

Original Article

Incidence of cyclophosphamide-induced hemorrhagic cystitis in Chinese Han population with autoimmune disease

Di Fu¹, Shanhui Ye¹, Chuyin Xiao¹, Yingying Xie¹, Jianquan Gao¹, Liuqin Liang², Xiuyan Yang²

¹First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, Guangdong, China; ²First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, Guangdong, China

Received November 27, 2015; Accepted March 25, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Cyclophosphamide (CTX), has been widely used for treating various human malignancies as well as autoimmune disease. Although CTX could rapidly decrease the response of immune system, it also could result in severe adverse effects, such as hemorrhagic cystitis (HC). In this study, we investigated the occurrence of HC in patients taking CTX from Chinese Han population and divided all cases into different groups according to the methods of CTX administration. In all cases involved in our study, macroscopic hematuria caused by the primary diseases was observed in 316 patients (47%) before the initiation of CTX treatment and the symptom in these patients gradually disappeared during follow-up examination, indicating the hematuria was unrelated with CTX-induced HC. Moreover, there were 5 macroscopic hematuria cases and 17 macroscopic hematuria cases were classified as urinary tract infection and can be treated by antibiotics. Only one female patient had been suspected as HC case but the symptom disappeared after switching CTX to mycophenolate mofetil. In conclusion, our data suggested the Chinese Han population may be highly resistant for CTX induced hemorrhagic cystitis.

Keywords: Cyclophosphamide, hemorrhagic cystitis, hematuria, autoimmune disease

Introduction

Cyclophosphamide, which is under the trade name of Cytoxan (CTX), is an oxazaphosphorine alkylating agent, has been generally used for threatening various human malignancies as well as autoimmune disease [1, 2]. As a nitrogen mustard alkylating agent from the oxazaphosphorine group, CTX could attach the alkyl group to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring which forms intra-strand and inter-strand DNA cross-links to interfere with DNA replication [3]. CTX itself is a prodrug and it has to be converted by host enzyme liver cytochrome P450 to form bioactive metabolites [4].

As a DNA replication inhibitor, CTX has been widely used for treating different cancers, such as lymphomas, brain cancer, leukemia [5, 6]. Besides, since CTX could rapidly decrease the response of immune system [7], it has been used for treating severe autoimmune diseases

if the disease-modifying antirheumatic drugs (DMARDs) have been ineffective, such as systemic lupus erythematosus (SLE) with severe lupus nephritis, severe rheumatoid arthritis, granulomatosis with polyangiitis and multiple sclerosis [2, 8-10]. However, CTX could also lead to severe adverse effects, such as hemorrhagic cystitis (HC) [11], as well as chemotherapy-induced nausea, vomiting and bone marrow suppression [12, 13].

Although the mechanism of CTX-induced HC is still elusive, it is believed that CTX caused oxidative stress mediated by reactive oxygen species (ROS) would be the major contributing factor for HC [14, 15]. The occurrence rate of HC was ranged from 4% to 36% in autoimmune patients receiving CTX. However, during the clinical practice, as SLE in the Asian population is more severe than it in the Caucasian, increased CTX dose without any prevention of HC such as co-administration with MES-na is widely used by doctors for treating individual patient

Occurrence of hemorrhagic cystitis in Chinese population

in Asia region. However, there is little report about the occurrence of HC from patients taking CTX in China may imply the Chinese Han population is more resistant for CTX-induced HC than other population. Thus, we conducted an investigation for the occurrence of HC in patients taking CTX from Chinese Han population. According to our result, only one female patient had been suspected as CTX induced HC case as the symptom disappeared after switching to other immunosuppressant. In conclusion, our data suggested that Chinese Han population may be highly resistant for CTX induced hemorrhagic cystitis, but it needs further validation.

Materials and methods

Ethics statement

Institutional Ethics Board approval was obtained from the Medical Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University. All participating patients were formally informed for the purpose of using their medical records and the written informed consents were obtained from all participants in this clinical trial.

Patients

A total of 672 adult patients receiving CTX treatment were included in this investigation. These patients were admitted to First Affiliated Hospital of Sun Yat-sen University or Guangzhou Medical University from January 2008 to December 2014. The clinical diagnosis for each patient was conducted as previously described [16-23]. To confirm the CTX-induced HC, the patients were also examined for infection, urinary calculi, tumor as well as other factors contributing to the post-renal hematuria.

