Radiation Stimulates Nitric Oxide Release from Isolated Protein of Patients with End Stage Renal Failure: In Vitro Study

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Received 12/4/2011 – Accepted 17/1/2012
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الخلاصة

من المعروف ان تعرض البروتين البشري الى الاشعة فوق البنفسجية يحصل له تمسخ . وان معلومات قليلة قد اوضحت اهمية انتاج اوكسيد النتريك بطريقة غير انزيمية من مصادره داخلية المنشأ .

هدفت الدراسة لكشف تأثير التشعيع بالأشعة فوق البنفسجية، ضوء احادي اللون وضوء الشمس على انتاج تحرر اوكسيد النتريك بتفاعل غير أنزيمي من بروتين مصل الدم المعزول من مرضى الفشل الكلوي في مرحلته النهائية.

عزل بروتين مصل ١٢ من مرضى الفشل الكلوي في مرحلته النهائية بطريقة الترسيب وتم تعريضه الى الأشعة فوق البنفسجية نمط A(طول الموجي ٣٦٥ نانومتر) ، الأشعة فوق البنفسجية نمط C (طول الموجي ٢٥٤ نانومتر)، ضوء احادي اللون (مصباح صوديوم ذو طول موجي ٥٨٩.٣ نانومتر) او اشعة الشمس لمدة ساعتين. تم قياس مستويات الانواع النتروجينية بصورة اوكسيد النتريك وبيروكسي نتريت في مصل المرضى وفي مستخلص البروتين قبل وبعد التشعيع . فقد لوحظ زيادة في انتاج اوكسيد النتريك بطريقة غير انزيمية من مستخاص البروتين عند تعرضة الى الأشعة فوق البنفسجية نمط A(763.8±1586 ماكرومول) ، الأشعة فوق البنفسجية نمط C (794.6±919.3ماكرومول)، ضوء احادي اللون (794.6±919.3 ماكرومول) مقارنة بضوء الشمس (531.4±687.7).

نستنتج ان الاشعة فوق البنفسجية او الضوء احادي اللون محفز حيد لانتاج اوكسيد النتريك من البروتين المعزول من مرضى الفشل الكلوي في مرحلته النهائية وان مثل هذا الفعل قد يتسبب بتأثيرات ضارة ونافعة.

مفتاح الكلمات: اوكسيد النتريك، بروتين المصل، الفشل الكلوي

ABSTRACT

It is well known that exposure of human protein to the ultraviolet radiation resulted in denaturation. Few data explore the importance of the production of non enzymatic nitric oxide from endogenous sources. This study is aimed to explore the effect of ultraviolet radiation, monochromatic light and day light on the production of non-enzymatic nitric oxide from isolated serum protein of patients with end stage renal failure. The sera protein of 12 patients with end stage renal failure were isolated by precipitation methods and exposed to UVA (365 nm), UVC (254 nm), monochromatic light (sodium lamp; 589.3 nm) or sun light for 2hours. Nitrogen species in term of nitric oxide and peroxynitrite were determined in patients sera, and in the protein aliquots before and after irradiation. Production of non-enzymatic nitric oxide was significantly observed in protein aliquot exposed to UVA (1586±763.8 µmol), monochromatic light (1047.7±397.7 µmol) and UVC (919.3±794.6) compared to that exposed to the sun light (687.7±531.4 µmol). It concludes that ultraviolet radiation or monochromatic lighting are good stimulator of nitric oxide production from isolated sera protein of patients with end-stage renal failure. Such effect may produce harmful as well as beneficial effect.

