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Report

Complex Formation Between Metronidazole and Sodium Urate: Effect on Photodegradation of Metronidazole

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Photodegradation of solutions of metronidazole in the presence and absence of sodium urate was studied. Photodegradation appeared to follow zero-order kinetics and was found to be dependent on the pH, buffer species, sodium urate concentration, and light source. Complex formation between metronidazole and sodium urate accounted for the photostabilization of metronidazole. The dissociation constant for this complex was calculated to be 3.4×10^{-3} *M*.

KEY WORDS: photodegradation; metronidazole; sodium urate; complex formation.

INTRODUCTION

Metronidazole is a light-sensitive, commonly used antifungal drug available in solution for injection, oral and vaginal tablets, and suppositories (1). It is also a hypoxic sensitizer that can be used as an adjunct to radiotherapy in patients with cancer (2). A survey of the literature has indicated that no data have been published on the photostabilization of this drug. Uric acid and its soluble salts were used by Asker and his associates (3–5) to stabilize FD&C Blue No. 2 and sulfathiazole solutions against photodegradation. Consequently, sodium urate was selected in this study to investigate its potential photostabilizing effect for metronidazole solutions.

MATERIALS AND METHODS

Chemicals

Metronidazole, sodium urate, and all the other chemicals used in this investigation were purchased from Sigma Chemical Co., St. Louis, Mo.

Exposure to Light

Solutions of metronidazole (MNZ) in the various buffers with and without sodium urate (SU) were placed in spectrophotometer tubes, covered with Parafilm, and exposed to the light source in the light-stability cabinet (Atlas HPUV from Atlas Electric Devices Co., Chicago). The cabinet provided fluorescent light, black light (UV-A), and artificial sunlight (UV-B). The light intensity was maintained at 10.5 W/m^2 for fluorescent light and 990 mW/cm^2 and 6.4 W/m^2 for black light and artificial sunlight, respectively. A number of tubes containing (MNZ) solutions with and without (SU) were covered with aluminum foil before they were exposed to fluorescent light in order to assess the effect of light exclusive of heat generated by the fluorescent light within the light-stability cabinet. Solutions of (SU) in the appropriate buffers were also exposed to the light source to serve as blanks. The analysis was done on duplicate samples and the difference between the duplicates was less than 1.4%.

Photodegradation of Solutions of Metronidazole

A solution of (MNZ) at a concentration of 9.93×10^{-5} *M* in phosphate buffer of pH 7 was exposed to artificial sunlight (UV-B) and the change in the UV spectrum was monitored as shown in Fig. 1. The effect of pH on the photodegradation of (MNZ) solutions in the presence or absence of (SU) was studied by using phosphate buffers having pH values from 6 to 10. At a pH below 6, (SU) did not dissolve completely. The effect of buffer species was investigated in phosphate (0.1 *M*), acetate (0.1 *M*), and citrate (0.05 *M*) buffers whose pH was adjusted to 7 by the addition of sodium hydroxide solution. The influence of changes in the concentration of either (MNZ) or (SU) on the photodegradation of metronidazole was studied in phosphate buffer of pH 7. The concentration range investigated was 4.97×10^{-5} to 14.90×10^{-5} *M* for (MNZ) and 2.1×10^{-3} to 10.4×10^{-3} *M* for (SU).

RESULTS AND DISCUSSION

Kinetics of Metronidazole Degradation

Photodegradation of (MNZ) solutions was expressed as the rate of change of absorbance at 320 nm. The absorbance at this wavelength involves only the intact drug. A set of UV spectra obtained during photolysis is shown in Fig. 1. This figure demonstrates the gradual decrease in absorbance at 320 nm during photolysis. The effect of (SU) on the rate of photodecomposition of (MNZ) in phosphate buffer of pH 7 is shown in Fig. 2. It is evident from this figure that the pho-