CTX dosing, administration and regimen

The dose, methods of administration and regimen of CTX for individual patient were determined by the chief physician. Additional application of other drugs such as hormone was determined by the same physician as well. Both oral administration and I.V. were used. For oral group, CTX was administered daily with the dose ranged from 50 to 150 mg. For I.V. group, 400 mg to 600 mg CTX was administered along with 40 mL NS on the weekly or biweekly basis.

For patients needing pulse intravenous cyclophosphamide therapy, 800 mg to 1000 mg CTX was administered along with 100 mL NS once every 3 or 4 weeks. After relieving of the symptoms, the following dose and regimen of CTX, and the requirement for replacing CTX with other immunosuppressants such as MMF, Imuran or MTX were all determined by chief physician as well.

The follow-up examination and endpoint event

After discharge of the patients from hospital, the follow-up examination was initiated to monitor the occurrence of HC. The urine examination was conducted for each patient weekly or biweekly. All the patients had been trained for self-identification of urethral irritation and hematuria. If the patient was suspicious for the symptoms mentioned above, urine examination would be conducted. Cystoscopy was used to confirm the occurrence of HC if needed. The severity of HC was graded according to the general standard issued by WHO as follows: Grade I, microscopic hematuria; Grade II, macroscopic hematuria; Grade III, macroscopic or macroscopic hematuria with blood clots; Grade IV, macroscopic hematuria with blood clots and urinary tract obstruction. If no HC occurring after stopping CTX for 3 months, the patients will be classified as record censored. The times of follow-up and the cumulative dose of CTX were recorded as well. If the patient was confirmed for HC, the follow-up would be ended.

Statistical analysis

SPSS 15.0 software was used to analyze the data. Single factor analysis of variance was used for the comparison between multiple groups; t test was used for the comparison between 2 independent groups.

Results

General information of patients

There were totally 672 adult patients (586 females, 87.2%) enrolled in this investigation. The average age for all patients was 39.1 ± 15.3 years and the average regimen of disease was 19.1 ± 16.8 months. Among all these patients, there were 389 cases of SLE, which included 235 cases of lupus nephritis, 68 cases of neuropsychiatric lupus, 56 cases of lupus vasculi-

Occurrence of hemorrhagic cystitis in Chinese population

Table 1. The regimen and accumulated dose of CTX for different autoimmune diseases

Disease	Average Regimen (x±s) (Months)	Accumulated Dose (x±s) (g)
SLE (n=389)	23.4±18.5	13.8±9.89
Rheumatoid arthritis (n=72)	17.2±14.2	10.9±9.01
Vasculitis (n=69)	12.8±8.95	16.2±8.15
Undifferentiated connective tissue disease (n=44)	13.7±14.8	7.31±4.21
Mixed connective tissue disease (n=28)	12.5±15.5	9.74±8.89
Sjogren's syndrome (n=22)	7.45±4.25	9.27±4.79
Polymyositis and dermatomyositis (n=20)	10.8±9.37	12.3±7.73
Systemic sclerosis (n=19)	11.3±6.72	13.5±7.95
Relapsing polychondritis (n=5)	7.00±4.64	9.79±8.15
Adult onset Still's disease (n=4)	10.3±3.59	8.23±4.53

Table 2. Administration of CTX and accumulated CTX dose

CTX administration	Average Regimen (x±s) (Months)	Accumulated Dose (x±s) g
Oral		
50 mg (n=132)	15.5±12.4	7.33±6.52
100 mg (n=230)	14.1±11.8	10.0±6.93
150 mg (n=7)	12.3±6.88	14.1±7.02
I.V.		
400 mg Weekly (n=94)	28.3±24.7	17.7±9.11
600 mg Weekly (n=119)	19.3±13.7	17.2±9.00
400 mg Biweekly (n=27)	20.9±10.7	11.3±5.05
600 mg Biweekly (n=19)	26.5±12.3	14.1±6.47
High-dose intravenous pulse (n=44)	32.5±25.7	22.4±14.2

4 cases received more than 50 g. The details of CTX administration and cumulative dose for patients with different disease were listed as **Table 2**.

The occurrence of hematuria

Before the initiation of CTX treatment, all patients were subjected to microscopic hematuria examination. While urinary tract irritation was not reported in any patients, microscopic hematuria had been identified in 316 patients (47.0%) which means microscopic hematuria in these patients was

due to primary cause rather than application of CTX. Moreover, the symptom of microscopic hematuria in these patients was gradually disappeared after imitation of CTX treatment.

