ENHANCED URINARY SPONTANEOUS VISIBLE LUMINESCENCE IN SMOKERS

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Spontaneous visible chemiluminescence from urine is significantly increased in smokers over the average levels observed in non-smokers, when measurements are carried out both at pH 1 or pH 7. The analysis of the effect of different additives to urine sample suggests that the same process or luminescent intermediates are responsible for the observed light emission in both groups of subjects. The difference in urinary luminescence found is attributed to the enhanced oxidative stress status induced by cigarette smoking.

Keywords: urinary luminescence; oxidative stress; cigarette smoking; lipid peroxidation; chemiluminescence.

INTRODUCTION

Oxidative stress has been suggested to be involved in the pathophysiology of various diseases and aging, but its assessment in clinical conditions has been limited by the lack of standardized non-invasive methods[1]. The measurement of low-level chemiluminescence is a useful procedure to assess either the generation of electronically excited states in biological systems[2,3] or their presence in biological fluids such as urine[4] Visible urinary chemiluminescence has been attributed to the spontaneous decomposition of oxidized metabolites, whose intensity could constitute a non-invasive measure of the *in vivo* oxidative stress status[4]. This view is supported by studies in human hyperthyroidism, condition involving oxidative stress related to thyroid calorigenesis[5], in which urinary chemiluminescence levels were higher than those in euthyroid subjects, being reverted to normal ranges after treatment with propylthiouracil[4]. Enhanced urinary light emission has also been observed in patients with Duchenne muscular dystrophy[6] a genetically determined disease which appears more closely related to oxidative stress to muscle than any other type of underlying muscle disturbance[7].

Cigarette smoking is another condition implying an altered oxidative stress status[8], with enhanced values of *in vivo* lipid peroxidation indicators[9,10]. With regard to urinary luminescence, Rose and Wallace have reported that hydrogen peroxide-induced luminescence was significantly higher in smokers than in non-smokers[11]. However, these data are difficult to interpret as the origin of the hydrogen peroxide-induced urinary light emission has not been established. Spontaneous urinary luminescence was also found higher in normal smokers than in normal non-smokers, but the reported values were not normalized (i.e., by creatinine evaluation) and the significance of the detected difference was not established[12]. Furthermore, from the results obtained, Gesler et al.[12] concluded that most of the observed luminescence corresponded to singlet oxygen dimol decay, in spite of the simultaneous measurement of both carbonyls and singlet oxygen emission.

In view of these considerations, the present work reports data on the visible luminescence emitted from urine samples from healthy smokers and non-smokers, in order to establish the magnitude of the induced difference, relating the results to the oxidative stress associated with cigarette smoking.

METHODS AND MATERIALS

Samples from the first urine of the morning were freshly employed or kept frozen till measurements. The samples were centrifuged at 3000 rpm for 10 minutes at room temperature , and visible luminescence was measured in a Beckman liquid scintillation counter in the out of coincidence mode, using the narrow tritium iso-set module[13] Urine samples with high absorbance at 450 nm were diluted with distilled water, to give a value of 0.3, to avoid excessive light absorption by the samples. The measurements were carried out using 3 ml of urine supplemented with either 1 ml of 0.1 M sodium phosphate buffer pH 7 or adjusted to pH 1 by addition of 1 M HCl. Measurements at pH 1 were done in 105 samples from 11 healthy non-smokers and 39 samples from 6 smokers . The non-smokers group comprised subjects both male and female of different ages. However, a non significant dependence with the sex and/or the age could be established. Measurements at pH 7 were carried out in a more homogeneous group of healthy university students (male, age between 19 and 25 years old). This study considers 48 measurements from 5 non-smokers, and 131 measurements from 13 smokers. All smokers consumed an average of 10 or more cigarettes daily. Creatinine levels were measured employing the procedure developed by Heinegard and Tiderstrom[14]. The effects of different additives was evaluated by measuring the luminescence of the samples prior and after their addition. Results shown correspond to the means ± standard deviation, for the number of determinations indicated. All chemicals used were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS AND DISCUSSION

Luminescence from urine samples was not significantly modified by sample freezing, either when expressed as cpm (Table 1) or as cpm/mg of creatinine (data not show). In the conditions used, the reported values correspond to lumines-

Table 1. Effect of sample freezing upon spontaneous urinary visible luminescence in non-smokers, measured at pH 1.