Key words: Nitric oxide. Serum protein, Renal failure

INTRODUCTION

In biological context, the UV radiation acclaim a special mention in terms of their impact on life [1]. Ultraviolet light (UV) is electromagnetic radiation with a wavelength shorter than visible light, but longer than soft X-rays. Exposure of proteins and polypeptides to ultraviolet radiation below 240 nm (UVC) produces peptide cleavage which may or may not be observable changes in conformation and optical rotary dispersion properties depending on the experimental conditions. The changes produce by irradiation of solid bovine serum albumin containing 5% water were influenced by the presence of oxygen. Ultraviolet radiant energy has been known for decades to produce denaturation of proteins either in solution or in the dry state with the formation of secondary protein derivatives. Relative oxidation of specific proteins concomitantly occurs in UVB-irradiated human epithelial keratinocytes [2]. Uremic patients were exposed to UV radiation in several situation. During hemodialysis treatment, patients were exposed to UV radiation when the continuous on-line hemodialysis measurements is monitored by the optical dialysis adequacy sensor using wavelength 280 nm [3]. In one study, UV lamps were inserted in a complex reverse osmosis water distribution circuit to lower the bacterial and endotoxin content [4]. Such treatment kept the bacterial count below one colony forming unit per milliliter and endotoxin level below 0.125 endotoxin unit [5]. And ultraviolet-based therapy (UVB) has been used to treat pruritus in chronic renal failure [6,7]. The phototherapy was started with narrowband UVB at 400 mJ/cm^2 and increased to 1500 mJ/cm^2 for 2-3 times per week [8]. UVA radiation from 0.5 J/cm^2 to 10 J/cm^2 also improved the intensity of pruritis in patients with chronic renal insufficiency [9]. Patients with nephrogenic systemic fibrosis (skin sclerosis) associated with end-satge renal failure treated with UV-A1 phototherapy, when the kidney transplantation is not an option or is delayed, showed substantial improvement [10].

This study is aimed to explore the effect of ultraviolet (UVA and UVC) radiation, monochromatic light (sodium lamp) and day (sun) light on the production of non-enzymatic nitric oxide from isolated serum protein of patients with end stage renal failure.

MATERIALS AND METHODS

This study was done in Department of Chemistry, College of Science, Baghdad University and Department Pharmacology, College of Medicine , Al-Mustansiriya University in cooperation with Dialysis Unit at Al-Yarmouk Teaching Hospital in Baghdad, Iraq from January to June 2009. The study protocol was approved by the Scientific

Committee of the College of Medicine, Al-Mustansiriya University. An informed consent form to participate in the study was obtained from patients or their peroxy. The criteria of inclusion were that patients had established chronic renal failure of whatever cause referred to peritoneal dialysis because of symptomatic renal failure, and estimated creatinine clearance ($_eC_{cr}$) is ≤ 15 mL/min. A total number of 12 patients (2 males and 10 females) their median age was 67.3 years were enrolled in the study. Venous blood was obtained from each patient and subdivided into two samples, the first for determination of serum creatinine, serum nitric oxide, serum peroxynitrite and the second sample for isolation the protein by precipitation method. In this study, a commonly used surrogate marker for actual creatiinine clearance is the Cockcroft-Gault formula which calculate an estimated creatinine clearance [$_eC_{cr}$] which in turn estimates glomerular filtration rate [11,12]:

$$_eC_{cr} = \frac{(140\text{-age}) \times \text{mass (kg)} \times \text{constant}}{\text{serum creatinine } (\mu\text{mol/L})}$$

when constant is 1.23 for men and 1.04 for women

Determination of serum peroxynitrite

Peroxynitrite mediated nitration of phenol was measured (an index of $ONOO^-$ release) as described by Beckman et al [13] cited VanUffelen et al [14]. Briefly, 50 µL was added to 5mM phenol in 50 mM sodium phosphate buffer pH 7.4 in a final volume of 3 mL. After incubation for 2 hours at 37ºC, 50 µL of 0.1 M sodium hydroxide was added, and the absorbance at 412 nm of each sample was immediately recorded. The yield of nitrophenol was calculated from ε = 4400 $M^{-1}.cm^{-1}$. All experiments were performed in duplicate.

Determination of serum nitric oxide

Nitric oxide donating activity was determined as described by Newaz et al [15] utilizing Greiss reagent. Briefly 0.5 mL serum was added to 50 µL HCl (6.5M) and 50 µL sulfunalic acid (37.5 mM). After incubation for 10 min, 50 µL naphthylethylenediamine dihydrochloride (12.5 mM) was added and incubated for further 30 min, centrifuged for 10 min at 1000g. The reference nitric oxide donating compound was lithium nitrite. The absorbance at 540 nm was immediately recorded. All experiments were performed in duplicate.

