Noninvasive methodologies for measuring carcinogen exposure in humans, based on the use of urinary markers, are being developed and validated for use in molecular epidemiological studies. A range of 3-alkyladenines can be determined in urine samples by an immunoaffinity purification-GC/MS approach [3-methyladenine, 3-ethyladenine, 3-2-hydroxyethyladenine, and 3-benzyladenine]. Using this method, recent results in human subjects suggest that urinary 3-alkyladenines are potentially useful markers of alkylating agent exposure, particularly where the backgrounds of such adducts are much lower than 3-methyladenine. Urinary excretion of benzylmercapturic acid has been studied in experimental animals as a marker of exposure to benzylating agents such as N-nitroso-methylbenzylamine. 3-Nitrotyrosine (NTyr) is formed in vivo in tissue or blood proteins after exposure to nitrosating and/or nitrating agents such as tetranitromethane. After turnover of proteins, NTyr is released and excreted in urine as metabolites 3-nitro-4-hydroxyphenylacetic acid and 3-nitro-4-hydroxynitrosophenylacetic acid, which are determined by GC with a thermal energy analyzer. The sensitivity and specificity, combined with ease of use, of these noninvasive biomonitoring approaches means that they may be readily incorporated into molecular epidemiological studies in which exposure to nitrosating and alkylating agents may be important risk factors.

Introduction

In the rapidly expanding field of molecular epidemiology, the precise determination of individual exposure to carcinogens is regarded as a desirable goal. It would, nevertheless, be a mistake to translate this objective into one of the determination of individual cancer risk [although this remains a popular idea (7)]. In contrast, it is clear that groups of people at high risk of developing a particular cancer may be identified using molecular markers of carcinogen exposure. A number of methods are available to determine human exposure to carcinogens and are based on the idea that initiation, via formation of mutagenic lesions in DNA, is a crucial (but not sufficient) step in multistage carcinogenesis (2). Thus, characteristic adducts can be determined in DNA extracted from a variety of sources inducing target tissues (obtained during surgery or at necropsy), or from nucleated blood cells such as lymphocytes. This approach has unequivocally demonstrated human exposure to a range of agents arising from food [e.g., aflatoxin B1 (3)], occupation [e.g., benzo[a]pyrene (4)], and lifestyle [e.g., tobacco smoking (5)].

Blood protein adducts to these same exposures have been exploited as surrogate markers of DNA damage (3,5). All of these methods require tissue or blood samples which may limit the numbers of samples available for analysis. In addition, levels of adducts in nontarget tissues may not necessarily be good indicators of target tissue exposure or even of whole-body burden. It is from this standpoint that the idea of using noninvasive methods based on urinary analysis has been developed. Of the many types of DNA damage arising from carcinogen exposure, DNA alkylation has been extensively studied and biomonitoring methods based on this phenomenon form the main focus of this review.

As mentioned above, measures of human exposure to agents resulting in characteristic DNA damage are among the most important types of information to obtain, and it is crucial to show that any indirect method (such as urinary analysis) can give relevant information. Work by Craddock and Magee (6) showed that exposure of rats, whose DNA was labeled with 14C, to 1H-labeled dimethylnitrosamine (NDMA) resulted in urinary excretion of doubly labeled N7-methylguanine (N7-MeGua). It was shown, unambiguously, that most of this urinary adduct was derived from liver, which is the major target organ for NDMA carcinogenesis. More recently, Gombar et al. (7) showed that coadministration of [14C]aminopyrine and nitrite (a combination that results in 14C formation of [14C]NDMA) resulted in excretion...
of [14C]N7-MeGua, which correlated well with liver DNA methylation. Moreover, excretion of [14C]N7-MeGua was detectable at doses where in situ methylation was undetectable due to practical problems in obtaining sufficient DNA from liver for adduct analysis at low levels of modification (whereas urinary N7-MeGua was derived from the whole organ and possibly other sites). Bennett et al. (8) showed in rats that urinary excretion of aflatoxin B1-guanine adduct correlated well with levels of adduct in the liver (which is the target organ). These results suggested that determination of excreted DNA adducts (or alkylpurines) that arise as a consequence of DNA repair mediated by DNA glycosylases (9) form the basis of noninvasive methods for determining of DNA damage following human exposure to alkylating carcinogens.

In a broader context, it may be useful to obtain information on the whole-body burden of alkylating agents and/or precursors. Many alkylating carcinogens react with glutathione (GSH) in vivo and give rise to mercapturic acids (MAs), which in many cases represent the major urinary metabolite (10). S2 alkylating agents (such as alkyl halides, epoxides, and alkylsulfonates) can give up to 50% of the administered dose as MAs, which has led to much interest in the use of MAs as urinary markers of exposure to S2 alkylating agents (II). In contrast, S1 alkylating agents such as alkylidiazonium ions (the presumed active metabolites of N-alkyl-N-nitroso compounds) tend to give much lower yields although fewer of these compounds have been studied in this respect (e.g., dimethylnitrosamine (J2), N-nitroso-di-n-butylamine (J3), and N-nitroso-N-methylbenzylamine (NMBA)).

From the point of view of human biomonitoring to alkylating carcinogens, mercapturic acids offer the possibility of noninvasively measuring the biological effective dose of alkylating species. There are indications, however, that the use of mercapturic acids must be approached with care. Many studies in humans have made use of methods to determine total urinary thiocysteines (which include MAs), and in many cases no differences could be detected between controls and exposed subjects with wide variation in individual levels (II). Aringer and Lidums (J4) have also drawn attention to the problems of dietary confounding in the total urinary thiocysteines method. As a consequence, it has been recognized that the determination of individual mercapturic acids is not only desirable because of sensitivity, but also adds to the specificity of the method, as in the case of acrylonitrite (15), benzene (J6) and ethylene oxide (J7). Individual MAs can be determined by a number of methods including GC (15), HPLC-electrochemical detection (18), HPLC-fluorescence of derivatives (17), and GC–MS (19).

