Acute reduction in secretory immunoglobulin A following smoking cessation

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Abstract

Smokers report an increase in upper respiratory infections in the early phase of stopping smoking. One possible cause is a depletion in secretory immunoglobulin A (S-IgA) which has been observed in one study. The present study sought to establish this finding in smokers using nicotine patches. Ninety-two smokers, trying to stop smoking, were assessed whilst smoking and for up to six weeks of abstinence. All smokers were prescribed 15mg 16-hour nicotine patches. Among abstinent smokers, changes in S-IgA concentration, secretion rate and saliva volume, were assessed. **During the preliminary analyses we observed that for the pre-smoking cessation measure a longer time since the last cigarette was significantly related to lower S-IgA levels (P=0.006). Consequently the main analysis, of changes in S-IgA from pre-cessation to post-cessation, was confined to those who had smoked within 0.5 to 1.5 hours of the pre-cessation measure (n=51).** There was a significant decline in S-IgA, relative to pre-smoking abstinence levels, following abstinence of one day (P= 0.027), but levels returned to pre-abstinence values after one week. There was no evidence of any significant changes in saliva volume following smoking cessation, relative to pre-cessation levels. Users of 15 mg patches are likely to experience a decline in S-IgA levels on the first day of smoking cessation, independently of saliva volumes, **and this decline in S-IgA is likely to occur acutely, within the first few hours of smoking abstinence.** This acute drop in S-IgA appears to stem from a factor other than depletion of nicotine from the body. The observed decrease in S-IgA may help to explain the increased susceptibility of smokers to upper respiratory tract infections in the immediate post-cessation period.

Keywords: S-IgA concentration; S-IgA secretion rate; Saliva; Immunoassay;

Smoking cessation, Nicotine patches

Introduction

Secretory immunoglobulin A (S-IgA) is the primary antibody at mucosal surfaces (Bosch et al 2002; Underdown and Mestecky 1994) and individuals with reduced IgA in their saliva are more susceptible to upper respiratory tract infections (Evans et al 1995). Smokers have been shown to have lower S-IgA levels than non-smokers (Barton et al 1990; Evans et al 2000), while non-smokers and ex-smokers have been shown to have similar levels of S-IgA (Barton et al 1990), suggesting that the immunosuppressive effects of smoking are reversed following smoking cessation.

Two studies have examined changes in S-IgA following smoking cessation. One study reported no changes in S-IgA concentration measured after three months of abstinence, relative to smoking levels (Hersey et al 1993). However, this study was limited by a small sample size (N=8) and absence of measures taken soon after cessation. The other study (N=20) observed a significant decline in S-IgA concentration levels following seven days of abstinence, relative to smoking levels, and then a return to pre-abstinence levels following two weeks of cessation (Griesel and Germishuys 1999). However, this study did not take account of the effects of changes in saliva volume following smoking cessation (Miletic et al 1996), or the recency of smoking at the pre-cessation measure (Navazesh 1993). Furthermore, this study did not assess changes in S-IgA during the first few days of abstinence or beyond two weeks of abstinence. In addition, nicotine replacement therapy (NRT) is now a standard smoking cessation treatment (West et al 2000), and it is not clear to what extent loss of nicotine may be responsible for the observed decline in S-IgA following smoking cessation. We have found that smokers using nicotine patches do report an increase in symptoms of upper respiratory tract infections (Ussher et al 2003). The present study examined changes in S-IgA following smoking abstinence of

one day, one week, two weeks and six weeks, among those using nicotine patches.

2. Method

2.1 Sample

Ninety-two men and women aged 18 to 65 years, smoking at least 10 cigarettes a day for at least three years, and who were motivated to stop smoking, were recruited through newspaper advertisements or referral from their physician. Smokers with a current psychiatric illness, substance misuse problem or pregnancy were excluded. Participants provided written consent and the local ethics committee gave its approval.

