Tracheobronchial Histopathology in Habitual Smokers of Cocaine, Marijuana, and/or Tobacco*

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Background: **Marijuana and alkaloidal cocaine ("crack") are the two most commonly smoked substances in the United States after tobacco. While regular tobacco smoking has been found to be associated with extensive microscopic alterations in bronchial mucosa, little information is available concerning the effect of crack cocaine and marijuana on tracheobronchial histopathology.**

Study objective: **To determine the relative impact of smoked substances (cocaine, marijuana, and tobacco) alone and in combination on the histopathology of the tracheobronchial mucosa and to assess whether the effects of habitual smoking of two or more substances (cocaine, marijuana, and/or tobacco) are additive.**

Design: **Observational cohort study.**

Subjects: **Fifty-three nonsmoking control subjects (NS), 14 current, habitual smokers of crack cocaine only (CS), 40 current, regular smokers of marijuana only (MS), 31 regular smokers of tobacco only (TS), 16 current smokers of both cocaine and marijuana (CMS), 12 current smokers of both cocaine and tobacco (CTS), 44 current smokers of both marijuana and tobacco (MTS), and 31 current smokers of cocaine, marijuana, and tobacco (CMTS).**

Methods: **After preliminary screening evaluation, including a detailed respiratory and general health questionnaire and routine pulmonary function studies, subjects underwent fiberoptic bronchoscopy with endobronchial biopsies of the mucosa of the primary carina and randomly selected secondary or tertiary carinae. Biopsy specimens were processed for light microscopy, stained with hematoxylin-eosin or periodic acid-Schiff, and examined to assess epithelial, basement membrane, and submucosal alterations by one or two pathologists who were masked to the smoking status of the subject.**

Results: **Smokers of cocaine, marijuana, or tobacco alone all exhibited more frequent abnormalities than NS in 10 (CS) or all 11 (MS and TS) of the histopathologic features assessed. For most features, MS and TS showed significantly more frequent alterations than NS ($p \leq 0.02$), while CS showed significantly more frequent abnormalities than NS in only three features ($p<0.05$) and nearly significant differences from NS in two additional features ($p \leq 0.09$). Alterations were noted *most* frequently in CTS (six features) and MTS (three features), while abnormalities were relatively infrequent in CMS. For 10 features, MTS had more frequent alterations than MS and TS. With a single exception, CMTS did not show more frequent alterations than CTS or MTS.**

Conclusion: **Marijuana and tobacco smoking each produces significant bronchial mucosal histopathology and the effects of marijuana and tobacco appear additive. Cocaine appears to lead to fewer significant bronchial mucosal alterations than marijuana or tobacco when smoked alone and does not add to the changes associated with marijuana. When smoked together with tobacco, however, cocaine appears to augment the bronchial injury caused by tobacco smoking.** ***(CHEST 1997; 112:319-26)***

Key words: airway injury; bronchial mucosa; crack cocaine; histopathology; lung; marijuana; tobacco

Abbreviations: CMS=smokers of both cocaine and marijuana; CMTS=smokers of cocaine, marijuana, and tobacco; CS= smokers of crack cocaine only; CTS=smokers of both cocaine and tobacco; H&E=hematoxylin-eosin; MS=smokers of marijuana only; MTS=smokers of both marijuana and tobacco; N/C=nuclear/cytoplasmic; NS=nonsmoking control subjects; TS=smokers of tobacco only

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Smoking, whether of tobacco, marijuana, and/or cocaine, remains the most common form of substance abuse worldwide. This popular practice continues to attract new users,[1] despite the growing evidence that smoking is hazardous to health.[2-4] According to a recent national survey, approximately 0.5% of young adults currently smoke crack cocaine regularly, while 2% to 3% and 21% are daily smokers

