[Chem. Pharm. Bull. 36(9)3692—3695(1988)]

Reversible Azomethine Bond Cleavage of Guanabenz in Acidic Solutions at Body Temperature

NOBUO INOTSUME and MASAHIRO NAKANO*

Department of Pharmaceutical Services, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto 860, Japan

(Received March 2, 1988)

A reversible hydrolytic reaction of guanabenz in acidic solutions at body temperature was studied by both spectrophotometric and high performance liquid chromatography (HPLC) methods. The cleavage reaction of guanabenz took place at the azomethine bond at 37 °C, and 2,6-dichlorobenzaldehyde and 1-aminoguanidine were produced in equilibrium with guanabenz. These degradation products of guanabenz, once formed in acidic solutions, do not revert to the parent drug when the pH value of the medium is raised to a neutral pH region.

Keywords——guanabenz; degradation; hydrolysis; reversible reaction

Guanabenz, (*E*)-[2,6-dichlorobenzylidene)amino]guanidine, is an α_2-adrenergic agonist that is safe and effective for treatment of mild to moderately severe essential hypertension.[1,2] Reversible ring-opening reactions of various benzodiazepines,[3-5] nitrofurantoin,[6] and dantrolene[7] at the azomethine bond have been examined in acidic media at body temperature. Such reactions of acid-labile drugs in a simulated gastric fluid may cause errors in the measurement of dissolution rates in acidic media due to chemical degradation of the drugs. They may also influence absorption of the intact drug from the gastrointestinal tract following oral administration of guanabenz. The present report describes kinetic studies on the hydrolysis of guanabenz in acidic media at body temperature.

Experimental

Materials——Guanabenz acetate (lot FAT 016) was generously supplied by Nippon Shoji Co., Osaka, Japan. 2,6-Dichlorobenzaldehyde and 1-aminoguanidine bicarbonate were purchased from Tokyo Chemical Industry Co., Tokyo, Japan. Organic solvents were distilled before use. Other chemicals were of reagent grade and were purchased from Wako Pure Chemical Industries, Osaka, Japan.

Determination of Degradation Products of Guanabenz in Acidic Media——After incubation of guanabenz in 0.1 N HCl at 37 °C for 10 h, the resultant solution was extracted with chloroform and the extract was subjected to thin layer chromatography (TLC) on SiO_2 using chloroform: *n*-hexane (1 : 1) as the developing solvent; spots were visualized under ultraviolet (UV) light. Each spot was scraped off and eluted with chloroform. The gas chromatography-mass spectrometric (GC-MS, QP-1000 GC-MS system, Shimadzu Manufacturing Co., Osaka, Japan) analysis of the extract was performed after evaporation of chloroform. The GC part was equipped with a 1 m × 3 mm i.d. glass column of 3% OV-17 on Gaschrom Q. The flow rate of helium was 60 ml/min. The temperatures of the injection port, column, the transfer line to the MS system, and the ion source of the MS system were 200, 135, 250, and 250 °C, respectively. Mass spectra were obtained in an electron impact mode at the electron energy of 70 eV.

In the high performance liquid chromatography (HPLC) method for the determination of guanabenz starting from guanabenz (1.40×10^{-4} M) or from an equimolar mixture (1.40×10^{-4} M) of 2,6-dichlorobenzaldehyde and 1-aminoguanidine in 0.1 N HCl at 37 °C, aliquots of incubated test solutions were sampled at predetermined times and directly injected into an HPLC system (LC-4A, Shimadzu) equipped with a detector set at 272 nm. A 250 mm column of Zorbax C_8 (Shimadzu-Du Pont, Kyoto, Japan) was used at the column temperature of 50 °C, with a mobile phase of acetonitrile: 0.01 M phosphate buffer, pH 2.0 (80 : 20, the lower limit of pH for the column employed), at the flow rate of 1.0 ml/min. The concentration of guanabenz remaining or that formed in the test sample was calculated from the peak area on HPLC chromatograms.

After equimolar amounts of the two solutions were mixed in the synthetic reaction starting from 2,6-dichlorobenzaldehyde and 1-aminoguanidine, the reaction mixture was immediately incubated and a series of UV spectra and HPLC assays were carried out at predetermined time intervals. The nuclear magnetic resonance (NMR) (JEOL FX-200, Tokyo, Japan) spectral changes of guanabenz with time in 0.1 N DCl at 38 °C were also recorded.