Subjects	Luminescence [10^3cpm]		Observation
	Frozen samples	Fresh samples	
I	11.7 ± 1.9 (12)*	10.9 ± 1.8 (6)	n.s.+
II	8.0 ± 0.8 (7)	8.2 ± 0.6 (5)	n.s.
III	9.7 ± 1.5 (7)	8.7 ± 2.1 (8)	n.s.

(*) Number of different urine samples for each non-smoker subject studied.
(+) Not significantly different, using Student't test for unpaired data.

Table 2. Average values of spontaneous urinary visible luminescence in smokers and non-smokers, measured at pH 1.

Smoking condition	Subjects	Number of samples	Luminescence [10^3cmp]
Non-smokers	I	12	11.7 ± 1.9
	II	7	8.0 ± 0.8
	III	8	9.7 ± 2.1
	IV	11	14.0 ± 2.3
	V	8	16.1 ± 3.4
	VI	11	15.7 ± 2.6
	VII	8	5.9 ± 2.0
	VIII	6	6.3 ± 0.8
	IX	5	5.0 ± 0.9
	X	5	11.4 ± 1.9
	XI	6	8.2 ± 0.9
Smokers	I	5	24.2 ± 3.6
	II	6	24.3 ± 1.8
	III	6	22.2 ± 2.2
	IV	7	21.1 ± 0.9
	V	6	15.6 ± 1.2
	VI	9	20.4 ± 0.9

Table 3. Effect of additives upon the spontaneous urinary luminescence in smokers and non-smokers.*

Additive	I / I°			
	n	Smokers	n	Non-smokers
Azide (10 mM)	5	0.83 ± 0.15	14	0.87 ± 0.16
EDTA (10 mM)	5	0.88 ± 0.19	14	1.04 ± 0.20
Desferrioxamine(0.6 mM)	5	0.90 ± 0.13	6	1.03 ± 0.10
Catalase (300 U)	8	0.88 ± 0.12	4	0.95 ± 0.05
Propylgallate (0.2 mM)	6	1.01 ± 0.13	4	1.15 ± 0.06
Diethyldithiocarbamate (0.15 mM)	5	0.57 ± 0.08	12	0.61 ± 0.09
Trolox (50 μM)	6	0.95 ± 0.08	4	1.01 ± 0.05

(*) Results are given as I/I°,where I and I°correspond to the luminescence measured in the presence and absence of the additives, respectively, for the indicated number of subjects (n). Measurements were carried out at pH 7.

cence emitted in the 400-600 nm range[4] which can be attributed to excited carbonyls emission[2]. The measured intensities, both at pH 1 or pH 7 remain constant over the time considered (c.a., 1 hour).

The influence of cigarette smoking on urinary luminescence was studied in two separate groups of healthy individuals. The first group of 17 subjects, comprising 11 non-smokers and 6 smokers, revealed luminescence levels, at pH 1, considerably higher in the smoking group (Table 2). This difference is sustained when the data are expressed per mg of creatinine (data not shown). Similar results were obtained, at pH 7, in a second group of male students of 19-25 years old, comprising 5 non-smokers and 13 smokers (Figs. 1 and 2). In the latter condition, luminescense values were expressed in terms of the creatinine content of the urine samples, in order to take into account possible dilution effects[14]. As can be seen in Figure 1, no correlation is established between urinary luminescence and creatinine levels, indicating that light emission is not closely related to urinary dilution, and that other factors (i.e., urine composition) could be the main determinants of the measured intensities. The data presented in Figures 1 and 2, comprising 131 observations in 13 smokers and 48 observations in 5 non-smokers, were subjected to statistical analyses using specialized software (SAS, Version 5.16) in a IBM 4381 installation under VM/CMS. Both the Kolmogorov-Smirnov test[15] applied to smokers group, and the Shapiro-Wilk test[16] used in non- smokers, rejected the probability of normality of the distribution of urinary luminescence, as well as that for creatinine levels or luminescence/creatinine ratios. Although the analysis by Spearman rank correlation test[15] gave a positive statistically significant association between luminescence and creatinine levels in both groups of observations, the low determination coefficients obtained ($r^2 = 0.39$ in smokers; $r^2 = 0.28$ in non-smokers) prevented any attempt of linear modelling (luminescence / creatinine showed no association with light emission and negative association with creatinine). Finally, by applying the Kruskall-Wallis test[15] it was verified that (a) there is no significant difference in urinary creatinine levels between smokers and non-smokers (aprox. $X^2 = 0.38$, P-value = 0.538), (b) there is a significant difference in spontaneous urinary luminescence between both groups (approx. $X^2 = 17.60$, P-value < 0.0001), and (c) a significant difference in the values of the luminiscence/creatinine ratios between smokers and non-smokers (approx. $X^2 = 7.28$, P-value = 0.007), reinforces conclusion (b).