of marijuana and tobacco, respectively.[1] Little information is available concerning the effect of smoked substances other than tobacco on histopathology of the tracheobronchial mucosa. In a series of previous reports of light microscopic changes in biopsy specimens of the proximal tracheobronchial mucosa of relatively small numbers of smokers of marijuana alone (MS, cumulative n=30), tobacco alone (TS, n=15), marijuana plus tobacco (MTS, n=17), and nonsmokers (NS, n=11) who underwent fiberoptic bronchoscopy,[5-7] we found that smoking marijuana alone was associated with at least as frequent histopathologic abnormalities as were evident in biopsy specimens from smokers of tobacco alone, and a trend was noted toward additive effects of smoking both substances. Since the latter reports, smoking of the alkaloidal form of cocaine (crack) has become more prevalent,[1] despite increasing evidence of pulmonary complications of crack cocaine.[4] We now report light microscopic findings in biopsy specimens of the tracheobronchial mucosa of smokers of alkaloidal cocaine alone (CS, n=14) or with marijuana (CMS, n=16), tobacco (CTS, n=12), or both marijuana and tobacco (CMTS, n=31), as well as in a larger group of NS (n=53), MS (n=40), TS (n=31), and MTS (n=44). This study did not examine the effects of these smoked substances on the alveolar epithelium or pulmonary interstitium. We hypothesized that heavy habitual smoking of cocaine or marijuana alone may lead to pathologic alterations of the tracheobronchial epithelial lining and that such changes are more extensive in smokers of cocaine or marijuana who also smoke one or more additional substances, that is, that the effects of smoking two or more substances (tobacco, marijuana, and/or cocaine) on tracheobronchial histopathology are additive.

Materials and Methods

Subjects

Male and female subjects 21 to 50 years of age were recruited for bronchoscopic study from a cohort of participants in an ongoing longitudinal study of the pulmonary effects of heavy, habitual smoking of marijuana and/or cocaine, with or without tobacco.[8,9] Participants in this study also included a comparison group of age-matched smokers of tobacco alone, as well as nonsmokers of similar age, residing in metropolitan Los Angeles. To be included in the bronchoscopic study, marijuana smokers were required to have a current smoking history of an average of ≥10 joints per week for ≥5 yrs. Moreover, cocaine smokers were included only if they had a history of current or recent (within 6 months) smoking of alkaloidal cocaine on a regular basis for ≥9 months and in an average amount of ≥1.0 g/wk during the past year. Exclusionary criteria included IV drug abuse ≥6 times per lifetime, smoking of other illicit substances ≥20 times per lifetime, a recent (within 3 weeks) upper or lower respiratory tract infection, or a history of chronic lung disease (*eg*, asthma, interstitial lung disease), previous or active tuberculosis, pneumonia within the past year, or significant occupational exposure to dust or fumes. All subjects underwent a detailed respiratory and drug use questionnaire, modified from the American Thoracic Society/National Heart, Lung, and Blood Institute respiratory questionnaire[10] and the National Institute on Drug Abuse national survey.[11] All patients also underwent routine pulmonary function testing, as previously described.[8,9]

Bronchoscopic Procedures

Prior to bronchoscopy, all subjects signed an informed consent form approved by the UCLA School of Medicine Human Subject Protection Committee indicating their willingness to undergo fiberoptic bronchoscopy, BAL (results of which will be presented separately), and bronchial mucosal biopsy. Screening procedures prior to bronchoscopy included a medical history, physical examination, 12-lead ECG, and coagulation studies. Subjects were advised not to smoke or eat for at least 8 h prior to bronchoscopy. Topical anesthesia of the upper airway was achieved with 2 to 4% lidocaine and 20% benzocaine. Subjects were sedated with IV midazolam and meperidine (administered in 0.5- to 1.0-mg and 25- to 50-mg aliquots, respectively) in accordance with UCLA Medical Center guidelines for conscious sedation. A flexible fiberoptic bronchoscope (Olympus; Melville, NY) was then orally introduced with video monitoring. The larynx, trachea, both mainstem bronchi, and their branches down to the third order were endoscopically examined. Pinch forceps biopsies were performed of the mucosa of the primary carina and four or less secondary or tertiary carinae of the right or left lung; biopsy specimens were not taken from areas showing the most severe visual mucosal alterations.

Specimen Processing and Microscopic Procedures

Bronchial mucosal biopsy specimens were fixed separately in 10% buffered formaldehyde or 95% ethanol and routinely processed for light microscopy, embedded in paraffin, cut (minimum of nine step cuts per block), and stained with hematoxylin-eosin or periodic acid-Schiff. Sections from biopsy specimens from the last 101 subjects to undergo study bronchoscopy were independently examined by light microscopy by pathologists (S.E.G.F., S.H.B.) who were masked to the clinical and smoking histories of the subjects; biopsy specimens from all 241 subjects were interpreted by one of these pathologists (S.E.G.F.). Specimens were initially examined to evaluate overall morphologic conditions and were excluded if mucosal epithelium was not adequately represented or artifactual damage was present. Intact specimens were systematically evaluated according to criteria modified from Auerbach et al[12] (Table 1). Specifically, tissues were examined to assess proliferative, metaplastic, and dysplastic changes in the epithelium, as well as inflammatory and connective tissue alterations in the epithelium, basement membrane, and subepithelium.

