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TECHNICAL NOTE

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Evidence of Hexavalent Chromium Ingestion

ABSTRACT: We describe the application of histochemical demonstration of chromium in a case of fatal ingestion of potassium dichromate in a suicide attempt. Using 2-(8-quinolylazo)-4,5-di-p-tolylimidazole (QTI), we could demonstrate chromium in the erythrocyte of the victim, in situ. This finding provides a means of proving the hexavalent chromium ingestion.

KEYWORDS: forensic science, potassium dichromate, poisoning, histochemistry, 2-(8-quinolylazo)-4,5-di-p-tolylimidazole (QTI)

Dichromate chemicals are widely used in the production of chrome pigments, wood preservatives, plating and anticorrosives in various chemical forms (1). Following ingestion, the hexavalent chromium is reduced ultimately to trivalent form, but this conversion, and perhaps the intermediate pentavalent chromium, combines to produce severe, free-radical damage to mitochondria (2). Acute toxicity of dichromate compounds is due to this strong oxidation action, and causes multiple organ damage, such as liver and kidneys (1–5).

In general, the morphological findings are non-specific in a poisoning case, and the diagnosis depends on an analytical toxicology. Recently, histochemical demonstration of chromium in rat liver has been reported using 2-(8-quinolylazo)-4,5-di-p-tolylimidazole (QTI, Fig. 1) (6). It is a sensitive chelating agent and provides approximately 10 times more sensitivity than the conventional method (6). However, there have been no reports of its application to a human poisoning case. Here we employed the histochemical staining using QTI in a case of potassium dichromate ingestion in a fatal suicide attempt and detected chromium in erythrocytes. This evidence indicated hexavalent chromium exposure.

Materials and Methods

Case History

A 61-year-old male ingested potassium dichromate in a suicide attempt. Despite immediate detoxification, he died 5 days later. Diffuse necrosis and severe hemorrhage of the liver and severe erosion with bleeding in the gastrointestinal tract were observed at postmortem examination. Postmortem blood chromium concentration was 2.0 μg/g, which is extremely higher than normal range (10 ± 4 ng/mL) (7). The cause of death was confirmed as multiple organ failure due to dichromate compound ingestion (8).

Procedures for QTI staining

QTI staining was performed according to a previous report (6). In brief, the staining solution contains QTI (1–2 mg), 0.5 mL dimethylsulfoxide and one drop of 0.5N NaOH in 30 mL deionized water. Tissue sections were immersed in the staining solution for 6–7 h at 30°C. The sections were then washed in water, air dried, immersed in xylene and mounted. This QTI also has a high affinity for copper, lead, and zinc in addition to chromium. To prevent interference by these metals, the deparaffinized sections were immersed in an alkylamine solution for 15 min, and then, QTI staining was performed (6,9). Control sections were obtained from other ischemic heart disease cases.

Results and Discussion

We observed positive staining of chromium in the erythrocytes, which was stained a bluish purple (Fig. 2), but no staining was observed in the kidney and liver, and also in the control section. This is the first report of the application of histochemical detection of chromium in a human poisoning case. It has been reported that degrees of staining intensity depend on the concentration, rather than volume of injected metal solution (10). The blood is exposed to a relatively high concentration of hexavalent chromium in a short period following a large amount of chromium ingestion, and hexavalent chromium is easily uptaken and accumulated by the erythrocytes in blood without adverse effect (3). This may be the reason for our histochemical detection of chromium in the erythrocyte five days later following ingestion. The reasons that we failed to detect chromium in the kidney and liver, are unknown.

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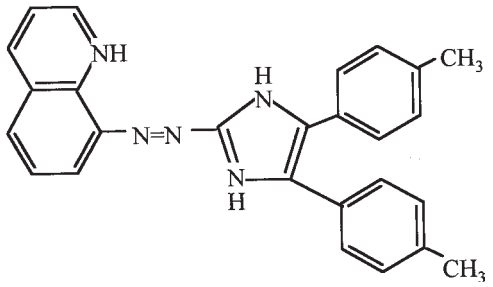

FIG. 1—*The chemical structure of 2-(8-quinolylazo)-4,5-di-p-tolylimidazole (QTI).*

