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Immunosuppressive therapy of cyclosporin A for severe benzene-induced haematopoietic disorders and a 6-month follow-up

Yuguo Song[a,b], Xuqin Du[a,b], Fentong Hao[a,b], Xiaoxin Gu[c], Zhenghua Zhang[d], Songquan Zhang[e], Chunsheng Li[a,b,*], Huiling Li[a,b], Jing Ma[a,b]

[a] Department of Occupational Medicine and Clinical Toxicology, Beijing Chaoyang Hospital, Capital University of Medical Sciences, Beijing 100020, China
[b] Beijing National Centre for Clinical Toxicology, Beijing, China
[c] Department of Occupational Medicine, Jinan Municipal Hospital of Occupational Disease, Shangdong Province, China
[d] Department of Occupational Medicine, Zibo Municipal Hospital of Occupational Disease, Shangdong Province, China
[e] Department of Occupational Medicine, Tsingdao Central Hospital, Tsingdao University of Medical Sciences, China

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ABSTRACT

Long-term exposure to benzene can potentially result in severe haematotoxicities, including pancytopaenia, aplastic anaemia and myelodysplastic syndrome, which are often accompanied by life-threatening symptoms and high mortality. Previous studies demonstrate that benzene-induced haematotoxicities are immune-mediated and that cyclosporin A is a prominent treatment in acquired aplastic anaemia. This study aims to evaluate the potential role of cyclosporin A immunosuppressive therapy for severe benzene-induced haematotoxicity. Between January 2002 and December 2008, 41 patients with severe benzene-induced haematopoietic disorders from five hospitals were enrolled in the study, 22 patients received cyclosporin A, supportive treatments and/or oral testosterone undecanoate, 19 patients were treated with supportive treatments and/or oral testosterone undecanoate as the control group, and a 6-month follow-up was conducted. The results showed that in the cyclosporin A group, 19 of 22 patients (86.36%) had responded to the treatments completely or partially with increased platelets, white blood cells and hemoglobulin counts by the fourth week ($P = 0.005$), the sixth week ($P = 0.001$) and the third month post-treatment ($P = 0.034$), respectively. However, in the control group treated by supportive methods, only 5 of 19 patients (26.32%) responded to the treatments partially ($P < 0.001$). Cyclosporin A in conjunction with supportive treatments may be an effective treatment modality for patients with severe benzene-induced haematopoietic disorders, which in turn implies that these haematotoxicities are immune-mediated.

1. Introduction

Benzene has been widely used in various industries as glues or solvents in China [1–4] and some developing countries [5]. It is estimated that there are 38,000 enterprises just in the shoe-making industry in China with approximately 2 million shoe workers employed [2]. However, long-term exposure to benzene can potentially result in severe haematopoietic disorders [6–9], including pancytopaenia, aplastic anaemia (AA) and myelodysplastic syndrome (MDS). These disorders are often accompanied by life-threatening symptoms (severe bleeding, infections and anaemia) and mortality of up to 26% (9/31) in human cases [4]. Benzene-induced haematotoxicities are still a very serious occupational environment problem in China [3] as well as a clinical challenge with a high mortality [1,4].

Previous studies [10–13] show that acquired aplastic anaemia is pathophysiologically immune-mediated in most cases and can be effectively treated using immunosuppressive therapy or allogeneic bone-marrow transplantation, and that environmental exposures are thought to trigger the aberrant immune response. Also, studies demonstrate that severe benzene-induced haematotoxicities are immunotoxic and autoimmune-mediated [5–8,13], and that autoimmune-mediated bone marrow injury is an early or predisposing event in the pathogenesis of benzene-induced persistent haematopoietic disease [14]. Like many other autoimmune diseases, these haematotoxicities are HLA-linked with immune-

Abbreviations: AA, aplastic anaemia; CBP, chronic benzene poisoning; CFU-E, colony-forming unit-erythroid; CFU-GM, colony-forming unit-granulocyte/macrophage; CSA, cyclosporin A; MDS, myelodysplastic syndrome; PCA, principal component analysis; PLT, platelet; PPE, personal protective equipment.

* Corresponding author at: Department of Occupational Medicine and Clinical Toxicology, Beijing Chaoyang Hospital, Capital University of Medical Sciences, No. 8, Baijiazhuang Road, Chaoyang District, Beijing 100020, China. Tel.: +86 10 85231051; fax: +86 10 85231524.
E-mail addresses: songrain123@hotmail.com (Y. Song), dreamchina12345@163.com (C. Li).

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related genes like HLA-DMB, HLA-DQA1, HLA-DPB1, ITGB2 and PFC down-regulated after chronic benzene exposure [15].

These findings that severe benzene-induced haematotoxicities are immunotoxic and autoimmune-mediated, taken together with the prominent treatment of AA with cyclosporin A (CSA) led us to investigate the potential role of the immunosuppressive therapy of CSA for these disorders. We retrospectively enrolled the patients with severe benzene-induced haematotoxicities that had visited our five hospitals from January 2002 to December 2008, investigated the effects of CSA, supportive treatments and/or testosterone undecanoate, and conducted a 6-month follow-up aiming to evaluate the potential role of CSA on severe benzene-induced haematotoxicities.

