Laser Photolysis of Bilirubin

G. SBRANA,⁽¹³⁾ M. G. MIGLIORINI, C. VECCHI, AND G. P. DONZELLI

Centro di Studio sulla Chimica e la Struttura dei Composti Eterociclici del CNR, Florence, Italy [G. S.]; Istituto di Chimica Fisica, Università di Firenze, Florence, Italy [M. G. M.]; Istituto di Puericultura, Università di Firenze, Florence, Italy [C. V. and G. P. D.]

Summary

Photodegradation of bilirubin *in vitro* has been investigated by using monochromatic light supplied by an argon ion laser selecting the 457.9, 488.0 and 514.5 nm wavelengths.

Bilirubin was examined in chloroform, in aqueous solutions and in human serum under different experimental conditions of concentration, laser power and time of irradiance. Photodecomposition was followed by optical density measurements on the absorption maximum of the electronic band at 460 nm.

The rate of degradation of bilirubin was found to be only slightly affected by the wavelength of the exciting lines provided they fall within the absorption band. In particular it was shown that any wavelength, λ , is equally effective if the corresponding absorbance, A_λ , exceeds a minimum value of 5-10%. In the aqueous solutions, light with $\lambda > 470$ nm has been found to be largely effective in the photodegradation of bilirubin *in vitro*.

Speculation

The results of bilirubin degradation by wavelengths longer than 470 nm *in vitro* suggest the use of green light in the clinical procedure in order to reduce damages to the tissues which can be caused when higher frequency radiations are employed.

Phototherapy has become an effective chemical procedure for hyperbilirubinemia in newborn infants. The efficacy of light in reducing the concentration of bilirubin in the blood is well known and many studies have been performed in the last years either *in vivo* (1, 3, 7, 12) or *in vitro* (2, 6, 10). However, treatment is still largely empiric and some questions arise concerning the most effective wavelengths, the intensity of the light, the time of irradiance, the bilirubin decomposition mechanism and the long-term biological effects.

In view of its clinical importance, many authors have investigated bilirubin photodegradation by using commercial lamps emitting white (400-700 nm) or blue (400-500 nm) light. The latter has been found to be more effective, in agreement with the occurrence of an absorption maximum at 460 nm in the visible spectrum of bilirubin due to the π - π^* electronic transition.

Blue light lamps are frequently employed in the clinical procedure although a suitable setting is necessary to avoid the undesirable effects that this light causes on the nursery attendants (11). Also light emission in the UV region is probably effective but it must be avoided owing to the potential dangerous side effects.

Since the electronic absorption band of bilirubin ranges from 400-500 nm, each wavelength emitted from a source in this region should be absorbed to some extent. However a different efficiency is, in principle, expected depending on whether or not light frequencies are used which occur in the vicinity of the absorption maximum.

Recently Lightner *et al.* (5) reported a detailed study on the photodegradation using a 10 nm bandwidth light in the 250-500 nm region. These authors concluded, in agreement with the results previously reported in the literature, that the most effective wave-

lengths are in the 400-470 nm range and in the region below 340 nm.

In this paper, we report a similar investigation on bilirubin irradiated *in vitro* with monochromatic light supplied by an argon ion laser; however we have reached a different conclusion on the efficacy of the longer wavelengths. Measurements have been performed by using the three lines of the laser at 457.9, 488.0 and 514.5 nm which occur on the peak, on the side and on the wing of the electronic band, respectively. Bilirubin was examined in chloroform, in aqueous solutions and in human serum under different experimental conditions of concentration, laser power and time of irradiance.

The spontaneous degradation in the dark was also checked in order to correct, if necessary, the experimental values obtained by exposure.

MATERIALS AND METHODS

Bilirubin IX α was supplied by Carlo Erba (Milan, Italy) and used without further purification. The following solutions were irradiated: (1) bilirubin in anhydrous chloroform ($1.6 \cdot 10^{-5}$ M), (2) bilirubin in saline solution, pH 10 ($1.4 \cdot 10^{-5}$ M), (3) bilirubin dissolved in a saline solution pH 10 containing 4% of human albumin ($2.3 \cdot 10^{-5}$ M). Human albumin was supplied by Immuno (Vienna, Austria). (4) Human serum of jaundiced infants diluted with saline solution up to a bilirubin concentration of $1.9 \cdot 10^{-5}$ M.

Standard fluorescence cells (4 ml volume) were used to irradiate the solutions and the laser beam was directed into the cell from the bottom. We have used the lines at 457.9, 488.0 and 514.5 nm emitted by an argon ion laser model 52 supplied by Coherent Radiation (Palo Alto, CA). Different laser powers, which varied for each line from 50 to 200 mW, were employed. The power of the beam was continuously checked by a power meter (model 210, Coherent Radiation, Palo Alto, CA) in order to keep it constant during the irradiance.