Data Analysis

For analysis, subjects were categorized into the following eight groups: marijuana smokers who were never or former tobacco smokers (MS); smokers of tobacco only (TS); marijuana smokers who were also current smokers of tobacco but never smoked cocaine (MTS); nonsmokers of marijuana, tobacco, or cocaine (NS); smokers of cocaine only (CS); smokers of cocaine plus marijuana but not currently (within the past 2 years) of tobacco (CMS); smokers of cocaine plus tobacco but not currently (within the past 2 years) of marijuana (CTS); and current smokers of

Table 1—*Criteria for Light Microscopic Evaluation of Bronchial Biopsy Specimens*

Criteria
○ Basal cell hyperplasia: >3 rows of basal cells
○ Stratification: flattening of surface epithelium with focal loss of cilia
○ Goblet cell hyperplasia: >25% of all epithelial cells are goblet cells
○ Cellular disorganization: alterations in the normal architectural orientation of epithelial cells
○ Nuclear variation: pleomorphism of nuclear size and shape and alterations in staining characteristics
○ Mitotic figures: present
○ Increased N/C ratio: >1/4, due to increase in nuclear size
○ Intraepithelial inflammation: ≥3 inflammatory cells per high-power field (×400)
○ Basement membrane thickening: >7 μm
○ Subepithelial inflammation: ≥5 inflammatory cells per high-power field
○ Squamous metaplasia: stratification involving all of the mucosa with total loss of cilia

cocaine, marijuana, and tobacco (CMTS). Thirteen subjects underwent bronchoscopy on two separate occasions 1.6 to 11.6 yrs apart. For these subjects, results of the most recent bronchoscopy were used in the analysis. The prevalence of histopathologic changes in the bronchial biopsy specimens was determined for subjects in each smoking category. Comparisons of these prevalence rates between pairs of smoking categories were performed using χ^2 or Fisher's Exact Test. Among smokers of only one substance, logistic regression was also used to determine the effect of the amount of each substance smoked on the presence of abnormalities. A sign test was used to test individual smoking groups against all the other groups combined to determine whether any smoking group consistently had higher prevalence rates for most features than any other group.[13] All analyses were performed using software (SAS[13] and SPSS[14]). A p value <0.05 was considered statistically significant.

To allow comparison across smoking categories of biopsy specimens from a maximum number of subjects in all smoking categories, the interpretation of the pathologist (S.E.G.F.) who had reviewed biopsy specimens from all 241 subjects was used in the main analysis. To obtain an estimate of interobserver variability in the interpretation of histopathologic alterations in bronchial mucosa from these forceps-biopsy specimens, agreement between the interpretations of the two pathologists for those 101 specimens that were examined by both was assessed using the kappa statistic.[14]

Results

Technically satisfactory biopsy specimens were obtained in 241 healthy volunteers (82.6% male; mean age, 35.4±8.3 [SD] years). Table 2 lists the number, gender, and mean age of the subjects in each smoking category and their frequency and duration of smoking marijuana, cocaine, and/or tobacco. Most subjects in all smoking categories were male. Smokers in all categories were slightly older than the nonsmokers, but smokers of cocaine, marijuana, and/or tobacco were of comparable age. Smokers of cocaine along with marijuana and/or tobacco admitted to similar amounts of crack use, whereas CS reported smoking less cocaine than the combined smokers of cocaine and one or two additional substances. Moreover, CMS smoked less marijuana currently than MS. Similarly, smokers of

Table 2—*Number (% Male), Mean Age (±1 SD), and Smoking Characteristics (Mean±1 SD) of Subjects by Smoking Category***

