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DNA methylation by N-nitrosomethylbenzylamine in target and non-target tissues of NMRI mice

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Abstract

Adult female NMRI mice received a single i.p. injection of N-nitroso(*methyl*-^{14}C)methylbenzylamine (2.5 mg/kg body weight). Multiple weekly applications of such a dose, by this route, have previously been shown to induce lung adenomas and forestomach carcinomas in all experimental animals. After a survival time of 6 h, DNA was isolated from various tissues and analysed for methylated purines by separation of the acid hydrolysate on Sephasorb columns. Highest concentrations of 7-methylguanine and O^6-methylguanine were present in hepatic DNA, followed by lung and forestomach. DNA methylation in the oesophagus was only 21% less than in forestomach. Since both tissues develop a high tumour incidence after oral administration of N-nitrosomethylbenzylamine (MBN), this observation suggests that despite their anatomical similarities the level of DNA modification required for malignant transformation differs considerably in these tissues. In the remaining organs, DNA alkylation was either considerably less (colon, glandular stomach, kidney) or not detectable (small intestine, spleen). These data indicate that following i.p. injection in mice, MBN is preferentially metabolised in a non-target organ (liver). Among the various other tissues investigated, highest levels of initial DNA methylation were present in forestomach and lung, i.e., the principal target organs of MBN for this route of application.

Introduction

Organ-specific tumour induction by N-nitrosomethylbenzylamine (MBN)* varies considerably in different species. Chronic administration of MBN to rats causes a selective induction of squamous cell carcinomas of the oesophagus. This effect is largely independent of the route of application, a 90–100% tumour incidence being observed after both oral administration in the drinking water (1) and weekly s.c. injections (2). Biochemical studies revealed that DNA methylation by MBN is considerably more extensive in the oesophagus than in any other rat tissue (3). Preferential bioactivation of the parent carcinogen in the target tissue has, therefore, been suggested to be responsible for its organ-specific carcinogenicity in the rat (3).

In mice, chronic administration of MBN in the drinking water (20 p.p.m.) causes the development of carcinomas of the oesophagus and forestomach in up to 100% of experimental animals (4). In contrast, weekly i.p. injections (2.5 mg/kg) were found to selectively induce forestomach carcinomas and lung adenomas but no oesophageal neoplasms (4). In the present study we have determined the initial extent of DNA methylation in various mouse tissues following a single i.p. injection of N-nitroso (*methyl*-^{14}C)methylbenzylamine ([^{14}C]-MBN). Experiments using ^{14}C-methylene-labelled MBN were not carried out since previous *in vivo* studies (6) showed no evidence for the formation of a benzylating intermediate.

Materials and Methods

Forty adult female NMRI mice (average body weight 20 g) received a single i.p. injection (2.5 mg/kg) of [^{14}C]MBN, synthesized as described by Skipper (5), at a specific radioactivity of 21 mCi/mmol and radiochemical purity of greater than 99%. Animals were killed 6 h later by exsanguination during light ether anaesthesia. Organs were rapidly removed, frozen in liquid nitrogen and stored at −70°C. DNA was isolated by phenol extraction from the combined tissues of all mice (7). After hydrolysis in 0.1 M HCl (37°C, 20–24 h), DNA purines were separated on Sephasorb columns (1 x 50 cm) eluted with 10 mM phosphate buffer (pH 5.5) as described earlier (3).

Results and Discussion

The present experiments were carried out with a single i.p. injection of 2.5 mg/kg of ^{14}C-methyl-labelled MBN. Long-term carcinogenicity studies in NMRI mice by Sander and Schweinsberg (4) have shown that weekly applications of such a dose, by this route, lead to the formation of forestomach carcinomas and lung adenomas in all experimental animals at a mean survival time of 17 months (total administered dose 57.5 mg/kg). Under these experimental conditions, no oesophageal tumours were induced. The present study was designed to determine in which mouse tissues MBN is metabolised to form a methylating intermediate, and whether the initial extent of DNA methylation correlates with the preferential sites of tumour induction.

The data in Table I summarize the extent of base

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*Abbreviations: MBN, N-nitrosomethylbenzylamine; [^{14}C]MBN, N-nitroso(*methyl*-^{14}C)methylbenzylamine; 7-MeG, 7-methylguanine; O^6-MeG, O^6-methylguanine.

Table I

Methylated purines in various tissues of NMRI mice and Wistar rats following a single injection of [^{14}C]MBN[a].

Organ	7-MeG[b]		O^6-MeG[b]		O^6-/7-MeG	
	Mouse	Rat	Mouse	Rat	Mouse	Rat
Liver	162.9 ± 20.2[c]	120.2	11.8 ± 0.4[c]	4.9	0.072	0.04
Lung	46.1[d]	64.9	4.8[d]	7.7	0.104	0.12
Forestomach	23.3	10.3	1.7	n.d.	0.073	–
Oesophagus	18.3	344.5	1.5	46.1	0.082	0.13
Colon	5.2[d]	n.d.	n.d.	n.d.	–	–
Stomach	2.5	2.3	n.d.	n.d.	–	–
Kidney	2.3	3.3	n.d.	n.d.	–	–
Small intestine	n.d.	n.d.	n.d.	n.d.	–	–
Spleen	n.d.	0.9	n.d.	n.d.	–	–

[a]Animals received a single i.p.(mice) or i.v.(rats) injection of [^{14}C]MBN (2.5 mg/kg) and were killed 6 h (mice) or 4 h (rats) later. Data on rats from Hodgson *et al.* (3).
[b]Expressed as fraction of guanine x 10^6.
[c]Mean of three DNA samples ± standard deviation.
[d]Mean of two determinations.
n.d., not detectable.

