Edited by

K. LETNANSKY

BIOLOGY OF THE CANCER CELL

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PROCEEDINGS OF THE FIFTH MEETING OF THE
EUROPEAN ASSOCIATION FOR CANCER RESEARCH (E.A.C.R.)
9–12 SEPTEMBER 1979
VIENNA, AUSTRIA

Edited by

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Institute for Cancer Research
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1980
KUGLER PUBLICATIONS AMSTERDAM

ISBN: 90-6299-003-7

Published by:
KUGLER PUBLICATIONS BV
P.O. Box 516, 1180 AM Amstelveen
The Netherlands

Set in the United Kingdom by H. Charlesworth & Co. Ltd., Huddersfield
Printed and bound in the Netherlands
by Intercontinental Graphics b.v., Dordrecht
and Stokkink's Boekbinderij b.v., Amsterdam

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FOREWORD

The publication of the Proceedings of the 5th Meeting of the European Association for Cancer Research gives me the opportunity to thank those persons responsible for the success of the meeting. In the first instance, the local organiser Doz. Dr. Karl Letnansky and the treasurer Prof. Dr. Viktor Dostel both played a major role in planning the scientific concept as well as in the practical organization of the meeting.

The Executive Committee of the Association and the Advisory Scientific Committee established the guidelines for the meeting. A discussion of the structure and function of the cell was chosen as the principal theme. This has resulted in a general reappraisal of the problems of carcinogenesis, DNA repair, and immune response, which will certainly stimulate future discussion. Contributions on the function of the cell surface and the structure and function of the nucleus have provided a broad survey of the present state of cancer cell biology.

I am convinced that this congress report will find its place amongst current publications and will generate constructive further discussion of the cancer problem.

H. Wrba

I. CARCINOGENESIS AND DNA REPAIR

CARCINOGENICITY OF ALKYLATING AGENTS

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INTRODUCTION

Although considerable advances continue to be made in the understanding of the mechanisms by which a wide variety of chemical agents induce tumours in experimental animals, progress appears to have been most rapid in the field of alkylating agents. This reflects both the relative ease with which the pathways of metabolic activation and reaction of the alkylating agents have been elucidated and the relatively simple modifications which the alkylating species introduce into cellular macromolecules. The identification of products of alkylation reactions *in vivo* has been greatly aided by the fact that while most chemical carcinogens introduce modifications which are unique to that particular agent, the alkylating agents consist of several different chemical classes all of which produce essentially the same type of modifications. Another important factor has been the availability of experimental systems in which the ability of a large number of alkylating agents to induce tumours in various laboratory animals has been described (for example see Druckrey et al., 1967; see also Magee et al., 1976). These systems have allowed comparisons of the reactions of agents of varying carcinogenic potency in target and non-target tissues or in susceptible or non-susceptible strains or species.

This is not an exhaustive review of the field but attempts to summarize the data which have led to and support the hypothesis that the alkylation of DNA at the O^6-position of guanine may be an essential factor in the carcinogenicity of alkylating agents (several extensive reviews have recently appeared on this subject: Pegg, 1977; Roberts, 1979; Margison & O'Connor, 1979).

PRODUCTION OF THE ALKYLATING SPECIES

Although reactions of higher alkylating agents are known, the present report restricts itself to the two simplest members of the series, i.e. methylating and ethylating agents on which the vast majority of work has been published.

In terms of their ability to react with cellular macromolecules, alkylating agents can be classed as those which can act directly or after spontaneous chemical breakdown (these include the classical alkylating agents of synthetic chemistry) and those which are not alkylating agents *per se* but which give rise to such after metabolic activation, i.e. modification by cellular enzymes (usually the cytochrome P_{450}-dependent microsomal mixed function oxidases). Examples of these classes together with the structural formulae of the simplest representatives are shown in Table I.