			Tobacco		Marijuana		Cocaine	
Category	N	Age, yr	cigs/d	pk-yrs	jts/wk	jt-yrs	g/wk	mo
NS	53	31.8	N/A	0.1	N/A	0.0	N/A	N/A
	(72)	(7.9)		(0.4)		(0.2)		
CS	14	36.9	N/A	1.0	N/A	28.7	1.5	121.5
	(79)	(11.0)		(3.6)		(52.2)	(1.0)	(58.1)
MS	40	34.5	N/A	0.4	20.9	91.9	N/A	N/A
	(78)	(8.0)		(2.2)	(26.0)	(246)		
TS	31	38.0	25.8	24.7	N/A	3.4	N/A	N/A
	(90)	(10.3)	(14.4)	(18.0)		(17.1)		
CMS	16	37.0	N/A	0.0	5.9	50.0	2.5	76.4
	(100)	(5.8)		(0.1)	(15.2)	(80.8)	(5.1)	(46.4)
CTS	12	40.2	16.3	13.9	N/A	21.3	2.0	108.8
	(75)	(6.3)	(10.2)	(9.3)		(39.6)	(1.6)	(73.9)
MTS	44	34.2	14.4	13.8	17.6	51.6	N/A	N/A
	(91)	(7.0)	(13.3)	(12.7)	(22.7)	(51.9)		
CMTS	31	38.4	17.2	17.8	3.7	35.4	2.4	92.4
	(84)	(7.2)	(14.3)	(17.4)	(2.2)	(59.6)	(2.1)	(67.2)

*cigs/d=number of cigarettes currently smoked per day; pk-yrs=the product of the number of packages of cigarettes smoked per day and the number of years of smoking; jts/wk=number of joints of marijuana currently smoked per week; jt-yrs=the product of the number of joints of marijuana smoked per day and the number of years of smoking; g/wk=number of grams of cocaine smoked per week; mo=the number of months cocaine was smoked regularly; N/A=not applicable.

tobacco in combination with marijuana and/or cocaine smoked less tobacco than TS.

Table 3 summarizes the frequency of acute and chronic respiratory symptoms reported by the subjects undergoing bronchoscopy. Compared with NS, CS or MS did not report a significantly higher frequency of symptoms of chronic bronchitis (cough and sputum for ≥3 months out of the year for ≤2 years) or a significantly higher incidence of acute bronchitic episodes, although the prevalence of wheeze was significantly higher in MS than NS ($p<0.05$). Moreover, few CMS reported acute or chronic respiratory symptoms. In contrast, compared with NS, a substantially larger proportion of smokers of tobacco alone or with cocaine and/or marijuana acknowledged symptoms of chronic bronchitis ($p<0.05$), and a variably higher proportion of subjects in the different tobacco-smoking categories complained of wheeze ($p<0.05$), multiple episodes of acute bronchitis and shortness of breath ($p<0.05$, CTS and TS vs NS), without evidence to suggest an additive effect.

Table 4 lists the results of pulmonary function tests for the subjects undergoing bronchoscopy. Mean values for spirometric indexes and single-breath diffusing capacity were within normal limits for subjects in all smoking categories. No differences were noted across the different categories in either percent predicted values or in the frequency of abnormality (data not shown) for any of the lung function measures, with the exception of diffusion of carbon monoxide, which was significantly lower in CMTS, compared to NS, MS, and MTS.

Figure 1 illustrates representative histologic appearances in biopsy specimens obtained from subjects in the different nonsmoking/smoking groups.

Table 5 lists the percentage of subjects in each smoking category with histopathologic abnormalities of the tracheobronchial mucosa with respect to each of the following characteristics: basal cell hyperplasia, stratification, squamous cell metaplasia (present and marked), goblet cell hyperplasia (present and marked), nuclear variation, mitotic figures, increased nuclear/cytoplasmic (N/C) ratio, epithelial inflammation (marked), basement membrane thickening, and submucosal inflammation. The significance levels for each histopathologic feature for each smoking group compared with the NS group by χ^2 or Fisher's Exact Test are also indicated.