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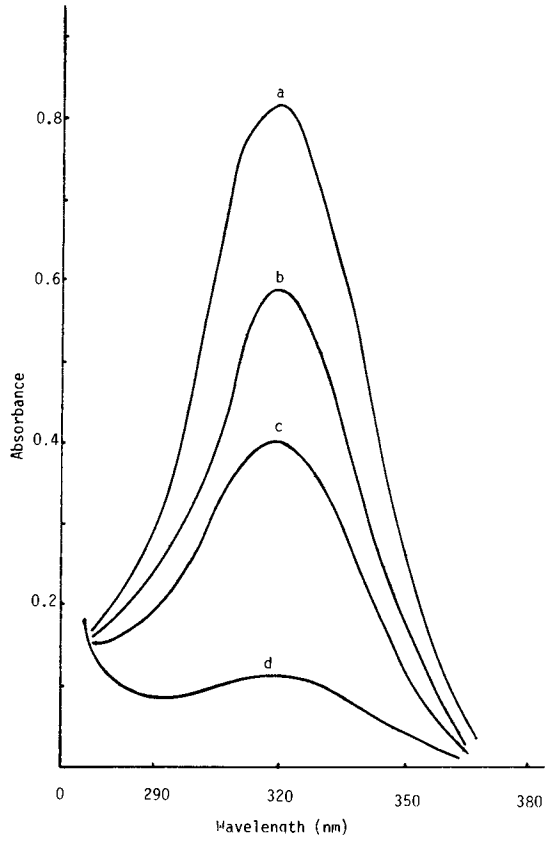

Fig. 1. Set of UV spectra obtained during photolysis of 9.93×10^{-5} *M* metronidazole in phosphate buffer of pH 7 under artificial sunlight. (a) 0 hr; (b) 4 hr; (c) 8 hr; (d) 14 hr.

todegradation of (MNZ) in the presence or absence of (SU) followed zero-order kinetics and that (SU) enhanced the photostability of (MNZ). The degradation rate constant was calculated from the slope of the line of absorbance versus time. The percentage of drug remaining was calculated from Beer's plot of concentration versus absorbance.

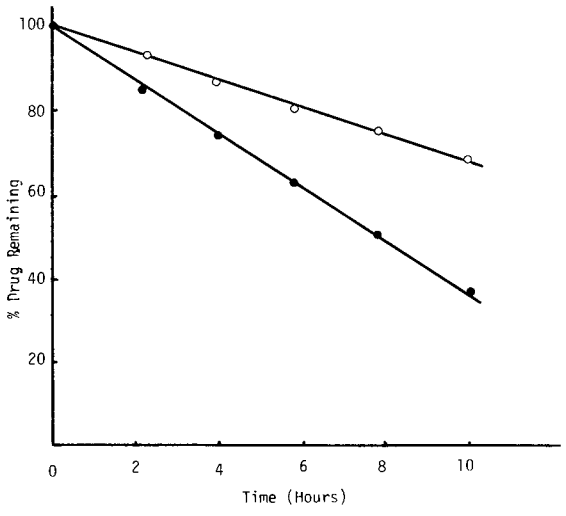

Fig. 2. Degradation of metronidazole in phosphate buffer of pH 7 under artificial sunlight. (●) Without sodium urate; (○) with 5.3×10^{-3} *M* sodium urate.

Effect of pH

Figure 3 shows that (SU) demonstrated its photostabilizing action for (MNZ) solutions in phosphate buffers having pH values from 6 to 10. As the pH of the solution increased, the stability of (MNZ) decreased in the absence of (SU). However, in the presence of (SU), the effect of pH on the reaction rate constant appeared to be significant only at pH values of 6 and 10. The effect of pH in the range of 7–9 on the rate constant remained practically unchanged. The percentage increase in the stability of (MNZ) by the addition of (SU) was found to be 20% at pH 6 and 72.5% at pH 10.

Effect of Buffer Species

Table I indicates that at pH 7, the catalytic effect of buffer species on the photodegradation of (MNZ) in the presence or absence of (SU) was citrate > acetate > phosphate. The effect of buffer species was determined irrespective of the ionic strength of the medium.

Effect of Light Source

Table II shows that fluorescent light was most detrimental to (MNZ) photostability, followed by artificial sunlight (UV-B) and then black light (UV-A). The rise in temperature obtained within the light-stability cabinet equipped with fluorescent light did not have any apparent effect on enhancing the photodegradation of (MNZ). No degradation was detected in solutions placed in the tubes that had been covered with aluminum foil and exposed to fluorescent light. (SU) exercised its photostabilizing effect for (MNZ) solutions exposed to either fluorescent or artificial sunlight but not for solutions exposed to black light.

