Concentrations of Thiocyanate and Hypothiocyanite in the Saliva of Young Adults

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Abstract

The study was conducted to determine thiocyanate (SCN^-) and hypothiocyanite (OSCN^-) concentrations in resting (RWS) and stimulated whole saliva (SWS) and stimulated parotid saliva (SPS) of 20 healthy young adults aged 21-29 y. Samples of saliva were collected at 12:30, immediately before lunch. Resting saliva was collected by expectoration, and stimulated saliva was collected during the uniform chewing of paraffin wax. Parotid secretion was collected using a modified Carlsson-Crittenden cup (Carlsson et al., *Am. J. Physiol.*, 26, 169-177, 1910). SCN^- concentration was determined by the ferric nitrate method (Betts et al., *J. Am. Chem. Soc.*, 75, 5721-5727, 1953) whilst OSCN^- was assayed using 2-mercaptoethanol as a reducing agent (Pruitt et al., *Caries Res.*, 16, 315-323, 1982). In RWS, SWS and SPS, the mean SCN^- concentrations (in mM) were $1.48 \pm 0.59(\text{S.D.})$, $0.90 \pm 0.56(\text{S.D.})$ and $1.24 \pm 0.65(\text{S.D.})$ whilst the mean OSCN^- concentrations (in μM) were $31.21 \pm 13.54(\text{S.D.})$, 24.90 ± 12.61 and $30.19 \pm 23.35(\text{S.D.})$ in the respective salivas. The presence of OSCN^- in the secretion collected from the parotid gland supported previous findings by Tenovuo and Pruitt (Tenovuo et al., *J. Oral Path.*, 13, 573-584, 1984), who suggested an endogenous glandular (eukaryotic) source of hydrogen peroxide (H_2O_2), since parotid saliva from healthy glands is devoid of bacteria and leukocytes.

Introduction

The salivary peroxidase system consists of salivary peroxidase enzyme, H_2O_2 and SCN^- . This system may contribute to the antimicrobial properties of saliva, as shown in numerous *in vitro* studies^[1-3]. The source of H_2O_2 in the oral environment may be leukocytes, oral bacteria and other host cells.

SCN^- concentration is related to dietary habits and smoking. The salivary glands concentrate SCN^- from blood through the epithelial cells of the striated duct and subsequently secrete SCN^- at relatively high concentrations^[4].

Salivary peroxidase enzyme catalyzes the oxidation of SCN^- by H_2O_2 to yield the antimicrobial oxidizing agent, OSCN^- :

OSCN^- is the major product at neutral pH, whereas at low pH, the major product is hypothiocyanous acid (HOSCN), which is in acid-base equilibrium with OSCN^- :

The antibacterial action is more effective at low pH, owing to the ability of uncharged HOSCN to penetrate microbial cell membranes more readily than OSCN^- , and thus attack groups of essential intracellular enzymes. However this neutral acid, HOSCN, decomposes rapidly^[5].

The concentration of OSCN^- found in saliva depends upon the method of collection. Upon stimulation, the OSCN^- concentration decreases and remains low throughout the period of stimulation.

OSCN^- has also been identified in parotid saliva, and since parotid saliva from healthy glands is devoid of bacteria and leucocytes, this suggests an endogenous glandular (eukaryotic) source of H_2O_2 ^[6].

The objectives of this study were to compare the assay methods which have been used to measure OSCN⁻ in saliva in view of the wide range of reported results, and to investigate the effect of stimulation on the concentrations of SCN⁻ and OSCN⁻ in whole saliva as well as to determine the levels of SCN⁻ and OSCN⁻ in stimulated parotid saliva (SPS).

Materials and Methods

Saliva collection

The subjects were 20 healthy dental students and dental personnel aged 21-29 y with a mean age of 24 y (S.D.=1.92). Samples of saliva were collected using a modified Carlsson-Crittenden^[7] cup immediately before lunch at 12:30. For resting whole saliva (RWS), the subjects were instructed to refrain from all tongue and jaw movements except when transferring saliva every 30 s into an ice-chilled graduated tube.