MS and TS both exhibited more frequent abnormalities than NS for *all* 11 histopathologic features assessed, and the differences from NS were statistically significant for most of these features (Table 5). For most features, MS exhibited more frequent abnormalities than TS, but the differences were not statistically significant. CS showed more frequent abnormalities than NS in 10 of the 11 features ($p<0.002$; sign test), and the difference in the prevalence of abnormality from NS was statistically significant ($p<0.05$) for three features (basal cell hyperplasia, squamous cell metaplasia, stratification)

Table 4—*Mean Spirometry Results (±1 SD) in Subjects Undergoing Bronchial Biopsy by Smoking Category*

Category	FEV_1, % Predicted	FVC, % Predicted	FEV_1/FVC, % Predicted	Dco,* % Predicted
NS	104.1	96.8	108.5	99.7
	(12.7)	(10.9)	(5.7)	(14.8)
CS	98.1	90.8	109.8	98.1
	(23.3)	(21.9)	(8.2)	(19.2)
MS	108.4	102.6	106.7	93.7
	(14.4)	(11.1)	(8.2)	(14.9)
TS	101.4	96.3	106.4	84.6
	(19.0)	(16.9)	(8.4)	(13.7)
CMS	98.1	90.1	104.8	90.9
	(14.5)	(12.7)	(7.8)	(17.5)
CTS	95.0	91.9	104.8	85.3
	(14.5)	(11.5)	(10.9)	(14.7)
MTS	105.7	103.8	102.8	91.8
	(11.8)	(11.0)	(7.5)	(17.2)
CMTS	93.2	90.1	104.8	75.3†
	(13.6)	(12.7)	(7.8)	(13.7)

*Dco=diffusion of carbon monoxide.
†Significantly less than values in NS, MS, and MTS ($p < 0.05$).

Table 3—*Prevalence of Chronic Respiratory Symptoms by Smoking Category, Percent*

Category	Cough	Sputum	Wheeze	Acute Bronchitic Episodes*	Shortness of Breath
NS	15	3	0	3	0
CS	15	15	8	8	0
MS	14	8	19	14	0
TS	24	29	28	14	17
CMS	7	7	0	0	0
CTS	25	25	18	9	25
MTS	20	23	31	14	6
CMTS	23	33	14	20	7

*More than one episode of increased cough and sputum lasting ≥3 weeks within the past 3 years.