2. Methods

2.1. Patients

All patients were enrolled in the following five hospitals: Jinan Municipal Hospital of Occupational Disease, Shangdong Province; Zibo Municipal Hospital of Occupational Disease, Shangdong Province; Tsingdao Central Hospital, Tsingdao University of Medical Sciences; Shangdong Province's Hospital of Occupational disease; and Beijing Chaoyang Hospital, Capital University of Medical Sciences, Beijing, China. Informed consent was obtained from each patient prior to CSA and/or androgen treatment and any clinical examinations. All the patients had been admitted into these hospitals between January 2002 and December 2008. The diagnostic criteria are based on the Diagnostic Criteria of Occupational Benzene Poisoning (GBZ 68-2002), published by the Health Ministry of the People's Republic of China [16], and the following criteria needed to be met: (1) a long-term occupational exposure to benzene; (2) one of the following diseases should be present: pancytopaenia (white blood cell WBC $< 4 \times 10^9$/L, platelet PLT $< 60 \times 10^9$/L, hemoglobin: female Hb < 110 g/L, male < 120 g/L), AA, or MDS which was diagnosed using WHO 2001 criteria [17]; (3) exclusion of other causes of abnormal blood counts, such as chloromycetin use and ionizing radiation; and (4) confirmation of the diagnosis by at least three physicians specializing in occupational diseases and clinical toxicology in line with China's prescribed occupational disease diagnosing procedures.

Patients were excluded if they had mild or moderate chronic benzene poisoning (only with a decrease in one or two peripheral blood cell lineages), or were undergoing chemotherapy for leukemia. Also, the cases with incomplete data were not included.

2.2. Treatment with CSA

When the patients received either CSA alone or CSA plus oral testosterone undecanoate (CSA, Huabei Pharmaceuticals Lmt., China; testosterone undecanoate, Organon Pharmaceuticals Lmt., Nanjing, China), the dosage of CSA was 3–4 mg/kg/day for the first 3 months, which was then maintained at 1–2 mg/kg/day, and decreased gradually after the peripheral blood cell counts recovered or showed improvement. The patients' plasma levels of CSA were monitored once every 1–2 months in the early treatment by high performance liquid chromatography in order to keep the concentration of CSA in a range of 150–250 μg/L. The dosage of oral testosterone undecanoate was 0.15 mg/kg/day in the first 3 months, and thereafter was maintained at 0.75–1 mg/kg/day for an additional 3 months.

Supportive treatment included haemopoietic growth factors, transfusions (PLT, RBC and human γ-globulin) and antibiotics. RBC were transfused when the Hb level was less than 60 g/L, and platelets were immediately transfused when the level was less than 10×10^9/L or 20×10^9/L in the presence of bleeding. In the CSA treatment group, 12 cases were subcutaneously given rhuGM-CSF (Schering-Plough, Cork, Ireland), 3–5 mg/kg/day, once every other day for 1–2 weeks; four patients were administered rhuEPO (Amgen-Roche, Thousand Oaks, CA, USA), 100 units/kg/day, by intravenous infusion 3 days a week for 1–2 weeks; five patients were given 1–4 units of packed red cells and 1–5 bags of PLT (one bag of PLT approximately equals the amount of PLT in 250 ml fresh blood); and seven patients received human γ-globulin as a continuous intravenous infusion, 3.75–5.00 g/day for 3–10 days. Six patients were diagnosed with upper respiratory tract infections and four patients with pneumonia and fever, and received antibiotics. In the control group treated by supportive care, 6 cases were subcutaneously given rhuGM-CSF; two patients were administered rhuEPO; six patients were given packed red cells or whole blood and bags of PLT, and two patients received human γ-globulin as a continuous intravenous infusion. Ten patients were diagnosed with upper respiratory tract infections and two patients with pneumonia and fever, and received antibiotics.

2.3. Clinical examinations

Routine peripheral blood examinations were repeatedly analysed (Beckman-Coulter® T540 blood counter), at least 2–3 times a week in the first month. The major lymphocyte subsets were also analysed (Becton Dickinson FACS CaliburTM flow cytometer, Software:Simul SET v3.1). Bone marrow aspirations and biopsies were obtained, along with colony assay data from the bone marrow haematopoietic progenitor cells, including colony-forming unit-erythroid (CFU-E) and colony-forming unit-granulocyte/macrophage (CFU-GM). Ham's test, Coomb's test and Rous' test on the patients were conducted. The concentrations of serum folic acid, iron and vitamin B_{12} were repeatedly analysed. After stabilization of the patients, an ultrasonic examination of the abdomen and a chest X-ray were performed. Enzymes of hepatic and renal function were periodically measured in order to monitor the possible side effects of treatment (a Synchron LX-20, Backman-Coulter, Lmt., USA).

2.4. Exposure and raw materials monitoring

After the patients were admitted into our hospitals, the workplaces were investigated as soon as possible in order to facilitate clinical diagnosis with the help of the local centres of disease control in China. The air concentrations of benzene and toluene in the workplaces or the used raw materials were analysed by gas chromatography with a flame ionization detector (GC-FID).

2.5. Follow-up and responses

Follow-up evaluations after CSA treatment were performed once every 1–2 months after the patients were discharged from our hospitals. All the participants treated with CSA were successfully solicited through the entry into our hospitals to perform re-examinations, as all the medical expenses were afforded by the related enterprises and our hospitals are well-equipped in clinical toxicology. At each evaluation, clinical assessments on the therapeutic effects and side effects were performed, and a complete blood count, PLT and enzymes of hepatic and renal function were repeatedly tested. Follow-up of patients in the control group was also conducted in a similar way.

2.6. Statistics

All statistical analyses were performed using SPSS for Windows version 16.0 (SPSS, Inc., Chicago, IL). All data were expressed as