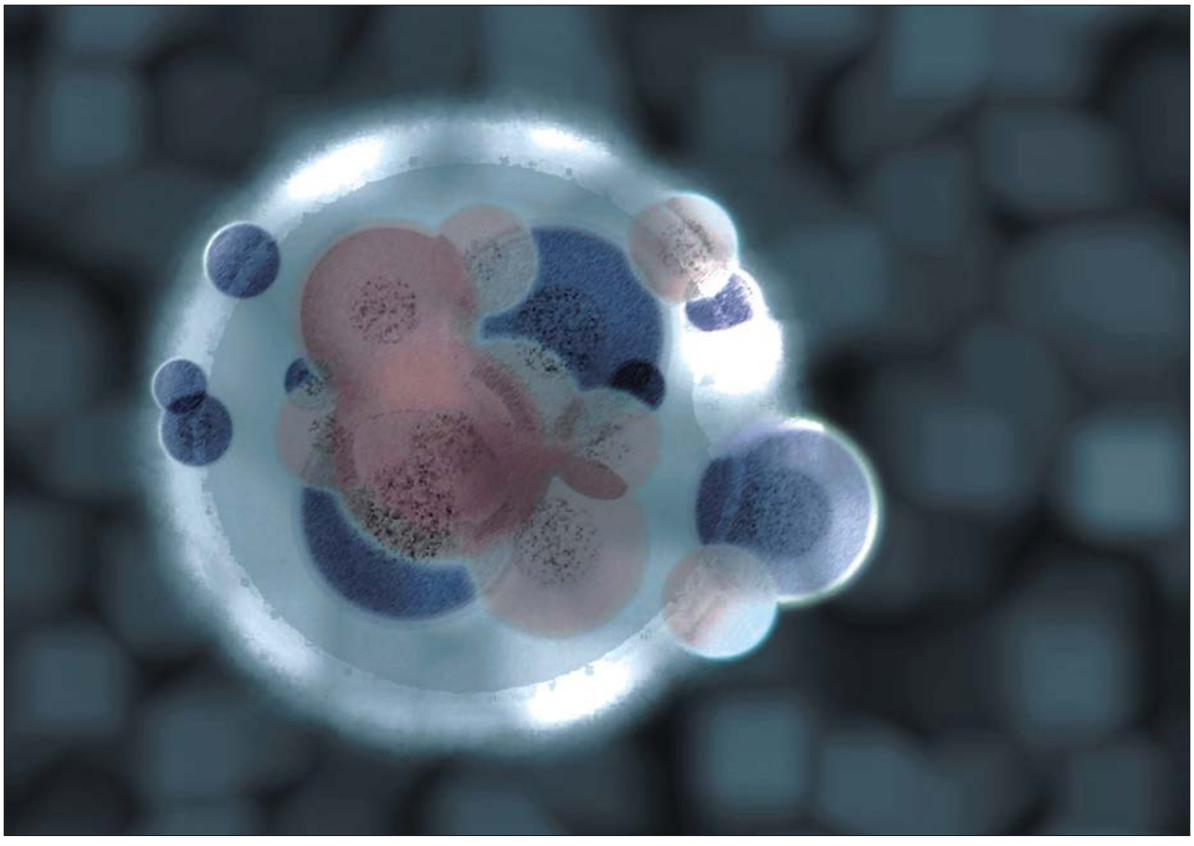

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Formation of Lumirubin During Light Therapy in Adults

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Abstract: The action of light-therapy in infants with hyperbilirubinaemia has been intensively investigated, the conversion of bilirubin to the water-soluble isomer cyclobilirubin (lumirubin) has been described in infants and in a 15 year old girl with Crigler Najjar syndrome. The purpose of present study was to see if lumirubin is formed during light therapy also in adults. While control measurements exhibited a steady decrease of lumirubin fluorescence, there was a steady increase of fluorescence during light therapy. Urine samples taken during light therapy at 10 min. intervals showed a highly significant ($p<0.01$) increase of 415 nm fluorescence when excited at 315 nm, compared to controls under dark conditions. Bilirubin fluorescence in plasma during light therapy showed a significant decrease after 50 min. These results show that lumirubin is formed and excreted during light therapy also in adults. In the last section possible implications of these findings for the action of light on biological rhythms and mood are discussed.