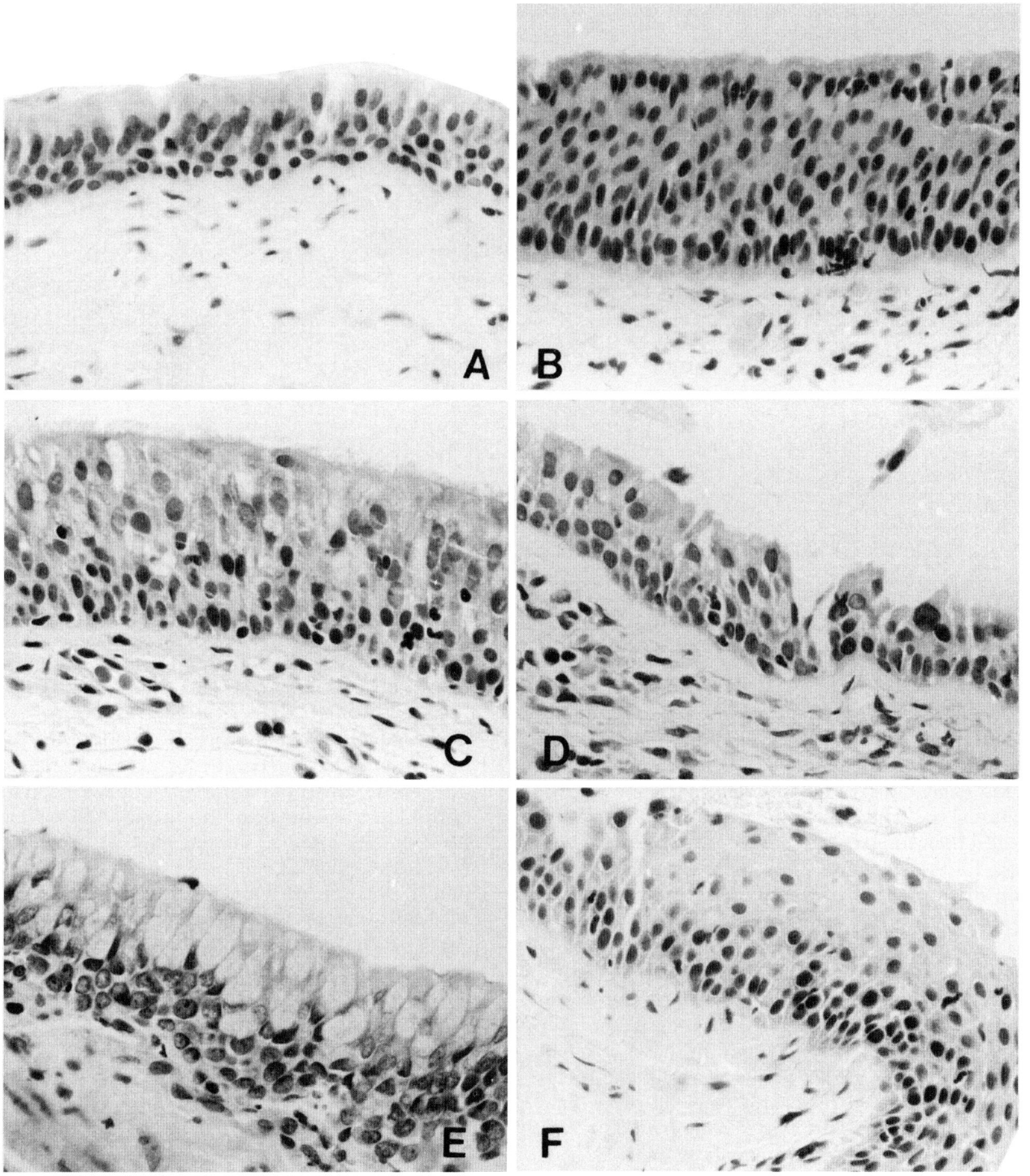

FIGURE 1. *Top left* (*A*): Normal bronchial biopsy specimen from an NS (hematoxylin-eosin [H&E], original magnification ×400). *Top right* (*B*): Prominent reserve cell hyperplasia and scattered intraepithelial inflammatory cells in a CMS (H&E, original magnification ×400). *Center left* (*C*): Prominent intraepithelial inflammation, variation in nuclear size and shape, cellular disorganization, and reserve cell hyperplasia in a CMTS (H&E, original magnification ×400). *Center right* (*D*): Markedly thickened basement membrane, increased N/C ratio in occasional epithelial cells, and moderate submucosal inflammation in a CMS (H&E, original magnification ×400). *Bottom left* (*E*): Extensive goblet cell hyperplasia in a CMS (H&E, original magnification ×400). *Bottom right* (*F*): Well-developed squamous metaplasia with focal hyperchromatic nuclei showing increased N/C ratio in a CTS (H&E, original magnification ×400).

Table 5—*Percentage of Subjects With Histopathologic Abnormalities in Bronchial Mucosal Biopsy Specimens by Smoking Category*

Category	NS (n=53)	CS (n=14)	MS (n=40)	TS (n=31)	CMS (n=16)	CTS (n=12)	MTS (n=44)	CMTS (n=31)
Basal cell hyperplasia	11.8	42.9‡	72.5*	53.3*	50.0†	91.7*	75.0*	73.3*
Stratification	9.8	35.7‡	50.0*	36.7†	28.6§	66.7*	54.8*	44.4*
Goblet cell hyperplasia	29.4	14.3	67.5*	76.7*	50.0	75.0†	81.8*	80.6*
Cell disorganization	33.3	57.1	58.3‡	53.3§	21.4	66.7‡	76.2*	44.4
Nuclear variation	35.3	64.3	52.5	56.7§	31.3	83.3†	70.5*	51.6
Mitotic figures	7.8	14.2§	15.0	23.3§	6.3	16.7	31.8†	22.6§
Increased N/C ratio	5.9	21.4	40.0*	30.0†	31.3‡	66.7*	54.5*	45.2*
Inflammation	62.7	78.6	85.0‡	76.7	56.3	91.7§	86.4†	80.6§
Basement membrane thickening	19.6	42.9§	65.0*	55.2*	43.8§	58.3‡	50.0†	64.5*
Subepithelial inflammation	88.2	100.0	95.0	90.0	75.0	75.0	95.5	64.5*
Squamous cell metaplasia	5.9	28.6‡	33.3*	31.0†	25.0‡	58.3*	41.9†	25.8†

*p<0.001.
†p<0.01.
‡p<0.05.
§p<0.10 (Note: in all p values, comparison of percentage of abnormality for designated histopathologic feature with percentage of abnormality among NS, χ^2 or Fisher's Exact Test).

and nearly significant ($p \leq 0.09$) for two additional features (mitotic figures and basement membrane thickening).

