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SH Reactivity of cigarette smoke and its correlation with carcinogenic effects on hamster lung cultures

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The "tar" component of cigarette smoke may not play the critical role in tobacco carcinogenesis attributed to it in the past. Results reported here point to a major role for gas vapour phase components and suggest a biological mechanism for their action.

I. Introduction

Epidemiological studies undertaken during the last 25 years have shown that smoking of cigarettes is one of the main causes of human lung cancer [17]. However, the question which specific components of cigarette smoke are responsible for pulmonary carcinogenesis cannot be answered conclusively at present. Elucidation of this interrelationship poses great problems mainly for the following reasons: the chemical composition of cigarette smoke is very complex because of its very large number of components, partly present as particles (particulate phase), partly as gases and vapours (gas vapour phase). Furthermore, there are inherent difficulties in the experimental replication of the voluntary human habit of inhaling puffs of fresh cigarette smoke. Nevertheless, the modelsystem of exposing human and animal lung cultures to puffs of fresh cigarette smoke under standardized conditions, developed by us, has proved to be a suitable bioassay for assessing the interrelation between chemical constituents of cigarette smoke and carcinogenic effects on the respiratory system [7, 9, 10, 14].

We report on a correlated chemical and biological study designed to examine the question whether differences in amounts of the particulate phase or differences in the amounts of the gas vapour phase in fresh cigarette smoke influence the growth of hamster lung cultures, especially the development of malignant transformation. In view of the fundamental importance of SH groups in cell metabolism and division [4], and since the gas vapour phase is known to contain constituents, such as free radicals or NO, which inhibit SH dependent enzyme systems [6, 15, 16], special attention was paid to the interrelation between quantitative differences of SH reactivity and biological effects of the cigarette smoke on these lung cultures (Table 1).

[1] Technical assistance.

II. Methods and Material

For this study primary cultures of hamster lung were exposed to puffs of fresh cigarette smoke in a CSM12 smoking machine under standardized conditions, as previously described [7, 9, 10]. Twelve different experiments were carried out comprising over 7000 hamster lung cultures. For each experiment a minimum of 2 sets of cultures consisting of 40 coverslips and 40 bottles with primary matched lung cultures were used. The sets comprised non exposed control cultures, and cultures exposed to 4 puffs daily (25 ml at intervals of 58 s.) of fresh smoke from 8 different types of cigarettes on three consecutive days per week for a period of 1 week up to 6 months. The smoke from the 8 types of cigarettes was analyzed by gaschromatography and/or chemically, and the SH reactivity (SH index) was determined as previously described [13] (Table 1).

Table 1
Effects of Quantitative Differences in Constituents and SH Reactivity of Cigarette Smoke on Growth of Hamster Lung Cultures

Type of cigarette	Particulate Matter			Gas Vapour Phase						SH index of smoke	Biological Effects (sequence of events)		
	TPM mg	Tar mg	Nicotine mg	CO mg	HCN µg	NO µg	TGVP r. d.	Acetaldehyde r. d.	Acrolein r. d.		Stage I Cytotoxicity	Stage II Atypical growth	Stage III Malignant transformation
C 1	4,8	4,4	0,4	5,0	35	33	<28	traces	traces	5,1	(+)	(+)	–
C 2	5,9	5,5	0,4	4,9	44	31	52	2,0	0,12	9,5	(+)	++	–
C 3	6,2	5,8	0,4	5,1	53	36	42	1,9	0,18	9,8	(+)	++	–
C 4	8,5	8,2	0,3	8,5	77	33	73	4,1	0,18	12,7	++ – +++	++ – +++	–
C 5	8,9	8,4	0,5	7,3	73	117	59	3,0	0,18	19,6	++++	++++	++++
Kentucky Standard	10,5	9,8	0,7	7,3	153	104	70	3,6	0,20	23,5	++++	++++	++++
C 6	6,5	6,1	0,4	7,5	51	354	59	1,9	0,08	29,9	++++++	++++++	++++++
C 7	5,9	5,6	0,3	6,8	40	378	68	2,2	0,10	35,7	++++++	++++++	++++++

– = negative ++ = mild ++++ = marked TPM = total particulate matter
(+) = doubtful ++ – +++ = moderate ++++++ = very marked TGVP = total gas vapour phase