These studies support the contention that smokers exhibit higher spontaneous urinary chemiluminescence than that of non-smokers, regardless of whether is carried out over the primary data or after their normalization by the amount of creatinine present in the urine. The difference observed between both groups could be due to the presence of a new emission mechanism in smokers, such as a significant hydrogen peroxide-induced luminescence and/or emission from a cigarrete-derived metabolite[12] or to an enhancement of the normal process. However, the fact that the effect of different additives to urine, including catalase, free-radical scavengers, iron chelators and decomposers of luminescent species, is rather similar in both groups (Table 3) would favour the latter possibility. The observed luminescence enhancement related to cigarette smoking is then compatible with the known increase in lipid peroxidation indicators reported in this condition[9,10,17] and with the proposed origin of the spontaneous visible luminescence[4,18]

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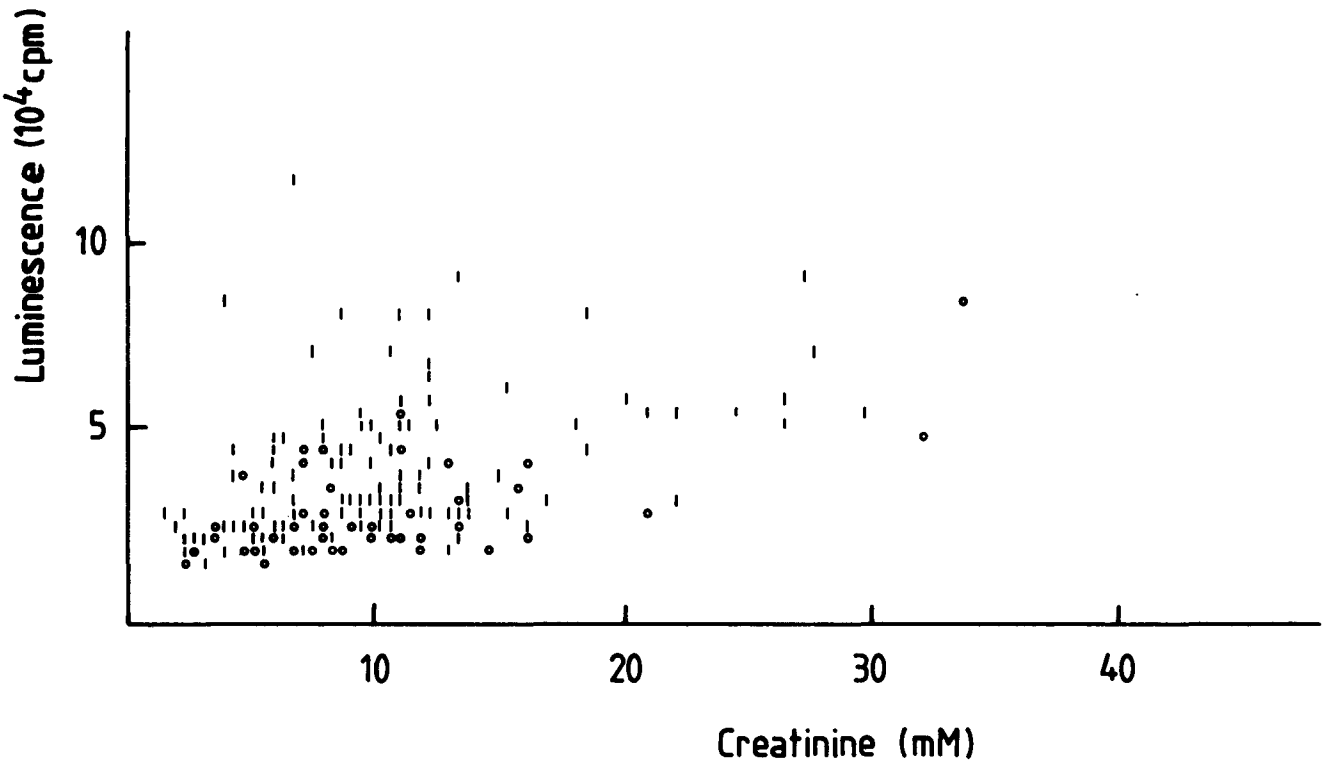

Figure 1. Luminescence intensity in urine samples measured at pH 7 as a function of the creatinine concentration. Values shown correspond to 131 observations in 13 smokers (I) and 48 obsevations in 5 non-smokers (o).

