An Approach for Decontamination of β-Lactam Antibiotic Residues or Contaminants in the Pharmaceutical Manufacturing Environment

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An effective procedure for decontamination of β-lactam antibiotic residues or contaminants in the pharmaceutical manufacturing environment was investigated. Decontamination with solutions of hydrochloric acid, sodium hydroxide, hydrogen peroxide and hydroxylamine as agents for degradation was assessed. According to the results, the β-lactam antibiotics were significantly degraded with sodium hydroxide and hydroxylamine. From the structural analysis of the degradation products of a cephem antibiotic, cefpodoxime proxetil, it was found that hydroxylamine degraded the β-lactam structure under mild conditions, while sodium hydroxide did not. Therefore, hydroxylamine was considered an appropriate decontamination agent for β-lactam antibiotics.

Key words β-lactam antibiotic; decontamination; hydroxylamine; contamination; degradation

Since contamination of highly potent or sensitizing pharmaceuticals may cause serious unexpected effects,[1,2] cross contamination is considered to be one of the most important issues in pharmaceutical manufacturing. β-Lactam antibiotics, as typified by penicillins and cephalosporins, have been known to cause serious anaphylaxis in rare cases,[3—6] therefore, pharmaceuticals contaminated with β-lactam antibiotics have a potential risk of induction of unexpected anaphylactic shock in some patients.

The regulatory authorities require pharmaceutical manufacturers to prevent cross contamination during the manufacturing process.[7—9] The residues of pharmaceuticals should be monitored with a validated analytical method and verified to be below a certain level of residue. General criteria for pharmaceutical residues have been proposed,[10—13] however, the criteria are dependent on the potency of each pharmaceutical. The U.S. Food and Drug Administration requires detection of β-lactam antibiotic penicillin G residues in other pharmaceuticals at the level of 0.03 ppm[14] at least.

An appropriate cleaning procedure at pharmaceutical manufacturing facilities is essential for preventing contamination. Detergents are generally used to achieve sufficient cleaning efficacy. However, while such a cleaning procedure is adequate for most pharmaceuticals, it is inadequate for some compounds. Regarding β-lactam antibiotics, residues in pharmaceutical manufacturing facilities should be reduced to as low a level as possible due to their sensitivity, as mentioned above. Thus, an effective cleaning procedure is necessary for β-lactam antibiotics in the pharmaceutical manufacturing facility.

Since the anaphylaxis of β-lactam antibiotics is related to the β-lactam structure, degradation of the β-lactam structure during the cleaning or decontamination process is considered to be an effective method for minimizing residues and contaminants. In this study, therefore, a method for the degradation of β-lactam antibiotics was investigated to develop an effective cleaning/decontamination procedure. The β-lactam antibiotic structure is known to degrade by hydrolysis in alkaline and acid solutions.[15] Hydroxylamine is also known to degrade the β-lactam structure by reacting with the β-lactam carbonyl carbon.[16] Oxidation of pharmaceuticals by peroxides is another well known degradation mechanism.[17] Hydrogen peroxide is used for degradation study in the development of pharmaceuticals in general. Therefore, the solutions of hydrochloric acid, sodium hydroxide, hydroxylamine and hydrogen peroxide at appropriate concentrations for application at pharmaceutical manufacturing facilities were evaluated as degradation agents for cleaning or decontamination.

Experimental

Materials and Reagents Cefpodoxime proxetil (cephem), cefmetazole (cephamycin) and CS-834 (carbapenem) were synthesized by Sankyo Co., Ltd. (Tokyo, Japan) and penicillin G potassium (penam) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). The chemical structures are shown in Fig. 1.

The 0.1 mol/l hydrochloric acid, 0.1 mol/l sodium hydroxide, 30% hydrogen peroxide, 50% hydroxylamine solutions, and ethanol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acetonitrile of HPLC grade and acetic acid from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and in-house Milli-Q water were used. Detergent was from Lion Corp. (Tokyo, Japan).