For those agents undergoing either spontaneous (e.g. *N*-nitrosamides) or enzyme-mediated (e.g. *N*-nitrosamines) breakdown, the ultimate alkylating species are usually highly reactive electrophiles. The location of the formation of the alkylating species within the animal is reflected in the tissue specificity of tumour production, the *N*-nitrosamines being carcinogenic only in those tissues capable of their metabolic activation while the *N*-nitrosamides can produce tumours in many different organs since they are randomly distributed and decompose to equal extents in all tissues (see Margison & O'Connor, 1979 and citations therein). The

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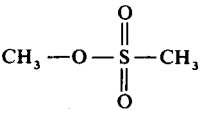
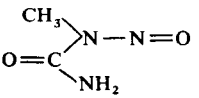
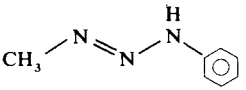
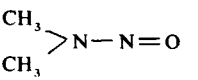
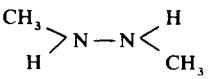
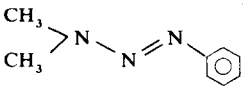
TABLE I

Classes of alkylating agents and structural formulae

DIRECT-ACTING		
Alkylalkanesulphonates		methyl methanesulphonate (MMS)
Alkylnitrosamides		*N*-methyl-*N*-nitrosourea (MNU)
Monoalkyltriazenes		3-methyl-1-phenyltriazene (MPT)
METABOLISM-REQUIRING		
Dialkylnitrosamines		*N*,*N*-dimethylnitrosamine (DMN)
Dialkylhydrazines		1,2-dimethylhydrazine (DMH)
Dialkyltriazenes		3,3-dimethyl-1-phenyltriazene (DPT)

dialkyl-phenyltriazenes are unusual in that, though requiring metabolic activation, they can produce tumours in tissues not possessing the appropriate activating enzymes (Kleihues et al., 1976) presumably because of the relatively high stability of the intermediate monoalkylphenyltriazenes which are themselves direct-acting agents (Margison et al., 1979).

ALKYLATION OF DNA

It is extremely difficult to provide a direct demonstration that alkylation of DNA leads to tumour production, however the majority of evidence indicates or can be interpreted to support the hypothesis that some alkylation-induced change in DNA is responsible for the initiation of malignant transformation. Many experiments have thus investigated whether a cause and effect relationship exists between carcinogenesis and the formation of specific DNA reaction products, based on the premise that initiation is a mutational event.

While we now know that there are 12 products of reaction of alkylating agents with DNA, the major and earliest recognized base reaction product is 7-alkylguanine. This product was originally thought to be responsible for the mutagenic and carcinogenic effects of alkylating agents (Lawley & Brookes, 1961). However, it was later found that the amounts of this product generated in DNA by a series of methylating or of ethylating agents bore no relationship to their capacity to produce tumours in the tissue examined (for example see Swann & Magee, 1968, 1971). 7-Alkylguanine was also produced by agents with a wide range of mutagenic potency and furthermore in experiments in which the template properties of alkylated bases in artificial polynucleotides could be examined *in vitro* it was shown that 7-alkylguanine formed a base-pair with its normal partner cytosine. These and other observations (see Margison & O'Connor, 1979) indicated that 7-alkylguanine was probably not responsible for the biological effects of alkylating agents.

During the period of study centred on 7-alkylguanine it was found that there was a minor product of guanine alkylation which was detected only after reaction with agents which were

TABLE II

Selection of alkylating agents arranged in order of increasing carcinogenicity and relative ability to attack DNA at the O^6-position of guanine

Agent	Carcinogenicity[a]	Alkylguanine ratio O^6/N7
Methyl methanesulphonate (MMS)	+/−	0.004
Ethyl methanesulphonate (EMS)	+	0.03
N,N-dimethylnitrosamine (DMN)	+++	0.11
N-methyl-*N*-nitrosourea (MNU)	+++	0.11
1,2-dimethylhydrazine (DMH)	+++	0.11
3,3-dimethyl-1-phenyltriazene (DPT)	+++	0.11
N-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)	+++	0.11
N,N-diethylnitrosamine (DEN)	++++	0.5–0.7
N-ethyl-*N*-nitrosourea (ENU)	++++	0.5–0.7

[a] Based very approximately on the dose required to produce tumours in any organ of any species.

mutagenic in T-even bacteriophage. It was suggested that this product, O^6-alkylguanine, would, because of the position of the alkyl group, cause anomalous base-pairing during transcription (Loveless, 1969). This suggestion was confirmed in experiments in which the coding properties of the lesion were measured (Gerchman & Ludlum, 1973; Abbott & Saffhill, 1979). Examination of the amounts of O^6-alkylguanine produced in animal tissue DNA after administration of agents of varying carcinogenic potency shows that the relative amount of alkylation at the O^6-position of guanine was indeed related to overall carcinogenicity (Table II). Thus, while essentially the same type of modifications are produced by all alkylating agents, the extent to which different sites are attacked varies and this is determined by the nature of the alkylating species and the mechanism by which it reacts (Lawley, 1974).