Effect of Sodium Urate and Metronidazole Concentrations

Figure 4 shows that the photostability of (MNZ) solutions increased appreciably with increases in (SU) within the range of 2.1×10^{-3} to 5.3×10^{-3} *M*. At higher concentra-

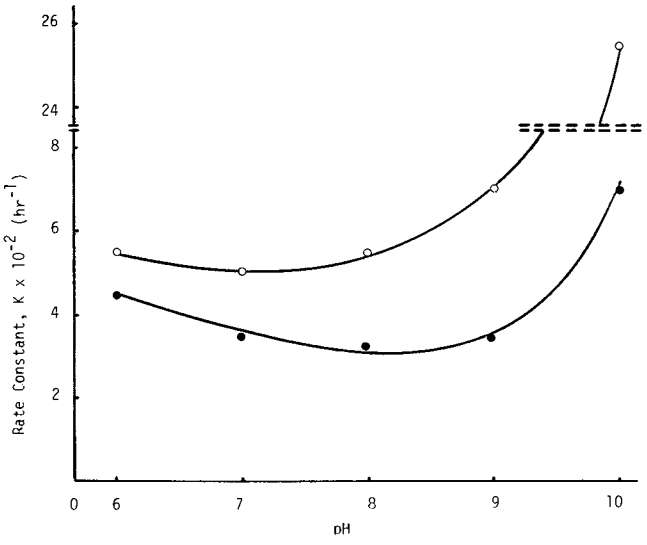

Fig. 3. pH–rate profile for the photodegradation of metronidazole in phosphate buffers under artificial sunlight. (○) Without sodium urate; (●) with 5.3×10^{-3} *M* sodium urate.

Table I. Effect of Buffer Species at pH 7.0 on the Photodegradation of Metronidazole[a] in the Presence and Absence of Sodium Urate[b]

Buffer species	Rate constant (absorbance unit) (hr^{-1}) of solutions ($\times 10^2$) Without (SU), K_c	With (SU), K_w	K_w/K_c
Phosphate	5.0	3.4	0.68
Acetate	8.3	4.3	0.52
Citrate	10.9	5.2	0.48

[a] Concentration of metronidazole, 9.93×10^{-5} *M*.
[b] Concentration of sodium urate, 5.3×10^{-3} *M*.

tions, the effect of increasing the concentration of (SU) was less pronounced. There was no apparent effect of changes in the concentration of (MNZ) on its rate of photodegradation.

Complex Formation

The presence of (SU) led to a change in the absorption spectrum of (MNZ) solutions. The 320-nm absorption peak of (MNZ) had shifted to 326 nm. This shift in peak as well as the decrease in absorbance at 320 nm by the addition of (SU) suggested a mechanism for the slowing down of photodegradation of (MNZ) through complex formation between (MNZ) and (SU).

According to Yagi and Matsuoka (6), these two phenomena indicated the formation of a complex between lactoflavin and phenol. The same criteria were found by Kowarski and Ghandi (7) to account for the complexation between menadione and cetylethylmorpholinium ethosulfate in the ground state.

The dissociation constant for the complex of (MNZ) and (SU) was calculated according to Yagi and Matsuoka (6) as follows:

$$\Delta i/\Delta s = n \quad (1)$$

where Δi is the difference between the absorbance of (MNZ) in phosphate buffer of pH 10 and in various concentrations of (SU), Δs is the difference between the absorbance of (MNZ) in the phosphate buffer and the maximal absorbance at the highest concentration of (SU), and n is the relative concentration of the complex. The absorbance was measured at 320 nm and the data are shown in Table III.

Table II. Effect of Type of Light on the Photodegradation of Metronidazole[a] in the Phosphate Buffer of pH 7.0 in the Presence and Absence of Sodium Urate[b]

Type of light	Rate constant (absorbance unit) (hr^{-1}) of solutions ($\times 10^2$) Without (SU), K_c	With (SU), K_w	K_w/K_c
Artificial sunlight	5.0	3.4	0.68
Black light	4.2	4.2	1.00
Fluorescent light	6.0	5.0	0.83

[a] Concentration of metronidazole, 9.93×10^{-5} *M*.
[b] Concentration of sodium urate, 5.3×10^{-3} *M*.