Photodecomposition of bilirubin was followed by optical density measurements on the absorption maximum of the band at 460 nm. Ultraviolet and visible spectra have been registered by the aid of an UV spectrophotometer Model SP8-100 PYE UNICAM (Cambridge, U.K.).

RESULTS

The effect of the light on bilirubin degradation is described by the curves reported in Figures 1 through 4. Because the length of exposure depends on the intensity of each laser line, the diagrams were drawn by plotting the optical density *versus* the energy ($W \times sec$) supplied to the solutions. The optical density has been corrected to account for spontaneous decomposition of bilirubin in the dark, which has been found to be different for different solutions.

Within the time of exposure, no bilirubin decomposition occurs in either the chloroform or the human serum in the dark. Only a slight spontaneous degradation is found for the albumin solution (~5% after 5000 sec). The concentration of bilirubin in the saline

solution decreases rapidly in the dark and is reduced by ~25% after 5000 sec. These results are in accordance with the well-known stabilizing effect of proteins. All the measurements were repeated several times in order to ensure a good reproducibility.

Bilirubin photodegradation has been studied (2) using high pressure mercury and blue fluorescent lamps emitting a wide spectral range. A considerably lower efficiency was found when fluorescent light was employed.

Figure 1 shows the effect of irradiating chloroform by laser monochromatic light. It is evident that the violet (457.9 nm) and blue (488.0 nm) lines are more efficient in degrading bilirubin when compared with the green line (514.5 nm). Actually the first two provoke a rapid decline of the bilirubin levels whereas the third one is much less efficient. These results closely agree with those reported for aprotic solvents (5).

Photodegradation of bilirubin in different aqueous solutions is shown in Figures 2, 3 and 4.

All the curves in Figure 2 have been obtained by irradiating bilirubin dissolved in a pH 10 saline solution. The curves exhibit a similar pattern, although the slope decreases in going from the green to the violet line. In particular, we wish to emphasize that

Fig. 1. Effect of different wavelength radiations on bilirubin photodecomposition in chloroform solution. Initial bilirubin concentration: $1.6 \cdot 10^{-5}$ M. Key: λ_{nm} ○, 457.9; ×, 488.0; ●, 514.5.

Fig. 2. Effect of different wavelengths on bilirubin photodecomposition in saline solution, pH 10. Initial bilirubin concentration: $1.4 \cdot 10^{-5}$ M. Key: λ_{nm} ○, 457.9; ×, 488.0; ●, 514.5. The values have been corrected for spontaneous degradation.

Fig. 3. Effect of different wavelength radiations on bilirubin photodecomposition in a 4% albumin-saline solution, pH 10. Initial bilirubin concentration: $2.3 \cdot 10^{-5}$ M. Key: λ_{nm} ○, 457.9; ×, 488.0; ●, 514.5. The values have been corrected for spontaneous degradation.

Fig. 4. Effect of different wavelength radiations on bilirubin photodecomposition in the human serum. Initial bilirubin concentration: $1.9 \cdot 10^{-5}$ M. Key: λ_{nm} ○, 457.9; ×, 488.0; ●, 514.5.

the 514.5 nm wavelength is about 100% more efficient than the 457.9 nm line, at least up to a 30% drop in concentration.

Bilirubin behaviour in a pH 10 saline solution with a 4% of human albumin is shown in Figure 3. These profiles differ substantially from those of Figure 2. In particular, the different slope suggests a slower photodegradation. This is probably due to the presence of albumin which partially inhibits the photooxygenation of bilirubin (8, 9). Also, in this case, the efficiency of the three lines progressively increases from the 457.9 nm to the 514.5 nm wavelengths.

The diagram of bilirubin in the human serum (Fig. 4) differs in part from those previously discussed. Actually, the three profiles are practically coincident up to a 30% drop in absorbance. At a larger drop, they start diverging and the curves due to the 514.5 nm and 488.0 nm wavelengths show a minor slope. This means that: first, up to 30% drop in concentration, bilirubin photodegradation is independent from the frequency of the exciting line and second, after 30% of the bilirubin is decomposed, the violet line becomes 20% more efficient than the blue and green lines.

DISCUSSION

Our data show that the rate of photodegradation of bilirubin is slightly affected by the wavelength of the exciting lines provided

Fig. 5. Visible absorption spectra of bilirubin in different solutions: a, chloroform; b, saline solution, pH 10; c, saline solution with 4% of human albumin, pH 10; d, human serum. Dotted lines indicate the laser exciting frequencies used in the irradiance.

they fall within the absorption band. For instance, the green line is equally effective even in the saline solution at pH 10 where the absorption maximum is shifted from 460 to 430 nm (4).