Isolation and determination of serum protein

Serum protein was precipitated with 0.15 M trichloracetic acid (1: 12 v/v), and then centrifuged at 3000 rpm for 5 minutes. Then chloroform (5 volumes) was added, vigorous shaking, and centrifuged at 10000 rpm for 10 minutes. The supernatant was discarded, then the precipitate

allowed to dry and frozen at -20ºC until use. The procedure of Bradford method [16] was used to determine the concentration of protein before and after exposure the aliquot of serum protein to the light

Exposure of dried serum protein to the light

A final concentration of precipitated protein 100 µg/ml was prepared by dissolving the dried protein in distilled water. A total volume of each sample 3 mL was exposed either to UVC (254 nm), UVA (365 nm) (Model C-65 Chromato-Vue Cabinet, 2330 V, 50 Hz, 80 A maximum, UVP, Inc, U.K.), sodium lamp (a monochromatically light averaging at 589.3 nm wavelength) or sun-light for 2 hours. The source of light was 4.5 cm far away from the upper surface and 15 cm far away from the bottom of test tube.

The effect of light on the protein is determined in percent of protein concentration recovery according to the following equation:

$$\text{Protein recovery (\%)} = \frac{\text{Protein concentration after light exposure}}{\text{Protein concentration before light exposure}} \text{ X } 100$$

Statistical analysis

The results are expressed as absolute number and whenever possible as mean ± SD. The data are analyzed using student's paired "t" test, two tailed taking $p \leq 0.05$ as the lowest limit of significance.

RESULTS AND DISCUSSION

Table 1 shows the characteristics of the patients. Hypertension is the major cause of chronic renal failure followed by diabetes mellitus. The median duration of illness was 4 years and 7 out of 12 patients had high blood pressure. The median creatinine clearance was 10.2 mL/min indicated that the patients in the end stage of renal failure.

Table 2 shows that the effect of sun light on the protein aliquot is negligible since the percent of recovery of protein concentration is 98.36%. Sodium lamp light is significantly reduced the concentration of protein compared to the sun light or UVA and UVC. There is no significant difference between the effect of UVA and UVC on the protein concentration. The generation of peroxynitrite as a result of exposed protein aliquot to light is observed in limited number of patients (Table 3). The highest value was 2.5 µmol after lighting with sodium lamp. On the other hand higher concentration of nitric oxide is released in the protein aliquot after lighting that reached to significant higher level after UVA radiation compared with UVC radiation or lighting with sodium lamp or the nature light of sun (Table 4). The detected levels of nitrogen species after lighting of protein aliquot are completely differed from correspond serum nitrogen species (Table 5).

Higher levels of serum peroxynitrite and lower serum levels of nitric oxide compared with corresponding values in protein aliquot is observed.

This study shows the effect of radiation is extended beyond the denaturation of protein to stimulate the release of nitric oxide which significantly observed with UVC, monochromatic light, and UVA compared with solar light.

The significant release of nitric oxide from isolated protein after exposure to the radiation may involve to increase the serum nitric oxide level which is decreased in patients with renal failure as demonstrated in this study [17]. And the vasodilatation effect of nitric oxide could explain the exacerbation of pruritis after exposure to the UVA or UVC [18] . The results of this study confirmed the previous studies that showed exposure of biological elements to the UVA and UVC is associated with release of nitric oxide. Suschek et al [19] demonstrated the significant continuous released of non-enzymatic nitric oxide production induced by UVA photolysis from endogenous nitric oxide stores of healthy human skin. Also, UVA illumination (25 Joules /cm^2) significantly elevates the intradermal nitric oxide production from intracutaneous photolabile nitric oxide derivative which contributed in lowering systemic blood pressure in healthy subjects [20]. Ultraviolet C radiation also contributed in release of nitric oxide. Fotiou et al [21] reported that UVC radiation of rat skin is considered as a potent stimulator of nitric oxide release in microvessels. Moreover, it was reported that the aggregates of denaturated protein stimulates intracellular production of nitric oxide [22]. This study adds a new data that not only UV-radiation stimulates the production of nitric oxide but monochromatic light also contributed in stimulation of non-enzymatic nitric oxide production. The limitations of the study include small sample size and to look for the effect of radiation on the enzymatic nitric oxide production. It concludes that UV radiation or monochromatic lighting are good stimulator of nitric oxide production from isolated sera protein of patients with end-stage renal failure. Such effect may produce harmful as well as beneficial effect.