Key words: Bilirubin, lumirubin, light therapy

INTRODUCTION

For many years light-therapy in infants with hyperbilirubinaemia has been a well established therapy[1,2].Numerous papers have shown that bilirubin is converted to more water-soluble photoproducts like lumirubin ZE- and ZZ-bilirubin and then eliminated by the kidneys[3]. According to Agati[4] the main photoproduct is lumirubin, which is eliminated rapidly. Formation of Lumirubin has also been reported for a 15 year old girl with Crigler Najjar syndrome[5]. No such report exists for light therapy in adults. This matter is all the more interesting as the mechanism of the action of light therapy in affecting biological rhythms, suppressing melatonin, and in affecting mood and energy has not yet been resolved. The common hypothesis that light-therapy acts on rods and cones and stimulates the suprachiasmatic nucleus to suppress epiphyseal melatonin formation has come under criticism. Mice with genetically degenerate rods and cones show light synchronized circadian rhythms[6]. The blind mole with its rudimentary eyes shows light synchronised cycles[7]. In 1995, Oren published his humoral phototransduction hypothesis as complementary explanation for the action of light on mood and cicadian rhythms. The good vascularisation of the retina would provide an intense illumination of the blood during light therapy and generate a signal of light that could be transported along the blood circulation and influence mood and circadian rhythms. As possible candidates for this signal of light he names bilirubin, biliverdin and melatonin. Especially bilirubin seems a good candidate to mediate mood effects[8]. The hypothesis of humoral phototransduction gained strong attention in 1998 from Campbell´s investigation in which he successfully shifted the circadian rhythm of students by applying light to the back of their knees. Others have failed to replicate this study[9,10]. Latest studies show that bilirubin metabolism is altered during the winter seasonal depression and normalized through light therapy[11] and that the suppression of melatonin release reaches a maximum at a wavelength of 464 nm[12], which coincides fairly well with the wavelength at which bilirubin is transformed into its photoderivatives[13]. These recent developments stimulated us to study the behaviour of bilirubin during light therapy in adults.

MATERIALS AND METHODS

Lumirubin: In experiment 1, five healthy individuals aged 37-74 year (mean 47) were randomly assigned to two groups, either starting with the illumination experiment followed by the control condition, or in the reverse order. After a dark period of one hour the first urine sample was taken, then the persons wearing a rissole were illuminated

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with a light therapy lamp at 40 cm distance and were instructed to look into the light once a min. Urine specimens were taken at baseline, 30 and 60 min. Control experiments were done under dark chamber illumination. Spectroscopic analysis of the urine samples was performed after thawing and dilution of 50 µl to 3 ml in a Hitachi P-4500 Fluorescence Spectrophotometer. In accordance with[17] we used the excitation wavelength of 315 nm and recorded the emission from 380 to 480 nm for lumirubin determination. The typical fluorescence peak of lumirubin around 415 nm (410-420 nm) was used for evaluation.

In experiment 2, urine samples were collected from a 45 year old healthy individual by use of single use Nelaton-catheters (Easy Cath 7 Coloplast) at 10 min. intervals and deep frozen at -20°C in 17 illumination, and 13 control experiments. Analysis of the lumirubin fluorescence were performed as in experiment 1.

Bilirubin: In experiment 3, 45 year old healthy volunteer and performed spectroscopic measurements of plasma bilirubin in seven experiments. After one hour of dark adaption, the person wearing a rissole was illuminated at 40 cm distance by a conventional light therapy lamp (Medilux 600).The person was advised to look into the lamp once a min. Blood was taken from an i.v. cannula under dark chamber illumination (the light was turned off for drawing the blood) in 10 min. intervals and centrifuged immediately in a Hettich EBA 8 centrifuge. Plasma samples were deep frozen at -20°C and stored. Analysis was performed after thawing without dilution in the Hitachi F-4500 Spectrophotometer. In good conformity with[16], excitation at 462 nm gave the typical bilirubin peak at 534 nm. For further evaluation we used the arithmetical means between 531 and 537 nm. The collected data were then used for statistical analysis.