During the CTX treatment, 17 patients developed microscopic hematuria which was characterized by increased count of both red and white blood cell in urine. As presenting of white blood cell in urine implied the infection of urinary tract, no further hematuria was developed in them during the remaining CTX treatment after application of antibiotics treatment.

There were 5 patients developed macroscopic hematuria during the CTX treatment. Two of them were SLE patients with transverse myelitis and were caused by urinary tract infections treating during the treatment of urinary retention with catheter. The hematuria was relieved by antibiotics and replacing of the catheter. The administration of CTX did not evoke repeated hematuria in these two patients after recover-

tis, 6 cases of antiphospholipid syndrome, 10 cases of overlap syndrome and 14 cases of unclassified SLE. For the other patients, there were also 69 cases of systemic vasculitis syndrome, which included 26 cases of Behcet's disease, 13 cases of polyarteritis nodosa, 9 cases of Wegener's granulomatosis, 7 cases of Takayasu arteritis, 5 cases of primary central nervous system vasculitis, 3 cases of giant cell arteritis and 6 cases of unclassified vasculitis. Moreover, there were 72 cases of rheumatoid arthritis as well. The detailed information for disease, case numbers, the regimen and cumulative dose of CTX were listed as **Table 1**.

Administration of CTX and cumulative CTX dose

The average cumulative dose of CTX for all patients was 12.9±9.34 g. There were 305 patients and 337 cases receiving the CTX dose of less than 10 g, or 10-30 g, respectively. Only 26 cases received the CTX dose of 30-50 g and

Occurrence of hemorrhagic cystitis in Chinese population

ing from hematuria. Moreover, two lupus nephritis patients and one scleroderma pulmonary hypertension patient developed macroscopic hematuria during the CTX treatment as well. The urine culture test confirmed the bacterial infection in urinary tract in these patients. Therefore, the antibiotics were applied to these patients until disappearing of the hematuria. After antibiotics treatment, no further hematuria was detected as well.

The occurrence of HC

In all patients enrolled in our study, only one female patient with lupus nephritis developed hematuria after weekly I.V administration of CTX for 9 times (600 mg CTX per IV). Microscope examination defined the hematuria as Grade II (macroscopic hematuria) without urinary tract irritation and urinary white blood cell. As the I.V. CTX alleviated the lupus nephritis and urinary protein level became negative in this patient, CTX was replaced by mycophenolate mofetil for avoiding further deterioration of HC. The macroscopic hematuria disappeared 9 weeks later after replacement of the drug. Since the patient refused to conduct cystoscopy examination for confirming hemorrhagic cystitis, this case was defined as the only suspected HC case in all patients involved in this investigation.

Discussion

The hemorrhagic cystitis, characterized by diffuse inflammation and hemorrhage in bladder, has been considered as one of the most severe side effects during the CTX administration for patients with life threatening autoimmune diseases. However, as hematuria could be caused by many factors, further examinations were required to exclude the microorganism infection and urolithiasis, as well as bladder hemorrhage due to the invasive tumor for the final confirmation of hemorrhagic cystitis. Previous study reported an incidence rate of HC was ranged from 4% to 36% in CTX receiving patients [24]. Large amounts of bleeding due to the necrosis of bladder mucosal may be life-threatening for patients and it was reported that the mortality rate for Grade III and Grade IV hemorrhagic cystitis patients could reach 30% to 40% [25].

According to previous reports focusing on CTX-induced HC, the HC symptoms were initiated mainly with macroscopic hematuria or macro-

scopic hematuria without frequent urination, urgency and urinary tract irritation. In a retrospective study based on 100 HC patients, macroscopic hematuria and urinary tract irritation were observed in 78% and 45% of patients, respectively [26]. In another follow-up study investigating 145 Wegener's granulomatosis cases, macroscopic hematuria and macroscopic hematuria were observed in 56% and 44% of total patients, respectively [27]. In 60 cases examined by cystoscopy, HC and HC related pathological changes caused by CTX was observed in 42 patients (72%) [27]. All these studies indicated HC was a common side effect in CTX receiving patients.