REPAIR OF O^6-ALKYLGUANINE AND THE ROLE OF DNA SYNTHESIS

(a) Single dose experiments

Although the relative amounts of O^6-alkylguanine produced in DNA correlated better with the carcinogenicity of alkylating agents than did 7-alkylguanine there were several observations principally concerning tissue specificity which could not be explained solely on this basis. For example, DMN reacts with the DNA of rat liver to a much greater extent than that of the kidney but single doses of DMN only produce liver tumours. Furthermore, MNU, which reacts with the DNA in all tissues to equivalent extents, produces tumours only in certain tissues such as the brain and the kidney.

One possible explanation for these observations became evident when it was found that while O^6-alkylguanine is stable in DNA *in vitro* it is enzymically removed from DNA *in vivo* (O'Connor et al., 1973; Lawley & Orr, 1970) and it was suggested that repair may also play a critical role in the carcinogenicity and tissue specificity of alkylating agents. In a number of experimental animal systems using single doses of various agents, the principal target tissue has subsequently been found to be that in which the O^6-alkylguanine removal system is least active, i.e. the effect observed was that this base persisted in the DNA of the target tissues to a greater extent than the non-target tissues (see Table III). The increased persistence is thought to increase the chance of a miscoding event taking place during DNA synthesis, which is presumably a step necessary to convert the lesion into a permanent heritable change in the base sequence of DNA. The critical role of DNA synthesis was clearly demonstrated in experiments in which tumours were produced in the livers of rats by a single dose of DMN only when it was given to animals during the peak of DNA synthesis following partial hepatectomy (Craddock, 1973). In a small number of the single dose experiments listed (Table III) DNA synthesis was suggested to play a more significant role in determining where tumours arise than did the

TABLE III

Experiments in which the persistence of O^6-alkylguanine in DNA has been compared in target (T) and non-target (NT) tissues after administration of single doses of various alkylating carcinogens

Agent[a]	Species	Tissues examined[b]	Persistence	Authors
ENU	rat(BD-IX)	*brain*, liver others	T>NT	Goth & Rajewsky (1974)
MNU	rat(BD-IX)	*brain,kidney*,liver	T>NT	Kleihues & Margison (1974)
DMN	rat(Wistar)	*kidney*,liver	T>NT	Nicoll et al. (1975)
DMN	hamster(Syrian)	*liver*,kidney,lung	T>NT	Margison et al. (1976)
MNU	mouse(C57Bl)	*thymus*,liver,lung,others	T>NT	Lawley (1976)
MNU	rat(BD-IX)	*brain*,liver,kidney,lung	T>NT	Kleihues & Bücheler (1977)
DMH	rat(Sp.Dawley)	*colon*,liver,kidney	NT>T	Rogers & Pegg (1977)
DMH	rat(BD-VI)	*colon*,liver,kidney	T>NT	Likhachev et al. (1977)
MNU	rat(Wistar)	*bladder*	T>NT[c]	Cox & Irving (1977)
DPT	rat(BD-VI)	*brain*,liver,lung,others	T>NT	Margison et al. (1979)
ENU	mouse(C57Bl)	*thymus*,brain,liver	T>NT	Frei et al. (1978)
DMH	rat(BD-IX)	*colon*,ileum,liver	NT>T	Swenberg et al. (1979)
DMN	hamster(Chinese)	*liver*,lung,kidney	T>NT	Swindell et al. (1979)

[a] Abbreviations see Tables I and II.
[b] Principal target tissue in italics.
[c] By comparison with Margison & Kleihues (1975).

relative persistence of the promutagenic lesion (Rogers & Pegg, 1977; Swenberg et al., 1979). This may also be true in experiments in which the susceptibility of a tissue to tumour production and the persistence of adducts in tissue DNA has been compared in two strains of mice (Den Engelse, 1974; DeMunter et al., 1979).