In general, the closer the exciting frequency is to the absorption maximum, the stronger the interaction is with the vibronic levels associated to the electronic transition. This implies that the efficiency of each wavelength should be proportional to the corresponding extinction coefficient of the electronic band. In our case the results obtained demonstrate that any wavelength, λ , is equally effective if the corresponding absorbance, A_λ , exceeds a minimum value of 5–10%.

This fact accounts also for the minor efficiency of the green line in chloroform. In fact, from the inspection of the electronic spectra reported in Figure 5, it is possible to deduce that the electronic absorption at ~ 460 nm has a smaller bandwidth in chloroform than in the aqueous solutions, so that the exciting line at 514.5 nm

occurs just at the edge of the wing where the extinction coefficient is almost negligible.

A further investigation, carried out by a helium–neon laser selecting the 632.8 nm exciting line (power ~ 15 mW), showed that no bilirubin decomposition occurs under experimental conditions far from the resonance.

The conclusions reached in the present investigation differ from those reported in the literature, especially as concerns the efficiency of the wavelengths longer than 470 nm in the phototherapy.

The logical extension of this research is to verify the applicability of exposing newborn infants to green light. Because the laser beam is not suitable for phototherapy, we have undertaken some preliminary investigations *in vitro* by using narrow-spectrum green fluorescent lamps with an emission maximum at about 520 nm. The rate of bilirubin photodegradation was found to be quite similar to that obtained by using the daylight fluorescent lamps usually employed in phototherapy.

Experiments are in progress in our laboratory in order to check the validity *in vivo* of the results discussed above. The use of the green lamps in the clinical procedure should offer some relevant advantages by decreasing the risk of side dangerous effects on the tissues due to higher frequency radiations.

REFERENCES AND NOTES

- Calvert, R. T., Hulshoff, A., Buice, R. G. and Kostenbauder, H. B.: Bilirubin Dynamics in the Gunn rat during phototherapy. *J. Pharm. Sci.*, **67**: 205 (1978).
- Garbagnati, E. and Manitto, P.: A new class of bilirubin derivatives obtained *in vitro* and their possible formation in jaundiced infants. *J. Pediatr.*, **83**: 109 (1973).
- Kostenbauder, H. B. and Sanvordeker, D. R.: Riboflavin enhancement of bilirubin photocatabolism *in vivo*. *Experientia*, **29**: 282 (1973).
- Lee, K. S. and Gartner, L. M.: Spectrophotometric characteristic of bilirubin. *Pediatr. Res.*, **10**: 782 (1978).
- Lightner, D. A., Wooldridge, T. A., Rodgers, S. L. and Norris, R. D.: Action spectra for bilirubin photodisappearance. *Experientia*, **36**: 380 (1980).
- McDonagh, A. F.: Thermal and photochemical reactions of bilirubin IX α . *Ann. N.Y. Acad. Sci.*, **244**: 553 (1975).
- McDonagh, A. F.: Phototherapy of neonatal jaundice. *Biochem. Soc. Trans.*, **4**: 219 (1977).
- Ostrow, J. D. and Branham, R. V.: Photodecomposition of bilirubin and biliverdin *in vitro*. *Gastroenterology*, **58**: 15 (1970).
- Pedersen, A. O., Schonheyder, F. and Brodersen, R.: Photooxidation of human serum albumin and its complex with bilirubin. *Eur. J. Biochem.*, **72**: 213 (1977).
- Sanvordeker, D. R. and Kostenbauder, H. B.: Mechanism for riboflavin enhancement of bilirubin photodecomposition *in vitro*. *J. Pharm. Sci.*, **63**: 404 (1974).
- Sisson, T. R. C. and Kendall, N.: Avoidance of undesirable effects of blue light in phototherapy. *J. Pediatr.*, **82**: 163 (1973).
- Vogl, T. P., Cheskin, H., Blumenfeld, T. A., Speck, W. T. and Koenigsberger, M. R.: Effect of intermittent phototherapy on bilirubin dynamics in Gunn rats. *Pediatr. Res.*, **11**: 1021 (1977).
- Requests for reprints should be addressed to: Dr. G. Sbrana, Istituto di Chimica Organica, via G. Capponi 9, 50121 Firenze, Italy.
- This research was supported by the Italian Consiglio Nazionale della Ricerche.
- Received for publication February 2, 1981.
- Accepted for publication June 15, 1981.