Stimulated whole saliva (SWS) was achieved by paraffin wax chewing (Orion Diagnostica, Finland) at a constant rate of one chew per second, and the saliva was then transferred into a tube every 15 s. Subjects switched chewing from one side of the mouth to the other every 15 s.

Parotid secretion was collected from one gland in each subject via a modified Carlsson-Crittenden cup placed over the orifice of Stensen's duct, and carried by polyethylene tubing (tubing TSR, Slaughter Ltd., Essex, U.K.) to a graduated tube standing in ice. Stimulation was provided by chewing paraffin wax on the side of the mouth where the collecting device was placed.

For the three types of saliva, the time taken to collect approximately 2.4 ml was noted.

Analysis of SCN⁻ and OSCN⁻

Aliquots of 200 μ l of saliva were utilized for estimation of OSCN⁻, which was analyzed immediately in duplicate. In addition, an 800- μ l sample of saliva was immediately centrifuged at 13,000 rpm for 20 min at 4°C and the supernatant stored at -17°C for later analysis of SCN⁻.

SCN⁻ concentration was determined by the ferric nitrate method^[8], whilst^[9] OSCN⁻ was assayed using 2-mercaptoethanol as a reducing agent, working at a pH of 8. The absorbances for SCN⁻ and OSCN⁻ analyses were read using a spectrophotometer (Uvicam SP1700, Ultraviolet, Cambridge, U.K.).

Detection of OSCN⁻ in saliva using 5-thio-2-nitrobenzoic acid (TNB) was first described by Thomas et al.^[10] and the method was subsequently modified by Pruitt et al.^[9] These two procedures were compared prior to the start of the study using samples of SWS collected from 10 volunteers working in the laboratory. TNB was first prepared by reduction of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) with agents such as borohydride^[10] or 2-mercaptoethanol^[9]. The assay of OSCN⁻ is based on oxidation of 2 mol of TNB to colorless DTNB by 1 mol of OSCN⁻.

Data analysis

The SPSSX was used to determine mean values, standard deviations and frequency distributions. In order to explore relationships between the salivary components, Pearson's correlation coefficients were calculated between the two sets of measurements.

Reproducibility

Reproducibility of SCN⁻ and OSCN⁻ measurements was also determined prior to the start of the study. For this, samples of stimulated saliva were collected from eight volunteers working in the laboratory. Pearson's correlation coefficients between the two measurements were calculated, and values of 0.99 and 0.96 were attained for SCN⁻ and OSCN⁻ estimations, respectively. There were no statistically significant differences between the means (data not shown).

Results

Table 1 shows that the two procedures used for assay of hypothiocyanite gave essentially the same results. There were no statistically significant differences between the values obtained.

Table 1 Comparison of two different techniques for assay of OSCN⁻

Subject number	Method of Pruitt et al. ^[10]	Method of Thomas et al. ^[9]
1	12.24	5.25
2	58.56	67.94
3	27.46	26.33
4	19.85	22.39
5	26.80	30.19
6	15.55	32.27
7	10.81	15.83
8	18.75	24.78
9	21.29	27.72
10	26.03	23.39
Mean	23.73	27.61
S.D.	13.55	16.15

Wilcoxon's signed rank test
T=12, P=0.11

(μM)

Although samples of saliva were collected from all of the 20 subjects participating in the study, the data were incomplete for thiocyanate due to analytical loss. Table 2 presents the mean flow rates and concentrations of SCN⁻ and OSCN⁻ in RWS, SWS and SPS of the participating subjects. In whole saliva the onset of stimulation was marked by a substantial increase in mean flow rate. The flow rate of SPS was comparable to that of RWS.