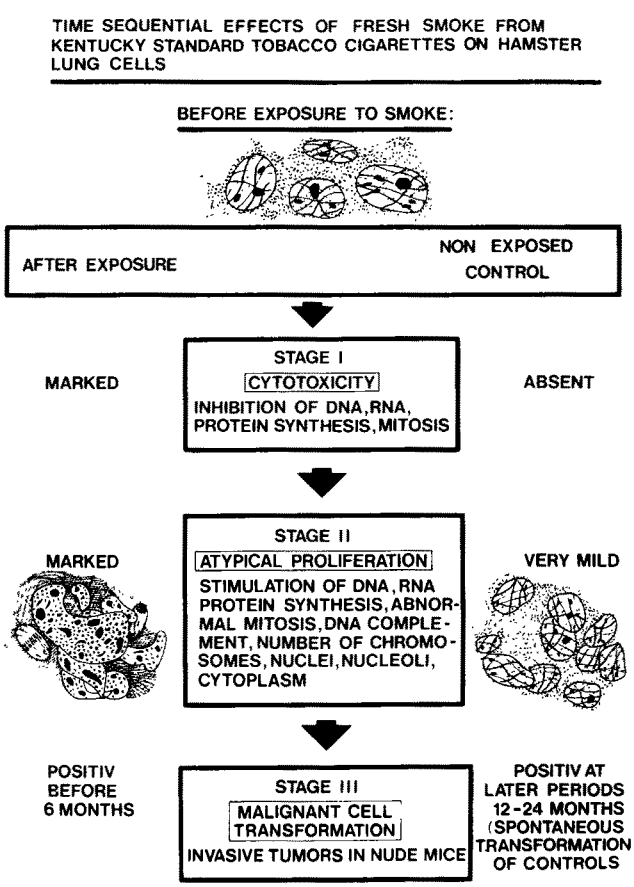
r. d. = relative delivery

All analytical values are calculated for 4 puffs

The assessment of the biological effects of the smoke on the cultures was done in living cultures and in fixed and stained preparations from a morphological and cytochemical point of view [7, 9, 10], without knowledge of the analytical results of the smoke. Cultures exposed to smoke from C_1-C_7 cigarettes were compared among each other, with those of non exposed control cultures and cultures exposed to smoke from Kentucky Standard cigarettes. The latter exposed cultures were used as a standard of reference because they displayed 3 main well defined stages of sequential morphological and cytochemical alterations, namely cytotoxicity, atypical growth, and malignant transformation (Fig. 1) within a period of 6 months [7, 9, 10, 14].

III. Results

Results were reproducible in all experiments. As can be seen from the data in Table 1, with exception of the cultures exposed to smoke from C_1 cigarettes which resembled control cultures, smoke from all other cigarettes produced alterations in the cultures. However, smoke from C_2-C_4 cigarettes evoked less abnormalities in growth, namely only atypical growth, than smoke from Kentucky Standard and C_5-C_7 cigarettes, which evoked malignant cell transformation. Relating these different biological effects to the analyzed chemical constituents of the smoke, it can be seen that amounts of TPM, tar and nicotine did not influence significantly the occurrence of atypical growth and/or malignant transformation in the hamster lung cultures. Smoke from C_1 cigarettes which did not evoke any alterations in the cultures had only a slightly lower tar content than smoke from C_2 and C_7 cigarettes which produced atypical growth and very marked malignant transformation respectively. Furthermore, smoke from Kentucky Standard cigarettes with a relatively high tar content led to less marked malignant transformation than smoke of C_6 and C_7 cigarettes having a significantly lower tar content. Therefore, these results obtained with fresh cigarette smoke do not support the widely accepted concept, based on skinpainting experiments with "tar" of cigarette smoke condensates, that it is mainly "tar" of cigarette smoke which is responsible for lung cancer of the human cigarette smoker [18]. On the other hand the amounts of gas vapour phase, and especially the SH reactivity of the smoke, seemed to play a role for the occurrence of atypical growth and of malignant transformation of the hamster lung cultures. Smoke from C_1 cigarettes which had the lowest amounts of TGVP, of acrolein and acetaldehyde, and the lowest SH index did not produce any alterations while smoke from all cigarettes with higher amounts of TGVP and higher SH index evoked atypical growth and/or malignant transformation. There exists especially a striking positive correlation between SH index and biological effects of the smoke on the cultures. The higher the SH index the greater the number and severity of the alterations, particularly of those of growth abnormalities. Smoke with relatively lower SH index produced atypical growth, while smoke with significantly higher SH index evoked malignant transformation. Furthermore, the malignant transformation was more marked and was observed at an earlier period (3 months) after exposure to smoke from C_6 and C_7 cigarettes which had a higher SH index than after smoke from C_5 and Kentucky Standard cigarettes (6 months) which had a lower SH index. It can also be seen, that there is a positive correlation between amounts of the SH reactive gas vapour phase constituent NO, and occurrence of malignant transformation. Only smoke with high amounts of NO evoked malignant transformation, while smoke with relatively low content of NO produced only atypical growth. Furthermore, the most marked transformation was observed after smoke with NO content above 350 μg.