Statistical analysis: The arithmetic mean of the pooled relative fluorescence intensities at defined points of time was converted into percent of the baseline values, and then compared using one and two-tailed, paired Student's t tests, either comparing illumination with baseline values, or the difference between illumination and control values, for statistical significance. The diagrams show the relative fluorescence intensities (RFI) in the course of time.

RESULTS

In experiment 1 five individuals aged 37-74 year (mean 47) were randomly assigned to two groups, either starting with the illumination experiment followed by the control condition, or in the reverse order. After a dark

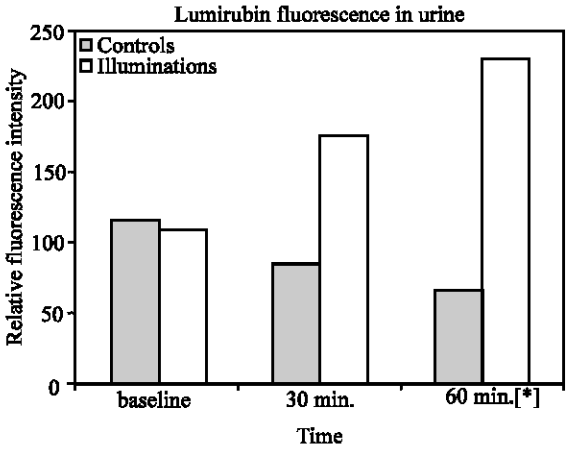

Fig. 1: Lumirubin fluorescence determined spectroscopically in five individuals by analysing 415 nm emission during excitation and control value reaches statistical significance [*] after 60 min

period of one hour the first urine sample was taken, then the persons wearing a rissole were illuminated with a light therapy lamp at 40 cm distance and instructed to look into the light once a min. Urine specimens were taken after 30 and 60 min. Under the conditions of the control experiment, performed under dark room illumination (<10 lux), and found constant decrease of the lumirubin fluorescence in urine not reaching statistical significance, gray bars in diagram 2. Illumination gave a constant increase of lumirubin fluorescence almost reaching significance at 60 min. (df=4, t=2.03>2.13), indicated by the white bars in Fig. 1. The difference between illumination and control experiments was significant after 60 min. at the 95% level (df=4, t=2.59>2.13). To get a better short time resolution of the behaviour of the lumirubin fluorescence in urine during illumination, in experiment 2 performed the same illumination protocol in a 45 year old subject, taking the urine samples at 10 min. intervals by using a catheder. In 17 experiments we saw B compared with baseline - a steady increase from 10 to 60 min. as shown by the white bars in diagram 3 reaching 95% significance after 30 min. (df=16, $t_{30\,min}$ = 2.17>1.75) and even 99%-level after 40 min. ($t_{40\,min}$ = 3.06>2.58, $t_{50\,min}$ = 3.18>2.58, $t_{60\,min}$ = 3.00>2.58). Lumirubin fluorescence in urine collected in 13 experiments in the dark (less than 10 lux) at 10 min. intervals showed B in relation to baselinevalue - a steady decrease significant at the 99% level (df=12, $t_{20\,min}$ = 3.05>2.68 $t_{30\,min}$ = 3.39>2.68) and 99.9% level ($t_{40\,min}$ = 4.67>3.93, $t_{50\,min.}$ = 4.95>3.93, $t_{60\,min}$ = 3.47>2.68). The difference of the lumirubin fluorescence of urine samples taken 40-60 min after dark

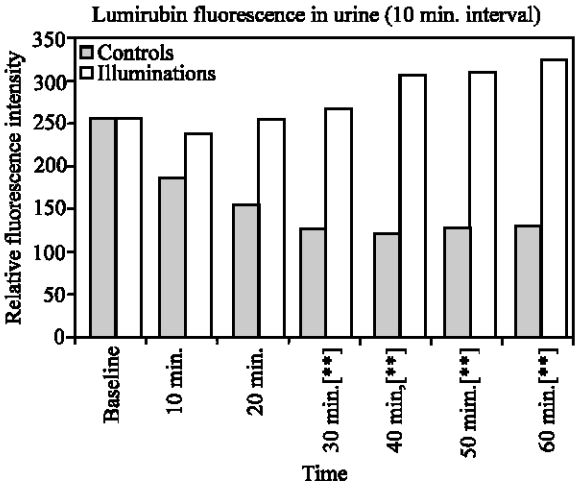

Fig. 2: Lumirubin fluorescence determined spectroscopically in repetitive measurements in the urine of a 45 year old individual shows a highly significant RFI increase during the illumination experiments and a highly significant decrease in the control group. The difference between illumination and control experiments is highly significant [**] after 30-60 min