Results and Discussion

The Nature of the Reaction

The spectral change of guanabenz in 0.1 N HCl at 37 °C is shown in Fig. 1A, while that due to reaction of 2,6-dichlorobenzaldehyde and 1-aminoguanidine is shown in Fig. 1B. The reactions starting from guanabenz in 0.1 N HCl at 37 °C reached equilibrium in about 3 d. The spectrum of an equimolar mixture of 2,6-dichlorobenzaldehyde and 1-aminoguanidine in 0.1 N HCl at 37 °C at time infinity was quite similar to that starting from guanabenz in 0.1 N HCl at 37 °C at time infinity, indicating a possible reversible nature of the reaction. Little spectral change was observed in a solution of guanabenz or a solution of equimolar 2,6-dichlorobenzaldehyde and 1-aminoguanidine at pH 7.4 at 37 °C within a 400 min period (not shown). Thus, the degradation products, once formed in acidic solutions, are not expected to revert to guanabenz when the pH value of the medium is raised to a neutral pH region. This behavior (irreversibility) is different from that of various benzodiazepines[3-5] and nitrofurantoin,[6] since these drugs easily revert to the parent drugs when the pH values of the media are raised to a neutral pH region.[3-6]

Structural Assignment of Degradation Products Formed from Guanabenz

One major spot at *Rt* 0.59 and three minor spots at *Rf* 0.20, 0.34, and 0.83 were observed in TLC of a solution of guanabenz incubated in 0.1 N HCl at 37 °C for 10 h, indicating that the reaction of guanabenz under these conditions takes place with some concurrent side reactions. The compound of *Rf* 0.59 was eluted from the GC-MS system at a retention time of 1.9 min and its mass spectrum was identical to that of authentic 2,6-dichlorobenzaldehyde as shown in

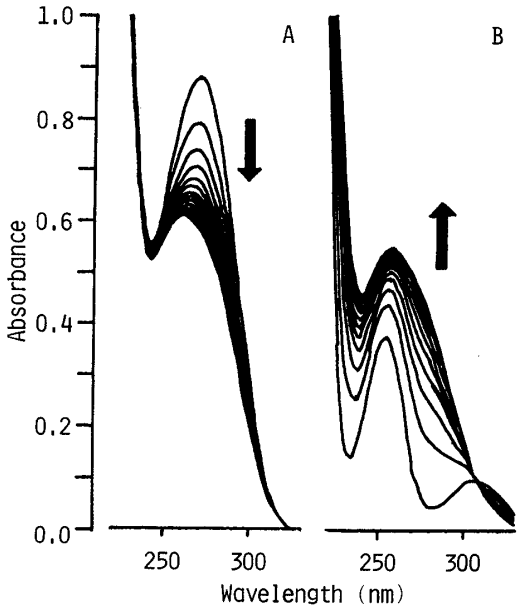

Fig. 1. Spectral Changes Starting from Guanabenz (A) and from an Equimolar Mixture of 2,6-Dichlorobenzaldehyde and 1-Aminoguanidine (B) in 0.1 N HCl at 37 °C

The scan interval was set at 100 min. Absorbance at 272 nm in A decreased with time, while that at 258 nm in B increased with time.

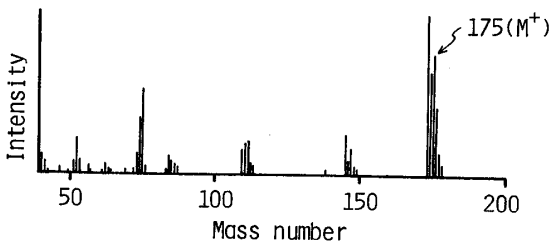

Fig. 2. Mass Spectrum of the Degradation Product of Guanabenz Extracted after Incubation of Guanabenz in 0.1 N HCl at 37 °C for 10 h