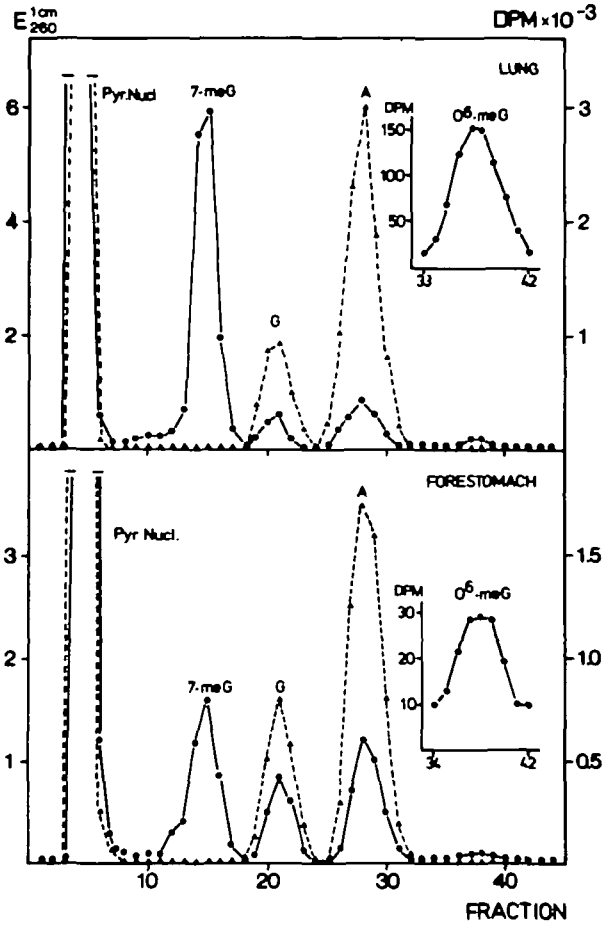

Fig. 1. Radiochromatographs of acid DNA hydrolysates from lung and forestomach. Mice were given a single i.p. injection of [^{14}C]MBN (2.5 mg/kg) and killed 6 h later. DNA was isolated by phenol extraction from the combined tissues of 40 animals. Neutralised hydrolysates were separated on Sephasorb HP columns eluted with 10 mM phosphate buffer (pH 5.5) at a flow rate of 1.3 ml/min (fraction volume 3.4 ml). ●——●, d.p.m.; ▲——▲, $E^{1\,cm}_{260}$

alkylation in various tissues. Representative radiochromatographs of DNA hydrolysates from lung and forestomach are shown in Figure 1. Highest concentrations of methylated purines were found in mouse liver. In the principal target tissues, i.e., lung and forestomach, levels of 7-methylguanine (7-MeG) and O^6-methylguanine (O^6-MeG) were considerably lower than in the liver but higher than in the remaining non-target organs. Comparison of the present data with those found in rats under similar experimental conditions (3) showed that in both species MBN methylates hepatic DNA to a similar extent (163 and 120 μmol 7-MeG/mol guanine). However, in rats DNA methylation in oesophagus was more than 30 times greater than in forestomach whereas in mice (Table I) the levels of DNA alkylation were higher in forestomach than in oesophagus.

The amount of 7-MeG produced in forestomach DNA of mice (Table I) was more than twice as high as that in rats. In contrast to mice, the promutagenic base, O^6-MeG, was not present in detectable amounts in the rat forestomach. This suggests that the high susceptibility of mouse forestomach to malignant transformation by MBN is due to its greater capacity for bioactivation of systemically administered MBN. However, the present data also indicate that a preferential bioactivation in the target tissue, which was evident in the rat (3), is not a prerequisite for tumour induction in the upper gastrointestinal tract of the mouse.

The observation that i.p. injections of MBN in mice do not induce oesophageal tumours although the initial extent of DNA methylation is only 21% less than that in the forestomach suggests that a different level of DNA modification is required for tumour induction in these tissues. Oral administration of MBN is likely to lead to a more extensive DNA methylation in the oesophagus due to direct uptake by the oesophageal mucosa and has indeed been shown to result in a 100% tumour incidence

in both oesophagus and forestomach (4). Lung tumours have been induced in mice by a great variety of chemical carcinogens. As with MBN such tumours are usually multiple adenomas. The present study indicates that MBN is metabolised to a significant extent in mouse lung but that the level of alkylation is still 30% lower than in rats following a similar dose of MBN (3). This indicates that the basic susceptibility for malignant transformation in the lung is considerably greater in mice than in rats.

In contrast to oesophageal tumour induction by MBN in rats, the organ-specificity of MBN in mice cannot be explained by a preferential bioactivation of the parent carcinogen in the target tissue. As shown for several other carcinogens, the greatest extent of initial DNA modification was present in liver, followed by the principal target organs. The reasons for the resistance of liver cells to single or multiple high doses of methylating carcinogens remain unclear. The presently available data on the formation and persistence of carcinogen-induced DNA lesions and the rate of cell turnover in target and non-target tissues do not allow the formulation of a unifying theory for the organ-specific carcinogenicity of alkylating agents.

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