However, in all cases involved in our study, macroscopic hematuria caused by the primary diseases was observed in 316 patients (47%) before the initiation of CTX treatment and the symptom disappeared during the follow-up period. Another 22 cases developed hematuria due to infection and the symptom was alleviated by antibiotic treatment. All these hematuria cases mentioned were not related with CTX administration. Even with the average cumulative CTX dose was 12.9 (12.9±9.34) g. In sum, no confirmed HC case was identified except one suspected, implying Chinese Han population may be highly resistant for CTX-induced HC.

Many reports suggested that occurrence of HC was correlated with regimen of CTX. In a long time follow-up study focusing on cumulative incidence of glomerular hematuria and its related risk factors on Wegener's granulomatosis patients, although data did not suggest cumulative regimen of CTX was an independent risk factor for HC occurrence, 40% patients with cumulative regimen for more than three years developed HC and the occurrence rate reached 80% in patients with cumulative CTX regimen for more than 10 years [28]. However, as there were 25 patients in this investigation receiving CTX for more than 5 years, none of them developed HC.

To evaluate if the administration methods could be a potential risk factor for CTX induced HC, all cases included in this investigation had been further classified into different groups according to the methods used for CTX administration. However, only one patient in the oral administration group was suspected for HC. Although it is still uncertain for the issue, there was study

Occurrence of hemorrhagic cystitis in Chinese population

suggested that patients received CTX via I.V may be more risky for HC than these who receive CTX orally [29]. In another report, oral administration of CTX (50-100 mg) for six months following with 19 months administration of 100 mg Azathioprine in Asia population did not result any HC or HC related complication, which was consisted with our observation [30].

In conclusion, our data suggested that HC induced by CTX was very rare in Chinese Han population. Whether Han population is more resistant to CTX-induced HC requires further validation by larger scale, multi-center and randomized controls-based investigations.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiuyan Yang, First Affiliated Hospital, Sun Yat-sen University, 58 Zhongshan Road 2, Guangzhou 510080, Guangdong, China. Tel: +86-020-83062314; Fax: +86-020-83062314; E-mail: xiuyanyang123@sina.com

References

- [1] Jurado JM, Sanchez A, Pajares B, Pérez E, Alonso L and Alba E. Combined oral cyclophosphamide and bevacizumab in heavily pre-treated ovarian cancer. *Clin Transl Oncol* 2008; 10: 583-586.
- [2] Steinberg AD, Kaltreider HB, Staples PJ, Goetzl EJ, Talal N and Decker JL. Cyclophosphamide in lupus nephritis: a controlled trial. *Ann Intern Med* 1971; 75: 165-171.
- [3] Foley GE, Friedman OM and Drolet BP. Studies on the mechanism of action of cytoxan. Evidence of activation in vivo and in vitro. *Cancer Res* 1961; 21: 57-63.
- [4] Huttunen KM, Raunio H and Rautio J. Prodrugs-from serendipity to rational design. *Pharmacol Rev* 2011; 63: 750-771.
- [5] Shanafelt TD, Lin T, Geyer SM, Zent CS, Leung N, Kabat B, Bowen D, Grever MR, Byrd JC, Kay NE. Pentostatin, cyclophosphamide, and rituximab regimen in older patients with chronic lymphocytic leukemia. *Cancer* 2007; 109: 2291-2298.
- [6] Young SD, Whissell M, Noble JC, Cano PO, Lopez PG and Germond CJ. Phase II clinical trial results involving treatment with low-dose daily oral cyclophosphamide, weekly vinblastine, and rofecoxib in patients with advanced solid tumors. *Clin Cancer Res* 2006; 12: 3092-3098.
- [7] Hurd ER and Ziff M. The mechanism of action of cyclophosphamide on the nephritis of (NZB x NZW)F1 hybrid mice. *Clin Exp Immunol* 1977; 29: 132-139.
- [8] Townes AS, Sowa JM and Shulman LE. Controlled trial of cyclophosphamide in rheumatoid arthritis. *Arthritis Rheum* 1976; 19: 563-573.
- [9] Novack SN and Pearson CM. Cyclophosphamide therapy in Wegener's granulomatosis. *N Engl J Med* 1971; 284: 938-942.
- [10] Makhani N, Gorman MP, Branson HM, Stazzone L, Banwell BL and Chitnis T. Cyclophosphamide therapy in pediatric multiple sclerosis. *Neurology* 2009; 72: 2076-2082.
- [11] Kim SH, Lee IC, Ko JW, Moon C, Kim SH, Shin IS, Seo YW, Kim HC, Kim JC. Diallyl Disulfide Prevents Cyclophosphamide-Induced Hemorrhagic Cystitis in Rats through the Inhibition of Oxidative Damage, MAPKs, and NF-kappaB Pathways. *Biomol Ther (Seoul)* 2015; 23: 180-188.
- [12] Singh G, Fries JF, Williams CA, Zatarain E, Spitz P and Bloch DA. Toxicity profiles of disease modifying antirheumatic drugs in rheumatoid arthritis. *J Rheumatol* 1991; 18: 188-194.
- [13] Lohrmann HP. The problem of permanent bone marrow damage after cytotoxic drug treatment. *Oncology* 1984; 41: 180-184.
- [14] Bhatia K, Kaur M, Atif F, Ali M, Rehman H, Rahman S and Raisuddin S. Aqueous extract of *Trigonella foenum-graecum* L. ameliorates additive urotoxicity of buthionine sulfoximine and cyclophosphamide in mice. *Food Chem Toxicol* 2006; 44: 1744-1750.
- [15] Kiuchi H, Takao T, Yamamoto K, Nakayama J, Miyagawa Y, Tsujimura A, Nonomura N, Okuyama A. Sesquiterpene lactone parthenolide ameliorates bladder inflammation and bladder overactivity in cyclophosphamide induced rat cystitis model by inhibiting nuclear factor-kappaB phosphorylation. *J Urol* 2009; 181: 2339-2348.
- [16] Fries JF, Hunder GG, Bloch DA, Michel BA, Arend WP, Calabrese LH, Fauci AS, Leavitt RY, Lie JT, Lightfoot RW Jr. The American College of Rheumatology 1990 criteria for the classification of vasculitis. Summary. *Arthritis Rheum* 1990; 33: 1135-1136.
- [17] Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-324.
- [18] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 172.