Considering all eight smoking categories, abnormalities were noted *most* frequently in combined smokers of tobacco along with either cocaine (for 6 of the 11 features) or with marijuana (for 3 of the 11 features) ($p<0.004$, sign rank test for CTS and MTS vs all other smoking categories [Table 5]). In contrast, for every feature, smokers of cocaine together with marijuana but *without* tobacco exhibited even less frequent abnormalities than smokers of marijuana alone. However, combined smokers of cocaine and tobacco had more frequent alterations in 9 of the 11 histopathologic features than smokers of tobacco only ($p<0.004$, sign rank test), despite lower amounts of tobacco consumption in the dual smokers. Moreover, MTS had more frequent abnormalities in 10 of the 11 histopathologic categories than smokers of either substance alone ($p<0.002$, sign rank test), despite lower amounts of both tobacco and marijuana consumption in smokers of both substances than in the smokers of one or the other single substance. Smokers of all three substances did not show more frequent alterations than smokers of tobacco and only one additional substance (with the exception of basement membrane thickening).

No dose-response relationship could be demonstrated between the current intensity or lifetime cumulative amount of smoking for any of the three substances and the presence of histopathologic alterations for any of the 11 features examined, with the single exception of mitotic figures, the frequency of which was increased with increasing amounts of current tobacco and marijuana smoking ($p<0.05$; logistic regression).

Agreement between the interpretations of the two pathologists who independently examined biopsy specimens from 101 of the 241 subjects was good for 9 of the 11 histopathologic features ($\kappa=3.85$ to 7.52; $p<0.001$), fair for submucosal inflammation ($\kappa=2.65$; $p<0.01$), and poor for intraepithelial inflammation ($\kappa<1.0$). With the exception of subepithelial inflammation, interpretations were the same (normal/normal or abnormal/abnormal) in an average of 74% of the subjects for all histopathologic features, with a range of 65 to 91% for specific features.

Discussion

We performed and evaluated bronchial mucosal biopsy specimens in smokers of one or more of three commonly smoked substances (cocaine, marijuana, and tobacco), as well as in nonsmoking control subjects. Of the 241 biopsy specimens analyzed, 158 were new tissue samples (including samples from 73 smokers of cocaine alone or with marijuana and/or tobacco) not included in our previously published reports.[5-7] Consistent with trends that were apparent from these earlier studies,[5-7] results now presented from a larger population of subjects from whom biopsy specimens were taken confirmed that habit-

ual smoking of marijuana alone caused at least as extensive histopathologic abnormalities in the tracheobronchial mucosa as tobacco alone, including metaplastic changes and nuclear alterations that may be premalignant. Moreover, the prevalence of abnormal histopathologic features was higher among smokers of both marijuana and tobacco than among smokers of either tobacco or marijuana alone for most categories of abnormality, suggesting an additive effect of marijuana plus tobacco, despite the fact that smokers of both marijuana and tobacco smoked less tobacco than smokers of tobacco only and less marijuana than smokers of marijuana only. Furthermore, because of the inclusion of far more cocaine smokers than had been studied previously, we are now able to report several new findings pertaining to the impact of habitual use of cocaine on airway abnormalities.

First, of those individuals who smoked only one substance, CS generally showed less frequent and less severe histopathologic alterations than were noted in MS or TS. However, for most features, the morphologic alterations in the bronchial biopsy specimens from CS were greater in frequency and severity than those noted in biopsy specimens from NS. The less frequent and extensive microscopic findings in the CS than among the smokers in other categories are consistent with the relatively low frequency of chronic respiratory symptoms reported by CS in comparison with the prevalence of symptoms of both acute and chronic bronchitis in TS and together with marijuana and/or cocaine (Table 3). Comparison of the amounts of cocaine, marijuana, and tobacco smoked per week or per day (Table 2) suggests differences of one or more orders of magnitude in exposure of the lungs to the smoke of these three substances. Consequently, the disparity in the prevalence of histopathology and symptoms of chronic bronchitis between CS and smokers in other categories might reflect the substantial quantitative differences in exposure of the lower airways to the smoke of cocaine compared to that of marijuana and/or tobacco.