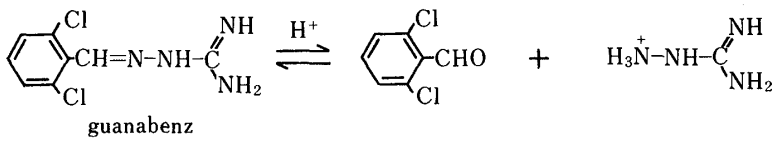

Chart 1. Reversible Azomethine Bond Cleavage of Guanabenz in Acidic Media

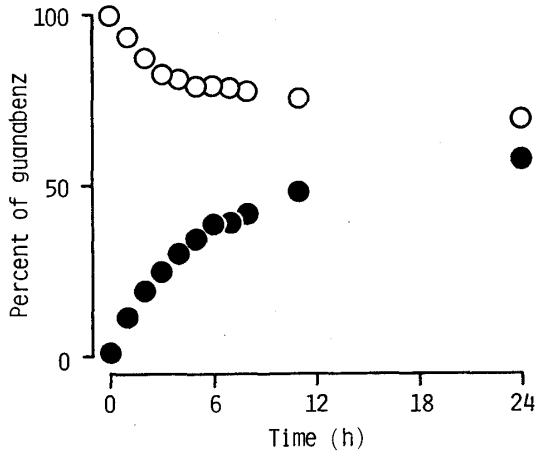

Fig. 3. Percent of Guanabenz Remaining Starting from Guanabenz (○) and from an Equimolar Mixture of 2,6-Dichlorobenzaldehyde and 1-Aminoguanidine (●), in 0.1 N HCl at 37 °C

Fig. 2. Other minor spots at *Rf* values of 0.20, 0.34, and 0.83 could not be identified because only trace amounts could be obtained.

In the HPLC experiment, the peak area of guanabenz at the retention time of 3.2 min decreased with time when guanabenz was incubated in 0.1 N HCl at 37 °C. When an equimolar mixture of 2,6-dichlorobenzaldehyde and 1-aminoguanidine was incubated in 0.1 N HCl at 37 °C, the peak at the retention time of 3.2 min appeared and its area increased with time. In the NMR experiment, the aldehyde proton at 10.4 ppm was not observed at time zero when quanabenz was incubated in 0.1 N DCl at 38 °C, but it subsequently appeared and its intensity increased with time (not shown).

Therefore, quanabenz is considerted to degrade reversibly to 2,6-dichlorobenzaldehyde and 1-aminoguanidine as shown in Chart 1. Soda *et al.*[8] reported on the epimerization of guanabenz by light to give the *Z*-epimer of guanabenz, but little information about the physicochemical characteristics of the *Z*-epimer of guanabenz was obtained and possible intermediate(s) of the reaction in 0.1 N HCl at 37 °C could not be identified.

Quantitative Aspects

The time course of intact guanabenz in 0.1 N HCl at 37 °C starting from guanabenz or from equimolar 2,6-dichlorobenzaldehyde and 1-aminoguanidine, determined by HPLC, is shown in Fig. 3. The reverse reaction to form the parent drug may take place within the HPLC system because the pH value of the eluate for HPLC was adjusted to pH 2.0 and pH dependence of the degradation reaction of the azomethine bond of guanabenz is considered to be similar to that of benzodiazepines, as reported previously.[4] The decrease in guanabenz with time starting from guanabenz at 37 °C and the increase in guanabenz with time starting from 2,6-dichlorobenzaldehyde and 1-aminoguanidine were observed at 37 °C. An approach to and eventual attainment of equilibrium are apparent.

General Discussion

Guanabenz is reversibly hydrolyzed at the azomethine bond to 2,6-dichlorobenzaldehyde and 1-aminoguanidine in an acidic solution which simulates human gastric fluid, in the same way as some other drugs.[3-6] Guanabenz once degraded in an acidic solution, however, is not

expected to be resynthesized from the degradation products when the pH value of the medium is increased to a neutral pH value which simulates human intestinal fluid. Thus, the reaction reported in this study is expected to affect the bioavailability of orally administered guanabenz.

Acknowledgement The authors are grateful to Prof. Morino, Kumamoto University Medical School, for his cooperation in NMR measurements.

References

1) P. Bonsanac, J. Dubb, and B. Walker, *J. Clin. Pharmacol.*, **16**, 631 (1976).
2) Y. Misu, K. Fujie, and T. Kubo, *Eur. J. Pharmacol.*, **77**, 177 (1982).
3) A. Walser, L. E. Benjamin Sr., T. Flynn, C. Mason, and R. Schwartz, *J. Org. Chem.*, **43**, 936 (1978).
4) M. Nakano, N. Inotsume, N. Kohri, and T. Arita, *Int. J. Pharmaceut.*, **3**, 195 (1979).
5) M. J. Cho, T. A. Scahill, and J. B. Hester Jr., *J. Pharm. Sci.*, **72**, 356 (1983).
6) N. Inotsume and M. Nakano, *Int. J. Pharmaceut.*, **8**, 111 (1980).
7) N. Inotsume and M. Nakano, *Int. J. Pharmaceut.*, **17**, 357 (1983).
8) Y. Soda, K. Kashima, M. Okuhira, N. Ito, T. Nakajima, H. Tatsumi, S. Uetai, and S. Igarashi, *Iyakuhin Kenkyu*, **16**, 93 (1985).