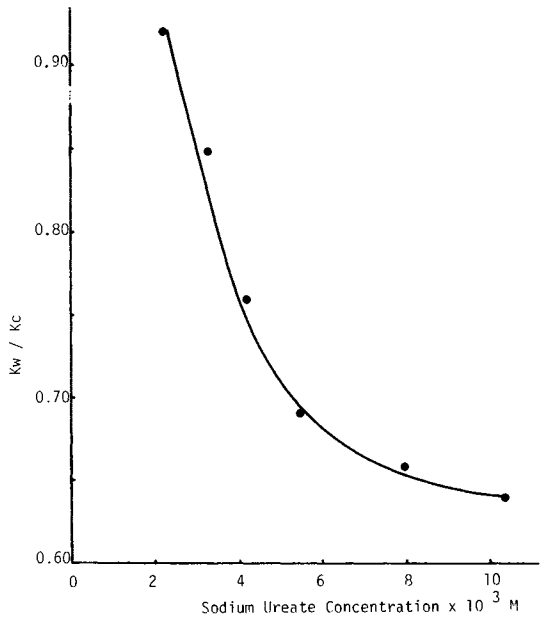

Fig. 4. Effect of sodium urate concentration on the relative photostability of metronidazole in phosphate buffer of pH 7 under artificial sunlight.

The quotient of the complex concentration (MNZ · U) and the metronidazole concentration (MNZ) is

$$\beta = \text{MNZ} \cdot \text{U}/\text{MNZ} \quad (2)$$

β is also proportional to n, and hence

$$\beta = \alpha n \quad (3)$$

where α is the proportionality constant.

Assuming that the reaction between metronidazole and sodium urate is bimolecular, the dissociation constant K is given by

$$K = [\text{MNZ}][\text{U}]/[\text{MNZ} \cdot \text{U}] \quad (4)$$

where [MNZ] and [U] are the concentrations of metronidazole and sodium urate, respectively, and [MNZ · U] is the concentration of the complex formed.

Since the concentration of metronidazole is negligible as compared to that of sodium urate, the amount of the latter bound in the ground state is also negligible compared to its total concentration. Therefore, the concentration of U = U_0, where U_0 is the initial concentration of sodium urate. $K = [(1-\beta)/\beta]\,[U_0]$ and

Table III. Relationship Between the Concentration of Sodium Urate (U_o) and the Relative Concentration of the Metronidazole–Sodium Urate Complex at pH 10.0 (Metronidazole Concentration, 9.93×10^{-5} *M*)

U_o ($M \times 10^3$)	Absorbance	Δi	$\Delta i/\Delta s = n$
0	0.836	—	—
1.05	0.780	0.056	0.459
2.10	0.760	0.076	0.623
3.20	0.745	0.091	0.746
4.20	0.730	0.106	0.869
5.30	0.714	0.122 = Δs	1.000

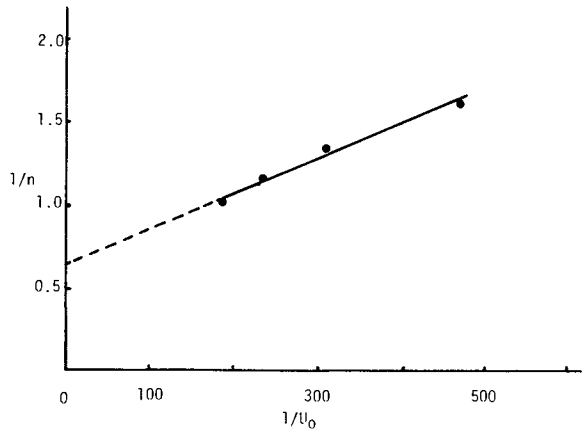

Fig. 5. Relationship between the reciprocal concentration of sodium urate, U_0, and the relative concentration of the complex, n.

$$K = \frac{1 - \alpha n}{\alpha n}[U_0] \quad (5)$$

or

$$\frac{1}{n} = \alpha K \frac{1}{U_0} + \alpha \quad (6)$$

If $1/n$ versus $1/U_0$ is plotted as shown in Fig. 5, a straight line is obtained whose slope = αK and intercept = α. Thus the equilibrium constant can be calculated. The dissociation constant was 3.4×10^{-3} M, and at a sodium urate concentration of 5.3×10^{-3} M, the concentration of the complex formed was 6.49×10^{-5}. Further studies are needed to assess the mechanism of complex formation between (MNZ) and (SU).

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