2.2 Design and treatment regimen

All participants were assessed one week before stopping smoking, and following smoking cessation of one day, one week, two weeks and six weeks. Participants attended six weekly smoking cessation treatment sessions involving individual cognitive-behavioural support (Jorenby et al 1995) and a follow-up session two weeks after the final treatment. Participants stopped smoking at their second visit and were then required to use one 15mg 16-hour nicotine patch on a daily basis (Dale et al 1995; Hughes et al 1999).

2.3 Measures

At the first visit data were collected relating to demographics and smoking characteristics; **including the Fagerström Test for Nicotine Dependence (Heatherton et al 1991) which is a self-report measure with six items relating to time to the first cigarette on awakening, difficulty of stopping smoking in no smoking areas, the cigarette that the smokers would most hate to give up, the number of cigarettes smoked per day, frequency of smoking in the first hours compared to the rest of the day, and whether or not the smoker smokes when**

they are ill and confined to bed. At the first visit smoking status was confirmed with expired air carbon monoxide (CO) using a Bedfont Smokerlyzer **(Hajek and Belcher 1991). Expired air CO gives an acceptable degree of discrimination of smoking status relative to a measure of cotinine (Jarvis et al 1987) and expired CO has been found to be highly correlated with both blood nicotine concentrations (Ashton et al 1981) and with plasma cotinine and saliva cotinine (Jarvis et al 1987).** Weekly self-reports of smoking abstinence were verified with expired CO (cut-off: 10ppm).

In order to obtain samples of S-IgA, unstimulated saliva was collected through the participants swallowing, placing a cotton roll under their tongue for exactly two minutes, then returning the roll to a salivette (Sarstedt Ltd.). Participants were asked to avoid food and drink (except water) for at least 30 minutes before providing saliva.

For the pre-cessation saliva sample, to minimise acute effects of smoking (Navazesh 1993), participants were required to avoid smoking for at least 30 minutes before this sample. The time since the last cigarette was recorded. Further saliva samples were provided after abstinence of one day, one week, two weeks and six weeks. On each occasion a single saliva sample was taken between 16.00-17.00 hours. For those unable to attend the clinic between these hours a salivette was provided, the sample was taken independently (16.00-17.00 hours), refrigerated and returned at the next appointment using an insulated cold pack.

Prior to assay all the saliva samples were stored at -20°C. To recover the saliva, samples were thawed, then spun at 3500 rpm for 10 minutes. **S-IgA concentration (µg/ml) was determined by single radial immunodiffusion (SRID) assay (LC-Partigen-IgA, Behring Diagnostika); as described in Zeier et al., 1996.** Saliva volumes (ml) were determined gravimetrically. S-IgA secretion rate (µg/min) was

calculated as S-IgA concentration x volume/2 (2 minutes sampling time).

Following the findings of Griesel and Germishuys (1999) it was hypothesised that there would be a decline in S-IgA levels, relative to pre-smoking cessation levels, up to and including the first week of smoking abstinence, followed by a return to pre-abstinence levels. Therefore, for the statistical analyses testing for a decline in S-IgA levels and saliva volume following smoking cessation the tests were one-tailed. For the remaining analyses the tests were two-tailed. SPSS version 10 was used throughout.

3. Results

At baseline 92 smokers provided data (see table 1). Following smoking cessation of one day, one week, two weeks and six weeks, 95.6% (88/92), 78.3% (72/92), 62.0% (57/92) and 51.1% (47/92) of the participants, respectively, maintained continuous abstinence and were therefore retained in the study. Of those abstinent for one day, one week, two weeks and six weeks, 70.4% (62/88), 63.9% (46/72), 71.9% (41/57) and 76.6% (36/47), respectively, provided S-IgA samples. In 12 cases there was insufficient saliva to execute the S-IgA assay. In other cases the salivettes were not returned.

3.1 Data transformations

At all measurement points the data for S-IgA concentration (μg/ml), S-IgA secretion rate (μg/min) and saliva volume (ml) were strongly positively skewed. Therefore logarithmic transformation **(log to the base of 10)** was applied to the S-IgA data, and square root transformation was applied to the saliva volumes. Following these transformations, in order to reduce high outliers, data were winsorised to the mean plus two standard deviations **(Howell 2002)**. There were 12 outliers from eight participants. Winsorising of the outliers did not affect the results of analyses for

changes in S-IgA or saliva volume following cessation as presented in section 3.3.