Apparatuses An Agilent 1100 HPLC system (Agilent technologies, CA, U.S.A.) was used for assay of the β-lactam antibiotics. The system was equipped with a G1379A online degasser, a G1312A binary pump, a G1329A autosampler, a G1316A column compartment and a G1315B diode array detector. An L-column ODS, 4.6 mm i.d.×150 mm (Chemicals Evaluation and Research Institute, Tokyo, Japan) was used as an analytical col-

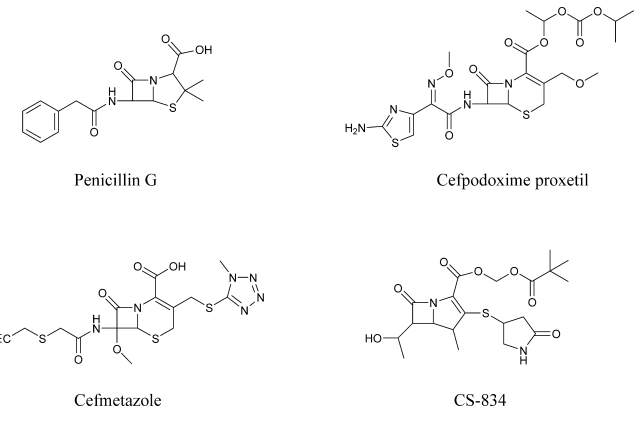

Fig. 1. Chemical Structure of β-Lactam Antibiotics

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umn. An aliquot of 10 μl of each sample solution was injected into the column maintained at a temperature of 40 °C, and chromatographed with a mixture of acetonitrile, water and acetic acid as a mobile phase. The ratio of acetonitrile and water in the mobile phase was adjusted from 150 : 850 (v/v) to 325 : 675 (v/v) so that all the chromatograph peaks were separated within a short time.

A Micromass Q-Tof 2 mass spectrometer (Micromass UK Ltd., Manchester, U.K.) was used for MS analysis. The mass spectrometer, equipped with an electrospray ionisation source, was operated in the positive ion mode. The cone voltage was set at 5 to 30 V. MS/MS was conducted by using argon as a collision gas with collision energy of 5 to 30 eV.

Preparation of β-Lactam Antibiotic Solutions The 1.0 mg/ml β-lactam antibiotic solutions were prepared by dissolving each β-lactam antibiotic in acetonitrile or a mixture of acetonitrile and water (1 : 1, v/v), respectively.

Preparation of Degradation Reagents The 0.01 mol/l hydrochloric acid and 0.01 mol/l sodium hydroxide solutions were prepared by diluting 5 ml of 0.1 mol/l hydrochloric acid and 0.1 mol/l sodium hydroxide, respectively, to a volume of 50 ml with water. The 0.1% hydrogen peroxide solution was prepared by diluting 0.33 ml of 30% hydrogen peroxide to a volume of 100 ml with water. The 1.0% hydroxylamine solution was prepared by diluting 2 ml of 50% hydroxylamine to a volume of 100 ml with water. The 0.5% and 0.1% hydroxylamine solutions were prepared by diluting 1 ml and 0.2 ml of 50% hydroxylamine to 100 ml with water, respectively. These solutions were used as the degradation agents for the β-lactam antibiotics.

To investigate the effect of pH on the degradation of β-lactam antibiotics with hydroxylamine, the pH of the hydroxylamine solution was adjusted with acetic acid or sodium hydroxide solution to 4.0, 5.0, 6.0, 7.0, 8.0 9.0 and 10.0, then diluted to a concentration of 0.1% with water. These pH-adjusted hydroxylamine solutions were used for the degradation of the β-lactam antibiotics.