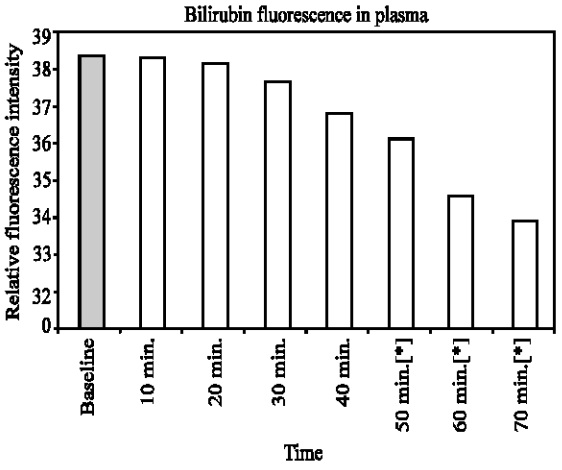

Fig. 3: Spectroscopy shows the decrease of bilirubin fluorescence determined in RFI by excitation at 462 nm and emission at 534 nm, in a 45 year old individual during 7 illumination experiments. Statistical significance is reached after 50 min. [*]. Grey bar representing preillumination values

adaption during illumination and in the dark is also very significant (df = 28, $t_{30\,min} = 2.58>2.47$, $t_{40\,min} = 3.37>2.47$, $t_{50\,min} = 3.40>2.47$, $t_{60\,min} = 3.06>2.47$) as shown in Fig. 2. In experiment 3 plasma fluorescence of bilirubin in seven measurements, during illumination with a light therapy lamp, gave a constant decrease from 10 to 70 min. (reaching statistical significance [*] after 50 min., df = 6, $t_{50\,min} = 2.40>1.94$, $t_{60\,min} = 3.12>1.94$, $t_{70\,min} = 2.71>1.94$), shown by the white bars in Fig. 3, with the grey bar giving the preillumination value.

DISCUSSION

The results clearly indicate urinary excretion of lumirubin during light therapy in adults with a steady increase of lumirubin fluorescence from 30 to 60 min. illumination. In experiment 2 the 30, 40, 50 and 60 min. values are very significant ($p<0.01$) above the corresponding values from the control experiment in the dark. In experiment 3, bilirubin fluorescence shows a constant decrease from 10-70 min. of illumination, meeting statistical significance ($p<0.05$) at 50, 60 and 70 min. These results demonstrate that bilirubin is reduced during light therapy, presumably (as in infants) by formation of the photoproducts 4Z,15Z bilirubin, 4Z,15E bilirubin, 4E,15Z bilirubin and lumirubin which is more water-soluble than bilirubin and the other photoisomers, and therefore rapidly eliminated by the kidneys. This reaction might just be a bypass of the bilirubin metabolism, but could as well have physiological functions. In some species biliverdin, the precursor of bilirubin is directly eliminated. The formation of bilirubin from biliverdin is energy consuming, it needs 2ATP to form a single molecule that is said to have no physiological function. On the other hand, there is this search for a signal of light that mediates the chronobiological and/or affective actions of light[14]. Bilirubin has already been a candidate for this role[8]. Extraocular phototransduction has been shown by Campbell[15] and most recent findings demonstrate changes of the nocturnal bilirubin-levels in seasonal affective disorder (SAD) patients, that are normalized by successful light therapy[11]. Furthermore, the action spectrum of melatonin suppression by light has its maximum near 464 nm[12] matching the wave-length 462+/-3 nm that gives optimal lumirubin formation[13] and that is used as excitation wavelength for spectroscopic bilirubin determination. All this indicates that bilirubin and its photoproducts are viable candidates for mediating physiological effects of light on circadian rhythms and mood, although more research has to be done to clarify their possible functions in these reactions.

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