3.2 Examining potential confounders of changes in S-IgA and saliva volume following smoking cessation

Nicotine patches

Following smoking abstinence of one week, two weeks and six weeks, a nicotine patch had been used, in the previous week, on a daily basis, by 91.3% (42/46), 92.7% (38/41) and 55.6% (20/36) of the participants, respectively. At six weeks of abstinence ANOVA did not reveal any significant differences, in relation to whether patches were used, for S-IgA concentration, S-IgA secretion rate, or saliva volume.

Location of saliva sampling

For the saliva sample taken following one day of smoking cessation a clinic visit was not scheduled and all participants took this sample at home. Pre-cessation, and following smoking abstinence of one week, two weeks and six weeks, the sample was taken in the clinic by 44.6% (41/92), 39.1% (18/46), 43.9% (18/41) and 61.1% (22/36) of the participants, respectively. Given that location of the sample is a potential confounder we carried out a series of ANOVAs comparing home versus clinic levels. Saliva volume was significantly higher for samples taken at home, relative to those taken in the clinic, after one week of smoking abstinence (F=12.4, df=1, 44, P=0.001, mean (SD) saliva volume (sqrt ml/min): home=1.3(0.6), clinic=0.7 (0.3)), but not at any of the other measurement points. **For S-IgA concentration and S-IgA secretion rate there were no significant differences for home versus clinic at any measurement point; therefore it is unlikely that the location of the saliva sample was a confounder in the analysis of changes in S-IgA. As a further check for the influence of the location of the saliva sample analyses were carried out of changes in S-IgA levels and saliva volume, pre-cessation to each post-cessation**

measure, only for those taking the pre-cessation sample at home (N=27). It was hypothesised that the findings for the 'home-only' sub-sample would be consistent with the findings for the larger sample (N=51).

Time since cigarette

Prior to the pre-cessation saliva sample all the smokers confirmed that they had not smoked for at least 30 minutes. The actual time since the last cigarette was recorded by 65.2% (60/92) of the smokers. The mean (SD) hours since the last cigarette was 1.07 (0.81) (range=0.5-4.5 hours). A longer time since the last cigarette was significantly related to both lower S-IgA concentrations and secretory rates at pre-cessation (Pearson's: R=0.349, P=0.006, R=0.296, P=0.022, respectively), but was not significantly related to saliva volume. Thus, smokers not having smoked for several hours might already be experiencing a state of tobacco withdrawal. On this basis, analyses of changes in S-IgA and saliva volumes, following smoking cessation, were restricted to those who reported that they had smoked within 0.5 to 1.5 hours of providing the pre-abstinence saliva sample (n=51, mean (SD) hours since last cigarette=0.79 (0.32)).

Stability of S-IgA measures

The stability of the S-IgA measures were examined through correlating S-IgA measures at consecutive time points. For S-IgA concentration (log μg/ml) the correlations reached significance for measures taken one day after smoking cessation versus one week after cessation (Pearson's R=0.403, P=0.009), one week post-cessation versus two weeks post-cessation (R=0.517, P=0.002), and two weeks post-cessation versus six weeks post-cessation (R=0.381, P=0.038). For S-IgA secretion rate (log μg/min) the correlations reached significance for one day

post-cessation versus one week post-cessation (Pearson's R=0.343, P=0.028) and for one week post-cessation versus two weeks post-cessation (R=0.433, P=0.012). For two weeks post-cessation versus six weeks post-cessation the correlation for S-IgA secretion rate approached significance (R=0.352, P=0.057). The correlations for one week pre-smoking cessation versus one day post-cessation were not significant for either S-IgA concentration (R=0.080, P=0.537) or S-IgA secretion rate (R=0.131, P=0.310). However, given the hypothesised reduction in S-IgA it was not anticipated that measures would be stable between pre-cessation and post-cessation. The finding that consecutive post-smoking cessation S-IgA values were moderately correlated indicates that the S-IgA measures were quite stable for any individual relative to other individuals and therefore did not just represent transient states.