Degradation of β-Lactam Antibiotics The degradation of the β-lactam antibiotics with the degradation agents was investigated by adding 9 ml of degradation agent to 1 ml of β-lactam antibiotic solution, respectively. After adding the degradation agent, the solution mixture was immediately shaken vigorously. The mixed solutions were then left standing for 5, 10 and 20 min, and then subjected to HPLC for assay.

Degradation of Solid State Cefpodoxime Proxetil To 10 mg of cefpodoxime proxetil, 50 ml of hydroxylamine solution was added and the mixture was reacted for 5, 10 and 20 min. After the degradation, 20 ml of 0.1 mol/l hydrochloric acid was added to quench the degradation reaction, the remaining cefpodoxime proxetil was dissolved by adding acetonitrile. Then the solution was diluted to 100 ml with acetonitrile. Separately, the hydroxylamine solution was diluted to a concentration of 1.0% with 10% ethanol or detergent and assessed as well. The detergent was used after diluting it with water to 1% (v/w). The resultant solutions were subjected to HPLC.

Results and Discussion

Degradation of β-Lactam Antibiotics The solutions of hydrochloric acid, sodium hydroxide, hydrogen peroxide and hydroxylamine at appropriate concentrations for application in the manufacturing facility were assessed as agents for the degradation of β-lactam antibiotic residues or contaminants in the pharmaceutical manufacturing environment. β-Lactam antibiotics were degraded with these solutions and any residues of β-lactam antibiotics were determined by HPLC. The results are summarized in Table 1.

Although penicillin G was degraded in 0.01 mol/l hydrochloric acid, other β-lactam antibiotics were relatively stable in both the 0.01 mol/l hydrochloric acid and 0.1% hydrogen peroxide solutions. On the other hand, cefpodoxime proxetil and CS-834 were completely degraded, and penicillin G and cefmetazole were also degraded in 0.01 mol/l sodium hydroxide. For the 1.0% hydroxylamine solution, all β-lactam antibiotics were degraded rapidly. According to the results, 0.01 mol/l sodium hydroxide and 1.0% hydroxylamine were considered appropriate for the degradation of β-lactam antibiotics in the pharmaceutical manufacturing environment. Regarding hydrochloric acid and hydrogen peroxide, the concentrations of solution were insufficient for degradation, thus no significant degradation occurred in this study.

Structural Analysis of Degradation Products With the degradation of cefpodoxime proxetil and penicillin G with 0.01 mol/l sodium hydroxide and 1.0% hydroxylamine, the peaks of these degradation products were detected in the chromatograms, respectively. Since the β-lactam structure is related to their anaphylaxis, it is considered that the degradation of the β-lactam structure is an effective method for the purpose of the decontamination of β-lactam antibiotics. Thus, accurate protonated molecule mass determination and MS/MS analysis were conducted to elucidate their chemical structures and confirm the degradation of the β-lactam structure. The results of the accurate mass determination of the protonated molecule and its MS/MS are summarized in Table 2.

For penicillin G, the degradation product in the sodium hydroxide solution (peak A) was elucidated as a β-lactam ring hydrolyzed compound and the one in the hydroxylamine solution (peak B) was elucidated as a hydroxamic acid compound, hydroxylamine adducted on β-lactam carbonyl carbon with cleavage of the β-lactam ring. The fragment ions observed in the MS/MS spectra were consistent with these

Table 1. Residual (%) β-Lactam Antibiotics after Reaction with Various Degradation Agents

Degradation agent	Time (min)	Residual (%)			
		Penicillin G	Cefpodoxime proxetil	Cefmetazole	CS-834
0.01 mol/l Hydrochloric acid	5	82.5	99.8	99.9	99.6
	10	74.7	100.1	100.6	98.8
	20	60.5	100.5	99.7	98.0
0.01 mol/l Sodium hydroxide	5	78.9	5.9	86.8	0.0
	10	70.3	0.0	75.3	0.0
	20	54.5	—	60.3	0.0
0.1% Hydrogen peroxide	5	96.1	99.6	97.6	96.0
	10	94.0	99.4	99.6	93.6
	20	91.2	99.5	98.6	88.4
1.0% Hydroxylamine	5	0.0	0.0	41.4	0.0
	10	—	—	18.9	—
	20	—	—	2.3	—

—: Not tested.