Table 2 Flow rate (FR) and concentrations of thiocyanate (SCN⁻) and hypothiocyanite (OSCN⁻) in RWS, SWS and SPS with standard deviations in parenthesis.

n=20

*n=19

	FR (ml/min)	SCN ⁻ (mM)	OSCN ⁻ (μM)
RWS	0.46 (0.24)	*1.48 (0.59)	31.21 (13.54)
SWS	1.62 (0.86)	*0.90 (0.56)	24.90 (12.61)
SPS	0.43 (0.33)	1.24 (0.65)	30.19 (23.35)

n=20

*n=19

Stimulation resulted in a decrease in the mean concentrations of both SCN⁻ and OSCN⁻ in whole saliva. The concentrations of SCN⁻ and OSCN⁻ in SPS were comparable to those in RWS.

Figs. 1, 2 and 3 show the correlation coefficients between SCN⁻ and OSCN⁻ in RWS, SWS and SPS. There were no statistically significant correlations between SCN⁻ and OSCN⁻ concentrations in RWS ($r=0.26$, $p>0.05$). However, the relationship between the concentration of SCN⁻ and OSCN⁻ in SWS was significant ($r=0.60$, $p<0.05$), the levels in SPS approaching the levels of statistical significance ($r=0.54$, $p=0.08$).

Fig. 1
Correlation between thiocyanate (SCN^-) and hypothio-
cyanite (OSCN^-) concentrations in RWS
n=19

Fig. 2
Correlations between thiocyanate (SCN^-) and hypothio-
cyanite (OSCN^-) concentrations in SWS
n=19

Fig. 3
Correlations between thiocyanate (SCN^-) and hypothio-
cyanite (OSCN^-) concentrations in SPS
n=20

Discussion

Comparison of the methods which have been used to measure OSCN^- in saliva indicated that they gave similar results. Slightly lower values were obtained when the assay was performed using the method of Pruitt et al.^[5] These differences were not sufficient to account for the wide range of OSCN^- values reported.

Earlier studies of SCN^- concentration in RWS, SWS and SPS are presented in Table 3. In this study, the SCN^- concentration in whole saliva was reduced on stimulation, consistent with a previous study conducted in adolescents^[11]. The levels of SCN^- in adults in this study are comparable to those reported by Pruitt et al.^[5] and Lamberts et al.^[12] The values in adolescents^[11] are lower than those in adults, and this may reflect age differences as well as smoking experience. Maliszewski et al.^[13] reported that SCN^- concentrations are higher in the saliva of smokers than in that of non-smokers. Lamberts et al.^[12] carried out thiocyanate estimations in two groups of subjects, smokers and non-smokers, and reported that SCN^- levels in RWS were higher in the former than in the latter. Tenovuo et al.^[14] reported an even lower mean SCN^- level of 0.38 mM in RWS of children aged between 0.8 and 3.8 y.

Table 3 Thiocyanate (SCN^-) concentrations (mM) in RWS, SWS and SPS in the present and other studies

Authors	n	age	mean $\pm$ S.D.
RWS			
Present study	19	21-29	1.48 $\pm$ 0.59
Jalil ^[11]	92	12-14	0.71 $\pm$ 0.42
Lamberts et al. ^[12]	29	17-29	1.46 $\pm$ 0.86
Pruitt et al. ^[5]	28	20-50	1.21 $\pm$ 0.66
Tenovuo et al. ^[14]	16	0.8-3.8	0.38 $\pm$ 0.18
SWS			
Present study	19	21-29	0.90 $\pm$ 0.56
Jalil ^[11]	93	12-14	0.34 $\pm$ 0.20
Grahn et al. ^[15]	50	19-21	1.71 $\pm$ 0.57
Tenovuo et al. ^[14]	35	17-61	1.57 $\pm$ 0.75
Tenovuo & Anttonen ^[16]	13	22-37	1.12 $\pm$ 0.55
SPS			
Present study	20	21-29	1.24 $\pm$ 0.65
Pruitt et al. ^[5]	28	20-50	0.76 $\pm$ 0.55

In SWS, the values obtained from the present adult population are lower than those reported by Grahn et al.^[15] and Tenovuo et al.^[16, 17]. However, half the subjects in these studies were smokers, in contrast to our subjects, few of whom smoked.