Table-1: The characteristics of patients enrolled in the study

Sex	
Male	2
Female	10
Age (year)	
Mean ± SD	60.75 ± 15.3
Median	57.5
Causes of CRF	
Hypertension	6
Diabetes mellitus	4
Polycystic kidney	1
Glomerulonephritis	1
Duration of CRF	
Range (year)	1-9
Median	4
Systolic BP ≥ 140 mmHg (No.)	5
Diastolic BP ≥ 90 mm Hg (No.)	2
Serum creatinine (µmol/L)	
Mean ± SD	601.7 ± 80.8
Median	612.6
Creatinine clearance (mL/min)	
Mean ± SD	11.81±5.312
Median	10.2

Table -2: The effect of UV radiation, sodium lamp light and sun light on protein recovery (%)

Patient's No.	UVC (254nm)	UVA (365 nm)	Sodium lamp light (589.3 nm)	Sun light
1	88.56	94.88	86.52	97.68
2	100	90.76	91.28.	98.8
3	96.4	95.24	88.8	99.6
4	98.36	90.52	82.6	99.12
5	88.72	97.12	89	98.72
6	94	90.84	87	98.64
7	91.8	90.76	89.48	98.68
8	90.8	89.92	84	96.24
9	94.72	90.52	93.32	98.76
10	91.08	84.28	83.28	97.88
11	98.28	91.36	90.84	97.88
12	88.4	88.48	85	98.4
Mean ± SD	93.42±4.135*	91.22±3.480*	87.59±3.419*†§	98.36±0.863

* $p < 0.001$ in comparison with day light
† $p < 0.001$ in comparison with UVC
§ $p < 0.01$ in comparison with UVA

Table -3: The level of peroxynitrite (µmol) in protein aliquot after exposure to UV radiation, sodium lamp light and sun light

Patient's No.	UVC (254nm)	UVA (365 nm)	Sodium lamp light (589.3 nm)	Sun light
1	1.59	0	1.59	2.045
2	0.0	0	2.5	0
3	0.454	0	1.59	0
4	0	0	0	0
5	0	0	0	1.59
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0.09	0
12	0	0	1.136	0

Table-4: The level of nitric oxide (µmoL) in protein aliquot after exposure to UV radiation, sodium lamp light and sun light.

Patient's No.	UVC (254nm)	UVA (365 nm)	Sodium lamp light (589.3 nm)	Sun light
1	666	3066	1746	1386
2	3066	1386	1526	486
3	986	1886	886	406
4	1786	1986	766	1226
5	586	2946	706	1066
6	586	1126	606	166
7	526	1386	1246	466
8	466	826	486	186
9	46	626	826	0
10	926	1146	1046	1226
11	506	1386	1366	246
12	886	1266	1366	1406
Mean ± SD	919.3±794.6	1586±763.8 *(p=0.0003) **(p=0.05) ***(p=0.03)	1047.7±397.7 *(p=0.038)	687.7±531.4

In comparison with *sun light, ** UVC, *** sodium lamp light

Table-5: Serum nitrogen species levels (μmoL) in patients with end stage renal failure

No. of patients	Serum nitric oxide (μmoL)	Serum peroxynitrite (μmoL)
1	0	153
2	0	235
3	0	92
4	0	494
5	0	263
6	0	174
7	300	85.9
8	8.5	212
9	17	102
10	505	130
11	632	145
12	492	145
Mean ± SD	162.87±246.52	185.90±111.808

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