Table 2. Result of Accurate Ion Mass Determination and Its MS/MS

	Protonated molecule $[M+H]^+$ (m/z)		Major fragment ion (m/z)
	Observed	Calculated	
Penicillin G	335.1078	335.1066	160.0, 176.1
Peak A	353.1136	353.1171	128.1, 160.0, 174.1, 309.1
Peak B	368.1302	368.1280	160.0, 176.1, 217.1, 335.1
Cefpodoxime proxetil	558.1323	558.1328	241.0, 382.1, 410.1, 428.1
Peak C	428.0703	428.0699	210.0, 241.0, 285.0, 324.1
Peak D	428.0697	428.0699	152.0, 241.0, 396.0
Peak E	591.1514	591.1543	413.3, 559.1

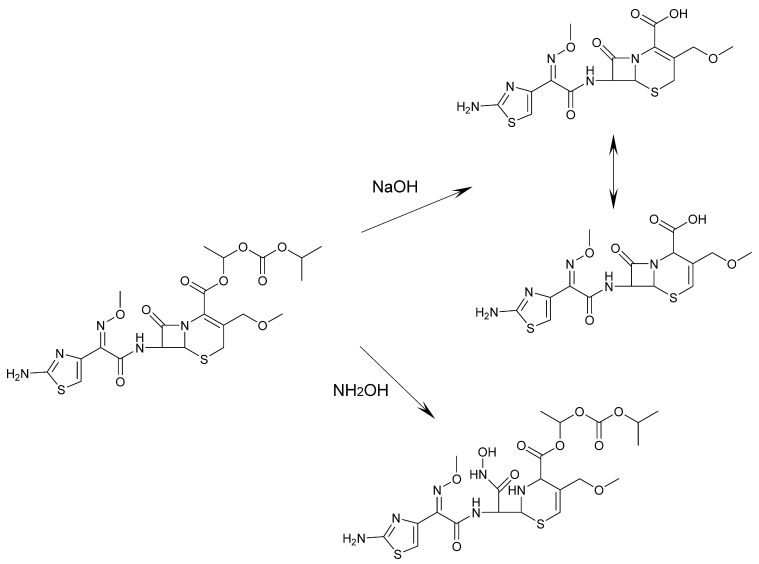

Fig. 2. Degradation Pathway of Cefpodoxime Proxetil

structures.

Regarding cefpodoxime proxetil, the major degradation products in the sodium hydroxide solution (peaks C and D) were indicated to be an ester hydrolyzed compound and the one in the hydroxylamine solution (peak E) was indicated to be a hydroxamic acid from their accurate protonated molecule mass. A fragment ion observed at m/z 241 for both peaks C and D indicated the presence of the β-lactam structure while no m/z 241 ion was observed for peak E. Instead of that ion, the fragment ions at m/z 413 and 559 observed for peak E indicated the presence of the ester side chain. Furthermore, the fragment ions at m/z 413 for peak D and m/z 559 for peak E, and changes in the UV spectrum pattern obtained by the diode array detector indicated that the double bond in the cephalosporin structure was isomerized from Δ^3 to Δ^2. The elucidated chemical structures and a possible degradation pathway are shown in Fig. 2.

From these results, it was found that the β-lactam structure was degraded by hydroxylamine in both penicillin G and cefpodoxime proxetil, whereas only ester hydrolysis occurred in the sodium hydroxide solution for cefpodoxime proxetil. Therefore, hydroxylamine is considered to be a suitable agent for the degradation of β-lactam antibiotics.