3.3 Changes in S-IgA and saliva volume following smoking cessation

The results of comparisons using ANOVAs between pre-cessation measures and each post-cessation measure for S-IgA concentration, S-IgA secretion rate and saliva volume are presented in table 2. **These analyses were restricted to those who had smoked within 0.5 to 1.5 hours of providing the pre-abstinence saliva sample (n= 51). Each analysis compared S-IgA and saliva measures for all those providing data at each post-cessation measurement point (one day: n=31, one week: n=25, two weeks: n=23, six weeks: n=17) with baseline levels.** Relative to pre-cessation levels, there was a significant decline in S-IgA concentration following one day of smoking abstinence. There was no evidence of a significant change in S-IgA concentration between pre-cessation and abstinence of one week two weeks or six weeks. At these time points S-IgA concentrations returned to pre-cessation levels. There was no evidence of any significant changes in S-IgA secretion rate or saliva

volume at any post-cessation measurement point, relative to pre-cessation.

As hypothesised, when only including those who took the pre-cessation saliva sample at home there remained a significant decline in S-IgA concentration between pre-cessation and one day of abstinence (n=15, F=5.8, df=1, 14, P=0.015, mean S-IgA concentration: pre-cessation=1.91 (0.44), one day=1.59 (0.46)); and there was also a significant decline in S-IgA secretion rate between these times (n=15, F=3.5, df=1, 14, P=0.041, mean S-IgA secretion rate: pre-cessation=1.63 (0.65), one day=1.25 (0.70). When using this 'home-only' sub-sample there was no evidence of any other significant differences between pre-cessation measures and any post-cessation measure for S-IgA concentration or secretion rate, or for saliva volume. **That the findings for the 'home-only' sample are consistent with the findings for the main analyses further confirms that the location of the sample is unlikely to have been a confounder in the main analyses.**

4. Discussion

This is the first study to examine changes in S-IgA levels during the first week of smoking cessation. It is also the first study to assess changes in S-IgA among those using NRT. The finding that S-IgA levels were lower at pre-cessation for those who had gone longer without a cigarette suggests that a reduction in S-IgA is likely to occur acutely, within the first few hours of smoking abstinence. Relative to pre-smoking cessation levels, the decline in S-IgA was evident following one day of smoking cessation and S-IgA returned to pre-cessation levels by one week of abstinence. This suggests that the observed decline in S-IgA during the first 24 hours of smoking abstinence is probably a transient tobacco withdrawal effect (Hughes 1992). As the vast majority of participants were using nicotine patches at this time this drop in S-IgA is not likely to be related to depletion of nicotine per se.

For the pre-smoking cessation measure a longer time since the last cigarette was significantly related to lower S-IgA levels; therefore the analysis of changes in S-IgA from pre-cessation to post-cessation was confined to those who had smoked within 0.5 to 1.5 hours of the pre-cessation measure. The analysis was also restricted to those who remained abstinent from smoking, those who provided S-IgA samples, and those who recorded the time between their last cigarette and the pre-cessation measure. Consequently, as is to be expected in these type of studies, the temporary reduction in S-IgA at one day post-cessation was observed when using a sub-sample of the original study population. Generalization of the findings are limited by the reduced sample size and the study requires replication with a larger sample. Our findings suggest that it is necessary that future studies of S-IgA record the time since the last cigarette for pre-cessation measures and also standardise the lower and upper boundaries for the time since the last cigarette; for example, the smokers could be required to have smoked within 30 to 90 minutes of the pre-cessation measure.

We did not find any significant differences in S-IgA levels for samples taking at home versus in the clinic at any measurement time; therefore it is unlikely that our findings were confounded by the location of where the sample was taken. Furthermore, when restricting our analysis to those smokers who took the pre-cessation sample at home the reduction in S-IgA following one day of smoking abstinence was still evident.