The SCN^- levels in SPS were higher than those reported by Pruitt et al.^[5]. No data have previously been presented on SCN^- levels in the parotid secretions of adolescents. The present study appears to indicate that SCN^- levels in both RWS and SWS in adolescents are lower than those in adults.

The OSCN^- concentration in whole saliva dropped upon stimulation of the saliva flow rate. According to Globerman et al.,^[18] the oxygen concentration in whole saliva decreases sharply upon stimulation, and this could result in decreased H_2O_2 production in saliva with a subsequent decrease of the OSCN^- level. Tenovuo et al.^[17] suggested that OSCN^- might be eliminated by reducing agents released into

saliva upon stimulation, and that the source of reducing agents could be salivary secretions, oral tissues or oral bacteria. Evidence for such reducing agents was provided by the 'negative' OSCN⁻ values reported, which indicated that DTNB was reduced to TNB. Although previous work carried out in our laboratory to estimate OSCN⁻ in SWS has yielded some negative OSCN⁻ values, no negative OSCN⁻ values were found in the saliva of the present subjects. In this study there was some evidence of an association between the concentrations of SCN⁻ and OSCN⁻, as seen in SWS. Hence, it may be assumed that one of the factors that may account for the decrease in the concentration of OSCN⁻ upon stimulation might be a reduction in the amount of SCN⁻ present.

As seen in Table 4, OSCN⁻ levels in RWS have varied considerably in different investigations. This variation was probably due to differences in the assay methods, pretreatment procedures or sample collection techniques. The values of OSCN⁻ in adolescents were slightly lower than in the adult population.

Table 4 Hypothiocyanite (OSCN⁻) concentrations (μ M) in RWS, SWS and SPS in the present and other studies.

Authors	n	age	mean $\pm$ S.D.
RWS			
Present study	20	21-29	31.21 $\pm$ 13.54
Jalil ^[11]	89	12-14	20.79 $\pm$ 10.58
Mandel et al. ^[19]	10	24-40	17.60 $\pm$ 17.70
Lamberts et al. ^[12]	29	17-29	47.10 $\pm$ 26.00
SWS			
Present study	20	21-29	24.90 $\pm$ 12.61
Tenovuo et al. ^[14]	30	17-39	27.00 $\pm$ 12.00
SPS			
Present study	20	21-29	30.19 $\pm$ 23.35
Mandel et al. ^[19]	10	24-40	15.20 $\pm$ 8.70

The OSCN⁻ concentration in stimulated whole saliva in this investigation was lower than that in resting whole saliva. Similar findings have been reported by other workers^[6,13].

The OSCN⁻ concentration in stimulated parotid saliva from the present adult population was comparable to levels in RWS. This finding differs from that reported by Mandel et al.^[19], where OSCN⁻ levels in SPS were found to be lower than in RWS. This may have been due to differences in the stimulation technique employed. The presence of OSCN⁻ in the secretion collected from the parotid gland suggests an endogenous glandular source of H₂O₂, since parotid saliva from healthy glands is devoid of bacteria and leukocytes^[5].

Conclusions

Comparison of the methods which have been used to measure OSCN⁻ in saliva indicates that these methods gave similar results.

Stimulation of the saliva flow results in a rapid decrease in OSCN⁻ levels in whole saliva. This effect may be due in part to decreased concentrations of SCN⁻, as well as to elimination of OSCN⁻ by reducing agents released into the stimulated saliva.

The antimicrobial activity of the salivary peroxidase system may be more effective during periods in

which saliva flow is unstimulated (such as during sleep).

The presence of OSCN⁻ in parotid secretions suggests an endogenous source of H₂O₂.

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