*o*-toluidine, disclosed three other incidents involving more than nine individuals.⁵⁻⁹

In Tokyo in 1946, at least six people who had taken 5-nitro-*o*-toluidine orally as a sweetener died of liver failure.⁵⁻⁷ The reports did not indicate the ingested amount but they described high fever, nausea, vomiting, liver swelling, jaundice, and bleeding tendencies. Three of these six fatal cases had fulminant hepatic failure, associated with the finding of liver atrophy at autopsy: microscopic findings in the liver included centrilobular necrosis, formation of pseudo bile ducts, and thrombosis associated with endothelial cell injury.⁶ One report described deposition of azo pigment in the stomach and liver.⁷ Also in 1946, two patients in Osaka survived ingestion of the compound,⁸ probably because they had regularly taken small quantities (daily intake in one case was 0.5 g over 20 days; in the other it was 0.08 g over 25 days). A case in Osaka in 1976 was reported as occupational liver injury,⁹ resulting from the manufacture of 5-nitro-*o*-toluidine from *o*-toluidine; this worker's exposures occurred over three months and he developed fulminant hepatic failure. While skin absorption was suspected, concentrations of compounds were surveyed in the air of the workplace: that of *o*-toluidine varied from 0.23 to 6.8 mg/m³ while that of 5-nitro-*o*-toluidine was 0.53 mg/m³, considered a trace amount.

A causal relationship between liver dysfunction and 5-nitro-*o*-toluidine exposure is difficult to prove conclusively, and the mechanism of hepatotoxicity of 5-nitro-*o*-toluidine is unclear. The link between liver dysfunction and 5-nitro-*o*-toluidine exposure was supported by our finding that the severity of liver dysfunction increased in relation to the numbers of times workers handled 5-nitro-*o*-toluidine. Causality was also supported by a similar latent period in each case from initial handling of 5-nitro-*o*-toluidine to occurrence of symptoms. When exposure to 5-nitro-*o*-toluidine finally ended, recovery and normalisation of liver function tests ensued. No additional workers were affected after cessation of exposure. Prior to exposure, liver function test results of all workers were normal, largely ruling out pre-existing liver disease. Hepatotoxicity from other chemicals, infectious agents, and medication were excluded. Considering that previous anecdotal reports also suggested hepatotoxicity of 5-nitro-*o*-toluidine in humans, a causal relationship between liver dysfunction and 5-nitro-*o*-toluidine exposure now appears evident.

With respect to the route of intake, we surmised that the powder was inhaled in our cases as during rest breaks taken within the enclosed work area the factory employees sometimes loosened straps holding respirators to their faces. This exposure to airborne 5-nitro-*o*-toluidine represented the only unprotected contact. Sealing of the work area presumably increased the 5-nitro-*o*-toluidine concentration in the air, permitting the affected workers to inhale a toxic dose during a short period. However, we could not measure concentrations of aerosolised 5-nitro-*o*-toluidine occurring in the work area shortly after loading of the mixing machines.

The exposure time for each worker was not recorded but could be estimated. Case No 7, who had the least exposure among the workers with liver dysfunction, was estimated to be exposed for approximately 15 hours. The six heavily exposed workers had at least four times as much contact. Nevertheless, the severity of liver dysfunction in case Nos 4 and 7 was similar. Although toxicity of many aromatic compounds is dose dependent and generally seemed evident in our study, dose dependence was not invariable in our cases. Imprecise estimation of exposure may be responsible for the discrepancy. We suspect that 15 hours is the minimum exposure duration capable of causing liver dysfunction under the workplace conditions in these cases.

In an experimental study of toxicity of 5-nitro-*o*-toluidine,³ hepatocellular carcinoma was observed in rats but was not preceded by evidence of hepatocellular damage. Three of our five hospitalised cases showed evident hepatocellular and cholestatic liver injury according to serum chemical data. Relatively non-specific pathological findings were similar to those seen in the recovery phase of other forms of acute liver injury. One case (No 3) showed fatty change and mild fibrosis, suggesting chronic liver disease such as alcohol induced damage. The abnormal PT values in this case also might suggest pre-existing liver disease. However, further history taking from case No 3 did not reveal a cause of fatty change such as heavy alcohol use or medications. Results of liver function tests in case No 3 were normal before and after the incident, and tests for hepatitis virus markers were negative. Causative agents of liver dysfunction except for 5-nitro-*o*-toluidine were carefully excluded but the reason why pathological findings in case No 3 differed from those in the others remains unknown. Kopelmann and colleagues^{10,11} described toxicity of 4,4'-diaminodiphenylmethane, an aromatic amino compound; the pathological features showed time dependent changes. Early findings included cellular infiltration and cholestasis surrounding the portal veins, as seen in cholangitis. After eight weeks, damage to the liver parenchyma was apparent; at 17 weeks histological appearances showed normalisation. The parenchymal damage was similar to typical findings in our recovery phase specimens; no liver specimens were obtained from our patients during the early phase.

Many aromatic compounds, including 5-nitro-*o*-toluidine, can induce formation of methaemoglobin in mammals. Circulating methaemoglobin in humans causes a feeling of euphoria and headache as early symptoms, and produces cyanosis at high concentrations. However, methaemoglobin formation induced by these compounds differs markedly between species; in fact, 4,4'-diaminodiphenylmethane does not cause methaemoglobinaemia in humans.¹ We did not measure methaemoglobin in our patients but no clinical sign of methaemoglobinemia such as headache, pallor, or respiratory distress was present in previous cases or in our own. Whether or not 5-nitro-*o*-toluidine causes methaemoglobinaemia in humans is unanswered; for now, the possibility remains that methaemoglobin may be involved in 5-nitro-*o*-toluidine toxicity in humans.

The current cases and those previously reported clarify some of the clinical features of 5-nitro-*o*-toluidine toxicity, and should broaden awareness of the risks. Although much remains uncertain about the mechanism of liver toxicity in humans as well as dose-symptom relationships, clinicians should be aware of this potential problem. Workers regularly exposed to 5-nitro-*o*-toluidine require monitoring of liver function. In cases of accidental exposure to this compound, evaluation of liver function and possibly methaemoglobin concentration is recommended.

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