IV. Discussion

The positive correlation between high SH reactivity and high NO content of the gas vapour phase of fresh cigarette smoke and malignant transformation is in good accordance with observations that atypical growth

and enhancement of pulmonary carcinogenesis occurred not only after exposure to whole smoke but also after exposure to the gas vapour phase only [7, 8, 9, 11, 12]. That high NO content of the gas vapour phase appeared to play a role in the malignant transformation is of special interest. NO is considered to be a potential precursor in the formation of N-nitrosamine, a carcinogenic substance found in cigarette smoke [3]. The present data indicating a positive correlation between SH reactivity and carcinogenic effects of cigarette smoke are also in accordance with recent observations that 3–4 benzpyrene carcinogenesis in mice can be reduced or completely inhibited by thiols [5], and that there is tumor rejection in animals treated with radioprotective thiols [1]. The results obtained in the present study appear also to add evidence for the hypothesis that one of the causes of human lung cancer may be the inhalation of components in cigarette smoke which react with thiols, thereby removing free cysteine from the bronchial epithelium [2].

Further chemical and biological investigations of gas vapour phase constituents with SH reactivity of cigarette smoke are urgently needed. Their chemical characterization and their elucidation of mechanisms of action in the process of malignant cell transformation may also be of help in attempts to provide cigarettes with less pathobiological effects.

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Summary

After hamster lung cultures were exposed repeatedly to puffs of fresh smoke from 7 types of cigarettes containing variable amounts of particulate and gas vapour phase components, atypical growth and/or malignant cell transformation were observed within a period of 3–6 months. A positive correlation was demonstrable between high SH reactivity and high NO content of the gas vapour phase and malignant transformation. There was no positive correlation for the other analyzed components of the smoke, including tar content.

Zusammenfassung

SH-Reaktivität von Zigarettenrauch und ihre Korrelation mit karzinogenen Wirkungen auf die Hamster-Lungenkultur

Die wiederholte Berauchung von Hamster-Lungenzellkulturen mit frischem Rauch von 7 Zigarettenarten mit unterschiedlichen Mengen Teilchenphase- und Gas-Dampf-Phase-Komponenten führte innerhalb eines 3–6monatigen Zeitraumes zu atypischem Wachstum und/oder bösartigen Zellveränderungen. Zwischen hoher SH-Reaktivität sowie hohem NO-Gehalt der Gas-Dampf-Phase und bösartigen Veränderungen wurde eine positive Korrelation nachgewiesen, während die anderen untersuchten Rauchkomponenten, darunter der Teergehalt, keine positive Korrelation zeigten.

Résumé

Réactivité au SH de la fumée de cigarettes et sa correlation avec les effets carcinogènes sur les cultures du poumon d'hamsters

L'exposition de cultures cellulaires de poumons d'hamsters à la fumée fraîche de 7 types de cigarettes avec quantités variables des facteurs chimiques de la phase particulaire et de la phase vapeur-gaz a resulté après une période de 3–6 mois dans une croissance atypique et/ou dans une transformation cellulaire maligne. Une corrélation positive a été démontrée entre une haute réactivité SH ainsi qu'une haute teneur en NO de la phase gaz-vapeur et la transformation maligne. Il n'y avait aucune correlation positive avec les autres facteurs chimiques analysés, dont la tenue en goudron.

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