The present findings are inconsistent with a previous report of a decline in S-IgA concentration following one week of smoking cessation, relative to smoking levels (Griesel & Germishuys 1999). Further studies are needed to compare changes in S-IgA levels following smoking cessation for those using NRT versus those not using

NRT, and among those using different types and doses of NRT.

Previously, ex-smokers have been shown to have higher S-IgA levels than smokers (Barton et al 1990). In the present study there was no evidence of an increase in S-IgA levels following six weeks of smoking cessation, relative to smoking levels. Further studies are needed to determine the time following smoking cessation at which S-IgA levels are significantly raised, relative to smoking levels.

The present study was limited by not including a control group of continuing smokers and future studies may benefit by including such a group so as to control for confounding events that vary across time. However, it is highly implausible that a group of smokers, on an arbitrarily defined day, would show a significant drop in S-IgA levels and then a rebound in S-IgA while continuing to smoke.

The present study does not report upper respiratory infections and it is possible that changes in the presence of these infections following smoking cessation (Ussher et al 2003) could influence S-IgA levels. Conversely, the observed decline in S-IgA, following one day of smoking abstinence, may increase vulnerability to upper respiratory infections during the first weeks of stopping smoking, and this may be of particular significance for smokers who have chronic upper respiratory conditions (Hillerdahl and Rylander 1984). Further studies are required to examine changes in S-IgA following smoking cessation among smokers who are immunocompromised, and to relate changes in S-IgA levels following smoking cessation to changes in respiratory symptoms.

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Table1. Baseline characteristics of study sample (N=92)

Characteristic	Mean (SD)	
Age, y	42.3 (11.4)	
Education, y	13.3 (3.5)	
Fagerström Test for Nicotine Dependence score		5.4 (2.0)
Smoking rate, cigarettes/d	20.6 (7.9)	
Expired carbon monoxide concentration, ppm		21.7 (9.0)
	No. (%)	
Female	64 (69.6)	
Married	53 (57.6)	
Employed	68 (73.9)	
Professional/managerial	42 (45.7)	
Caucasian	80 (87.0)	

Table 2. Comparisons for S-IgA concentration and secretion rate, and for saliva volume, between pre-cessation levels and each measure following smoking cessation

(Only including those smoking within 0.5 to 1.5 hours of the pre-cessation measure, N=51.)

Comparisons using ANOVA		N	F	df	P
Mean (SD) S-IgA concentration (§inverse of log μg/ml)					
Pre-cessation	Post-cessation				
70.79 (2.69)	one day: 45.71 (2.45)	31	4.0	1, 30	* 0.027
58.88 (2.82)	one week: 56.23 (3.24)	25	0.1	1, 24	0.393
58.88 (3.24)	two weeks: 52.48 (2.40)	23	0.0	1, 22	0.479
58.88 (2.82)	six weeks: 56.23 (3.24)	17	0.2	1, 16	0.335
Mean (SD) S-IgA secretion rate (§inverse of log μg/min)					
Pre-cessation	Post-cessation				
34.67 (4.17)	one day: 23.44 (4.07)	31	1.3	1, 30	0.127
25.12 (4.57)	one week: 25.70 (4.79)	25	0.0	1, 24	0.448
25.12 (5.25)	two weeks: 33.11 (3.47)	23	0.7	1, 22	0.212
25.12 (4.90)	six weeks: 31.62 (2.40)	17	0.4	1, 16	0.264
Mean (SD) saliva volume (§square of sqrt ml)					
Pre-cessation	Post-cessation				
1.02 (0.10)	one day: 1.10 (0.10)	31	0.3	1, 30	0.293
0.85 (0.10)	one week: 0.90 (0.08)	25	0.2	1, 24	0.337
0.94 (0.09)	two weeks: 1.02 (0.07)	23	2.5	1, 22	0.063
0.92 (0.11)	six weeks: 0.94 (0.04)	17	0.1	1, 16	0.418

*P value significant at <0.05 (one-tailed)

§Transformed values have been adjusted such that log values have been inverted and square-root values have been squared.