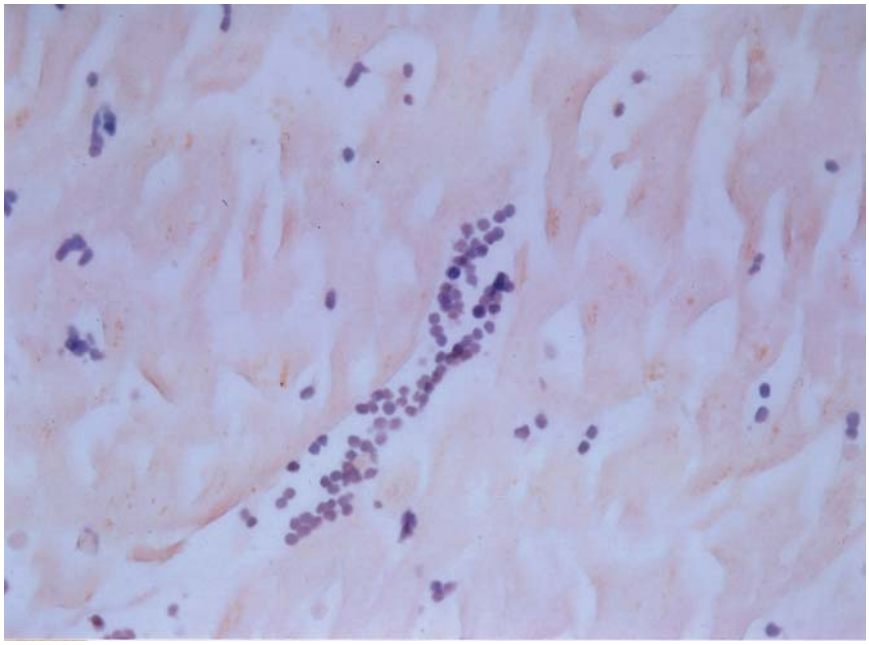

FIG. 2—*Positive staining by 2-(8-quinolylazo)-4,5-di-p-tolylimidazole (QTI). Erythrocyte is stained a bluish purple. It indicates the presence of chromium in erythrocyte (X100).*

The presence of chromium in erythrocytes becomes an indicator of the extent of hexavalent chromium exposure during the lifetime of the erythrocyte (3). Generally, it is difficult to prove the existence of hexavalent chromium in the tissues, because of its rapid transformation to a trivalent form (3). It is easy for hexavalent chromium to cross the erythrocyte membrane, but difficult for trivalent chromium (3). It has been reported that more than 90% of added hexavalent chromium were uptaken by erythrocytes, but almost none of added trivalent chromium were uptaken in vitro experiment (11). Following the uptake by erythrocytes, chromium firmly binds to hemoglobin beta-chains in the trivalent form (12), but trivalent chromium can't enter the erythrocyte (3). Our chromium detection in the erythrocyte by this histochemical method is also proof of the ingestion of hexavalent chromium.

References

1. Ellenhorn MJ, Barceloux DG. Dichromate. In: Ellenhorn MJ, Barceloux DG, editors. Medical toxicology diagnosis and treatment of human poisoning. New York: Elsevier 1988;1020–2.
2. Michie CA, Hayhurst M, Knobel GJ, Stokol JM, Hensley B. Poisoning with a traditional remedy containing potassium dichromate. Hum Exp Toxicol 1991;10:129–31.
3. Barceloux DG. Chromium. J Toxicol Clin Toxicol 1999;37:173–94.
4. Pascale LR, Waldstein S, Engbring G, Dubin A. Chromium intoxication with special reference to hepatic injury. JAMA 1952;149:1385–9.
5. Kaufman DB, DiNicola W, McIntosh R. Acute potassium dichromate poisoning treated by peritoneal dialysis. Amer J Dis Child 1970;119:374–6.
6. Sumi Y, Itoh MT, Yoshida M, Suzuki T. Histochemical staining of chromium with 2-(8-quinolylazo)-4,5-di-p-tolylimidazole. Eur J Histochem 1998;42:271–6.
7. Tanaka T, Nose T, Hayashi Y, Sugiyama K, Funakawa K, Ishizawa M. Concentration of chromium in biological materials (blood, serum, urine and scarp hair) from the chromium mine workers. Medicine and Biology 1980;101:277–81.
8. Kinoshita H, Koizumi M, Ijiri I, Ameno S, Seki K, Kumihashi M, et al. A fatal case due to potassium dichromate ingestion. Jpn J Toxicol 2000;13:407–9.
9. Sumi Y, Muraki T, Suzuki T. The choice of a masking agent in the histochemical staining of metals. Histochem J 1983;15:231–8.
10. Sumi Y, Muraki T, Suzuki T. Histochemical staining of cadmium with benzothiazolylazonaphthol derivatives. Histochemistry 1982;73:481–6.
11. Gray SJ, Sterling K. The tagging of red cells and plasma proteins with radioactive chromium. J Clin Invest 1950;29:1604–13.
12. Pearson HA, Vertrees KM. Site of binding of chromium 51 to haemoglobin. Nature 1961;189:1019–20.

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