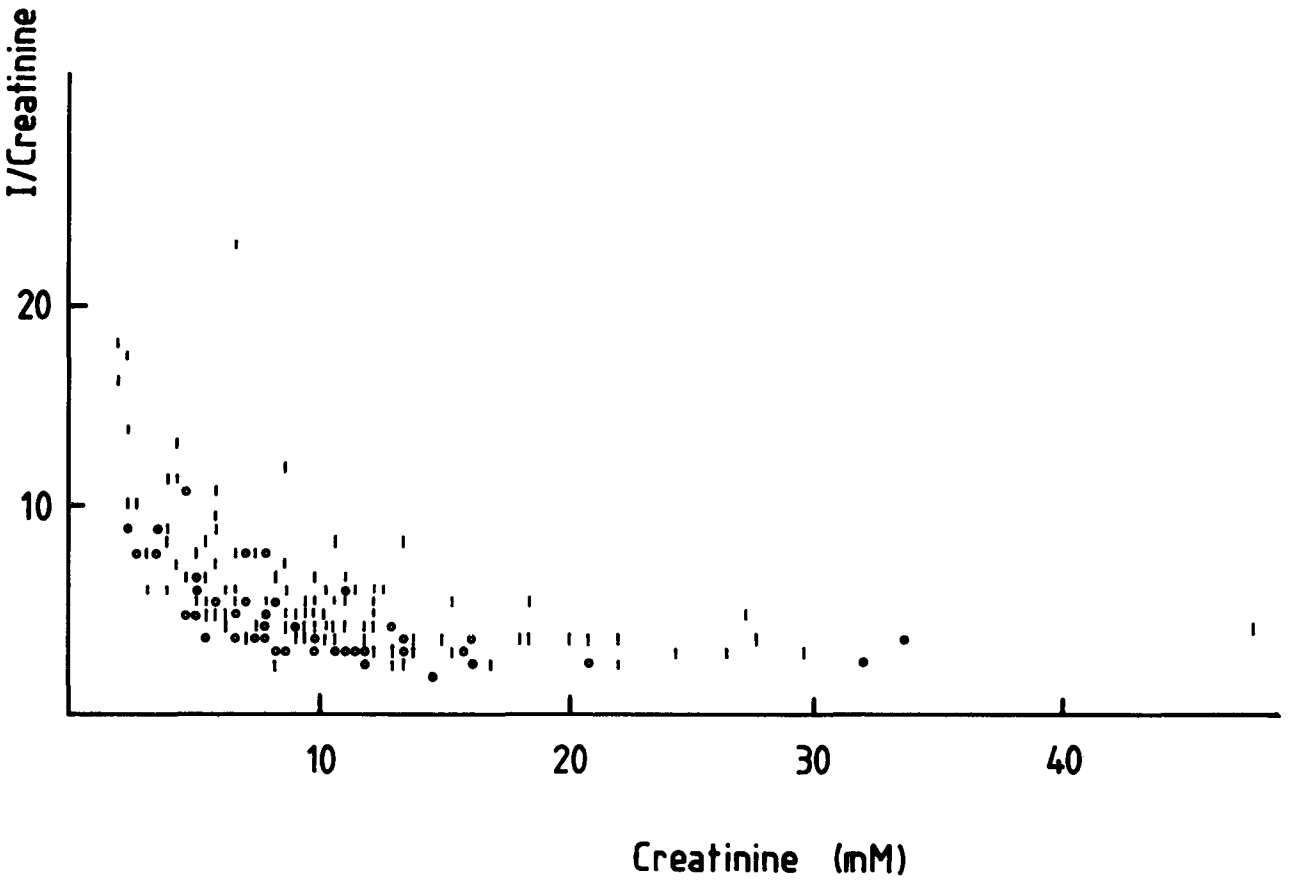

Figure 2. Luminescence intensity in urine samples measured at pH 7 divided by the millimolar concentration of creatinine in the sample, as a function of the creatinine concentration. Values shown correspond to 131 observations in 13 smokers (I) and 48 observations in 5 non-smokers (o).

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