Second, although the number and severity of morphologic alterations were generally greater in smokers of more than one substance (for example, in marijuana plus tobacco smokers), compared to single-substance smokers, for most features (6/11), the findings were more extensive when cocaine, rather than marijuana, was the additional substance in conjunction with tobacco. For example, basal cell hyperplasia was noted in 92% of CTS and only 75% of MTS. Similarly, stratification, squamous metaplasia, nuclear variation, and increased N/C ratio were noted in a higher proportion of CTS than MTS. These observations may represent an additive or potentiating influence of cocaine with the effect of tobacco on bronchial histopathology. In contrast, cocaine does not appear to augment the effect of marijuana on airway histopathologic features, while the effects of marijuana and tobacco appear to be additive. Possible mechanisms as to how cocaine might exacerbate the effect of tobacco are not known and warrant further study. Since cigarette-related chronic bronchitis is associated with increased polymorphonuclear leukocytes in both bronchial tissue[15] and airway lining fluid,[16] recent evidence that cocaine administered short term *in vivo* causes acute activation of polymorphonuclear leukocytes[17] suggests at least one possible mechanism, whereby cocaine smoking might aggravate tobacco-induced airway injury.

Third, for all histologic features, the frequency of alterations in the smokers of *both* marijuana and cocaine was less than that in the MS. These observations are suggestive of an interaction between marijuana and cocaine in the direction of amelioration of the effect of marijuana by cocaine, in contrast to the apparent additive effects of tobacco and cocaine. However, the less frequent alterations in the dual smokers of cocaine and marijuana than in the MS may simply be due to sampling differences related to the relatively small numbers of CMS studied (n=16), in comparison with MS (n=40). In addition, the substantially lower intensity of current marijuana smoking reported by CMS (<1 joint per day) than by MS (approximately three joints per day) may have accounted for the less extensive bronchial mucosal histopathologic condition evident in the CMS group compared with the MS.

Thirteen subjects had biopsy specimens taken twice during the course of our study (1982 to 1996) with an average interval between separate biopsies of 4.9±3.0 (SD) years. Eight of these 13 subjects retained the same smoking status (2 NS, 2 MS, 3 TS, and 1 MTS). In the remaining five subjects, smoking status changed as follows: CS→CMTS; MS→CS; MS→MTS; MTS→MS; and MTS→TS. Histopathologic alterations remained infrequent in the continuing nonsmokers and generally remained the same or worsened in the persistent smokers of marijuana alone, tobacco alone, or marijuana plus tobacco in those who had additional biopsies. In the two smokers who added another substance, an increasing number of features became abnormal, while in the two dual smokers who quit smoking one substance and the one subject who quit smoking marijuana but started smoking cocaine, the frequency of abnormalities remained unchanged. Too few subjects were restudied, however, to establish a definite pattern of progression in those who continued to smoke, started smoking an additional sub-

stance, or substituted one substance for another to detect any definite regression of histopathologic alterations in dual smokers who eliminated a single substance. A larger number of subjects will need to undergo repeated bronchoscopic mucosal biopsies to ascertain the impact of continuation or cessation of smoking of one or more substances on bronchial mucosal histopathologic features.

In conclusion, based on bronchial histopathology findings in 241 NS and smokers of cocaine, marijuana, and/or tobacco, cocaine leads to more frequent bronchial morphologic alterations when smoked alone than were noted in NS but generally less frequent abnormalities than were found in smokers of MS or TS. When smoked in conjunction with tobacco, cocaine appears to augment the bronchial damage induced by tobacco smoke, but no additive effect was noted between cocaine and marijuana. Marijuana and tobacco are each associated with strikingly significant bronchial histopathologic features when smoked as single substances, and the effects of marijuana and tobacco appear additive. Findings from the present study need to be interpreted with caution, however, since the smoking status of the subjects who underwent bronchoscopy was based on self-report, and the validity of the reported frequency and duration of smoking of cocaine, marijuana, and/or tobacco was dependent on the accuracy and integrity of the subjects' recall. Furthermore, since most crack smokers smoke more than one substance, the number of smokers of cocaine alone or with only one other substance (either marijuana or tobacco) was limited. Moreover, in the smokers of cocaine in combination with either marijuana or tobacco, it was not possible to match precisely the quantity of each substance smoked to that of control smokers of cocaine, marijuana, or tobacco alone. Therefore, further studies are required to assess the effects of cocaine when smoked by itself and in conjunction with other substances on airway histopathology. Moreover, the pathogenetic mechanisms whereby cocaine appears to augment the noxious effects of tobacco warrant additional investigation.

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