Effect of pH on Degradation of β-Lactam Antibiotics with Hydroxylamine Since the degradation of β-lactam antibiotics with hydroxylamine is nucleophilic addition of hydroxylamine on the β-lactam carbonyl carbon, the pH of the medium is considered to affect the nucleophilic reaction. Thus, the effect of pH on the degradation was investigated with cefpodoxime proxetil and by adjusting the pH of 0.1% hydroxylamine solution to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. According to the results, the degradation progressed at pH beyond 7.0, while it only slightly progressed at pH 4.0 and 5.0. The residue of cefpodoxime proxetil was 99.5% at pH 4.0, and 65.8% at pH 7.0 and 40.3% at pH 10.0. The degradation also progressed with pH-unadjusted hydroxylamine, the residue of cefpodoxime proxetil being 53.0% after the degradation. Since the pH of the hydroxylamine solution was 9.2, the nucleophilicity of hydroxylamine was maintained and had reacted with the β-lactam carbonyl carbon.

Effect of Concentration of Hydroxylamine Solution The effect of the concentration of hydroxylamine solution on the degradation of β-lactam antibiotics was investigated with cefpodoxime proxetil. The degradation of cefpodoxime proxetil was performed with hydroxylamine solution of concentrations of 0.1, 0.5 or 1.0%. The residue was 69.9% after 5 min, 56.4% after 10 min and 39.3% after 20 min for the 0.1% hydroxylamine solution, 0.7% for the 0.5% hydroxylamine solution and 0.0% for the 1.0% hydroxylamine after 5 min. From these results, a concentration of 0.5% would be necessary for the degradation of β-lactam antibiotics.

Degradation of Solid State Cefpodoxime Proxetil In pharmaceutical manufacturing environments, residues or

Table 3. Degradation of Solid State Cefpodoxime Proxetil with Hydroxylamine Solution

Concentration	Time (min)	Residual (%)
0.1% Hydroxylamine	20	74.8
0.5% Hydroxylamine	20	36.0
1.0% Hydroxylamine	5	16.4
	10	8.8
	20	3.5
1.0% Hydroxylamine/10% ethanol	10	6.6
1.0% Hydroxylamine/detergent	10	1.9

contaminants of pharmaceuticals are present in solid state. Therefore, the reactivity of hydroxylamine with a solid state β-lactam antibiotic was investigated. To confirm the reactivity with solid state, water insoluble cefpodoxime proxetil was used. Cefpodoxime proxetil was degraded with concentrations of 0.1, 0.5 and 1.0% hydroxylamine solution. As shown in Table 3, the degradation proceeded even in solid state, and was further advanced by the addition of ethanol or detergent. This degradation was considered to occur on the surface of fine particles of cefpodoxime proxetil. With the degradation of cefpodoxime proxetil, the soluble degradation product formed on the surface, and then further degradation took place. Hence the degradation would be stimulated by adding the surfactants ethanol or detergent. Consequently, it is expected that hydroxylamine would be effective for the degradation of antibiotic residues or contaminants in actual pharmaceutical manufacturing environments, and the combined usage of ethanol or detergent as surfactants would give favorable results for insoluble β-lactam antibiotics.

Conclusion

The solutions of hydrochloric acid, sodium hydroxide, hydrogen peroxide and hydroxylamine were assessed as agents for degradation of β-lactam antibiotic residues or contaminants in the pharmaceutical manufacturing environment. According to the results, the β-lactam antibiotics were significantly degraded with sodium hydroxide and hydroxylamine. However, from the structural analysis of the degradation products of cefpodoxime proxetil, it was found that only hydroxylamine degraded the β-lactam structure itself while sodium hydroxide only hydrolyzed the ester side chain. Therefore, hydroxylamine was considered as an appropriate agent for decontamination of β-lactam antibiotics. The effects of pH and concentration of hydroxylamine solution on the degradation were studied, and the degradation of solid state cefpodoxime proxetil was also investigated. These results indicate the potential of hydroxylamine as an agent for decontamination of β-lactam antibiotics in the pharmaceutical manufacturing environment.

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