Occurrence of hemorrhagic cystitis in Chinese population

- [19] Bohan A and Peter JB. Polymyositis and dermatomyositis (first of two parts). *N Engl J Med* 1975; 292: 344-347.
- [20] Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980; 23: 581-590.
- [21] Johnson SR. New ACR EULAR Guidelines for Systemic Sclerosis Classification. *Curr Rheumatol Rep* 2015; 17: 506.
- [22] Ota A. [Diagnosis and therapy for adult Still's disease]. *Nihon Naika Gakkai Zasshi* 2007; 96: 2206-2213.
- [23] Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS, Pillemer SR, Talal N, Weisman MH; European Study Group on Classification Criteria for Sjögren's Syndrome. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002; 61: 554-558.
- [24] Elliott RW, Essenhight DM and Morley AR. Cyclophosphamide treatment of systemic lupus erythematosus: risk of bladder cancer exceeds benefit. *Br Med J (Clin Res Ed)* 1982; 284: 1160-1161.
- [25] Rubin JS and Rubin RT. Cyclophosphamide hemorrhagic cystitis. *J Urol* 1966; 96: 313-316.
- [26] Stillwell TJ and Benson RC Jr. Cyclophosphamide-induced hemorrhagic cystitis. A review of 100 patients. *Cancer* 1988; 61: 451-457.
- [27] Knight A, Askling J, Granath F, Sparen P and Ekblom A. Urinary bladder cancer in Wegener's granulomatosis: risks and relation to cyclophosphamide. *Ann Rheum Dis* 2004; 63: 1307-1311.
- [28] Talar-Williams C, Hijazi YM, Walther MM, Linehan WM, Hallahan CW, Lubensky I, Kerr GS, Hoffman GS, Fauci AS, Sneller MC. Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. *Ann Intern Med* 1996; 124: 477-484.
- [29] Kimura M, Tomita Y, Morishita H, Takahashi K. Presence of mucosal change in the urinary bladder in nonhematuric patients with long-term exposure and/or accumulating high-dose cyclophosphamide. Possible significance of follow-up cystoscopy on preventing development of cyclophosphamide-induced hemorrhagic cystitis. *Urol Int* 1998; 61: 8-11.
- [30] Mok CC, Ho CT, Chan KW, Lau CS and Wong RW. Outcome and prognostic indicators of diffuse proliferative lupus glomerulonephritis treated with sequential oral cyclophosphamide and azathioprine. *Arthritis Rheum* 2002; 46: 1003-1013.