The ability of a large single dose of DMN to induce tumours in the liver of Syrian hamsters but not in that of normal rats appears to be related to the very much lower capacity of the hamster liver to remove O^6-alkylguanine from DNA. However, the hepatotoxic effect of the dose of DMN could also be a critical factor since regenerative DNA synthesis takes plaee in the hamster liver at a time when very little O^6-alkylguanine has been removed from hepatic DNA (Margison et al., 1979). A similar situation may arise in the livers of Chinese hamsters which are also susceptible to tumour induction by a single dose of DMN (Swindell et al., 1979).

(b) Multiple-dose experiments

Although tumours can be produced by single doses of alkylating agents many carcinogenesis experiments have involved repeated application of the agent in the diet, drinking water or by injection. Such dose schedules have been used in a small number of experiments in which the fate of O^6-alkylguanine has been examined (Table IV). In most cases a specific accumulation of this base was observed and this was most extensive or only detectable in the DNA of the principal target organ. However, in the experiments involving chronic administration of DMN to rats, which results in a high incidence of liver tumours, O^6-methylguanine was found to accumulate in the DNA of the lung and kidney while none was found in that of the liver (Margison et al., 1977). Further investigations of this system have shown that the capacity of the liver to repair O^6-methylguanine is in fact enhanced by chronic administration of DMN (Montesano et al., 1979). Similarly the removal of O^6-ethylguanine from rat liver DNA is enhanced by chronic administration of DEN using a dose schedule which results in a high incidence of liver tumours (Margison et al., 1979). More recently, administration of *N*-acetylaminofluorene in the diet has also been found to enhance the removal of O^6-methylguanine produced by a subsequent dose of DMN (Buckley et al., 1979). Whether this enhanced repair plays a causative role in tumour production by these dose regimes (possibly by inducing error-prone repair) has yet to be established. However, since chronic administration of 3,3-dimethyl-1-phenyltriazene, which also enhances O^6-methylguanine removal from

TABLE IV

Experiments in which the accumulation of O^6-methylguanine in DNA has been compared in target (T) and non-target (NT) tissues during chronic administration of various alkylating agents

Agent[a]	Species	Tissues examined [b]	Accumulation	Authors
MNU	rat(BD-IX)	*brain,kidney*,liver	T>NT	Margison & Kleihues (1975)
DMN	rat(BD-IV)	*liver*,lung,kidney	NT>T	Margison et al. (1977)
MNU	rat(Wistar)	*bladder*	T>NT[c]	Cox & Irving (1977)
DMN	rat(Wistar)	*kidney*,liver	T>NT	Nicoll et al. (1977)
DPT	rat(BD-IX)	*brain*,liver,others	T>NT	Cooper et al. (1978)

[a] For abbreviations see Tables I and II.
[b] Principal target tissue in italics.
[c] By comparison with Margison & Kleihues (1975).

hepatic DNA (Cooper et al., 1978), produces not liver tumours but a high incidence of central nervous system tumours, it is possible that increased DNA synthesis in the target tissues as a consequence of the toxicity of the agent may be responsible for tumour production.

Summary

The carcinogenicity and tissue specificity of alkylating agents is determined by a number of factors, the importance of which in the eventual production of tumours varies according to the experimental system. The indispensable factor appears to be the generation of (possibly a critical number of) miscoding lesions in DNA and this is determined by the nature of the agent and the dose. Repair reactions may reduce the chance of malignant transformation if they occur before DNA synthesis can take place on the damaged template. Chronic administration of alkylating agents enhances repair but increased DNA synthesis may, under certain conditions, counteract this effect and increase the frequency of malignant transformation.

The precise mechanism by which O^6-alkylguanine is removed from DNA has yet to be elucidated as has whether there is a system for the repair of the miscoding lesion O^4-alkylthymine which is produced in DNA to much lower extents than O^6-alkylguanine (O'Connor et al., 1979).

Correlations of the type described are based on whole-tissue measurements of DNA reaction products. However, with the development of radioimmunological methods it should be possible in the future to examine the formation and removal of DNA adducts in the various cell types within a tissue which have different susceptibility to malignant transformation.

ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Research Council and the Cancer Research Campaign. Thanks are due to Ms Gillian A. Simpson for typing the manuscript.

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