Endogenous formation of N-nitroso compounds has been shown to occur in humans, using N-nitrosamines excreted in urine as a marker (20,21). Carcinogenic N-nitroso compounds appear to be formed mainly in the stomach by the reaction of certain nitrogen-containing compounds with nitrite under acidic conditions. However, endogenous nitration may also occur in other organs such as the lung (22) and skin (23) after exposure to oxides of nitrogen and in inflamed or infected tissues by activated macrophages and bacteria (24,25), although such reactions have not yet been demonstrated to occur in tissues in vivo.

We are currently developing and validating noninvasive methodologies, based on the use of urinary markers, for eventual use in human biomonitoring studies. This approach is divided into three parts: a) an assessment of DNA damage by measurement of excreted alkylpurines b) the determination of specific urinary mercapturic acids, and c) the evaluation of precursors, e.g., endogenous nitration potential using 3-nitrotyrosine metabolites. Recent results in these areas are reviewed in this paper.

**Urinary Alkylpurines**

Alkylation at N-3 of adenine is a major route of DNA-adduct formation for many alkylating carcinogens (26). The resulting 3-alkyldioxadenosines are unstable and rapidly depurinate either spontaneously or via the action of specific DNA glycosylases to give the corresponding 3-alkyladenines (3-alkAde) (9).

We have recently shown that 3-methyladenine (3-MeAde) can be rapidly quantitated in human urine by immunochemical and/or GC–MS methods (27,28). In a recent study of cancer patients receiving methylnitrosourea (MNU, at a total single dose of 300 or 600 mg) as part of a combination chemotherapy, 24-hr urine samples were collected from each patient immediately before and after MNU administration. Analysis of urinary 3-MeAde showed, in every case, increased excretion of this marker of methylation after treatment. Overall, a dose-dependent excretion of 3-MeAde was observed, and preliminary experiments suggest some correlation between methyl adducts (7-methylguanine and O6-methylguanine) in lymphocyte DNA and urinary 3-MeAde excretion (Shuker et al., manuscript in preparation).

The use of 3-MeAde as a marker of methylating agent exposure to low levels of methylating agents is complicated by a relatively high urinary background. However, recent studies have indicated that simple dietary manipulation can virtually eliminate this background (27). In an experiment designed to examine the effect of cigarette smoking on urinary methyl adduct excretion, three healthy volunteers (current or ex-smokers) agreed to collect 24-hr urine samples for 10 consecutive days. During this period, they consumed normal diets, and smoked if they wished, on days 1, 2, 9, and 10. On days 3–8 they consumed a balanced liquid diet and bottled water, which was shown to contain a very low amount of preformed 3-MeAde (27). On days 5 and 6, the subjects were allowed to smoke their normal brand of cigarette ad libitum. Urines were analyzed for 3-MeAde levels, and the results are shown in Figure 1. It is clear that the dietary control rapidly lowers and stabilizes urinary 3-MeAde excretion (interestingly, a slight excess of excretion over intake is always observed) and that cigarette smoking markedly increases levels of this marker. In view of the apparent ubiquitous occurrence of preformed 3-MeAde, an analysis of tobacco smoke (from each brand used) was undertaken, and a small amount was detected. However, in no case did this background account for more than 10% of the observed increase. The rapid rise and fall of urinary 3-MeAde, is consistent with endogenous DNA methylation and rapid repair by tobacco smoke constituents, such as tobacco-specific nitrosamines (Prevost and Shuker, manuscript in preparation).

We have now developed a method for analyzing several different 3-alkyladenines simultaneously. Immunoaffinity gel was prepared by coupling monoclonal antibody EM-6-47, which
FIGURE 1. Excretion patterns of 3-methyladenine (3-MeAde) in three smokers on controlled diets. (Hatched bars), 3-MeAde preformed in cigarettes; (solid bars), 3-MeAde preformed in diet.

cross-reacts with a range of 3-alkyladenines (29), to protein A-Sepharose CL-4B. Columns were prepared in a similar way to that described recently (28). The full details of the preparation and characterization of these immunoaffinity columns will be described elsewhere (Prevost et al., manuscript in preparation). Thus far, analytical procedures for 3-MeAde, 3-ethyladenine (3-EtAde), 3-(2-hydroxyethyl)adenine (3-HOEtdAde), and 3-benzyladenine (3-BzAde) have been developed. Deuterated internal standards (d1,3-MeAde, d2,3-EtAde, d3,3-HOEtdAde, and d4,3-BzAde) were synthesized by standard methods and were routinely added to urine samples (5 mL) before immunoaffinity purification. Selected ion monitoring using a low resolution quadruple GC-MS system (Hewlett Packard 5970A) was used to quantitate the 3-alkAde. A representative GC-MS trace of four 3-alkyladenines with corresponding internal standards is shown in Figure 2. Using this approach a number of studies have been carried out, and the main conclusions are summarized below:

1. Background levels in humans of 3-EtAde (0.5 nmole/24 hr) and 3-HOEtdAde (6-10 nmole/24 hr) are much lower than 3-MeAde (50-100 nmole/24 hr), with 3-EtAde levels being very stable and little affected by changes in diet.
2. In two human volunteers, the metabolism of some 3-alkyladenines was studied using deuterated compounds. Recoveries of d1,3-MeAde, d2,3-EtAde, d3,3-HOEtdAde, and d4,3-BzAde in the 24 hr after oral administration were 93, 70, 93, and 23%, respectively.
3. Preliminary results with 3-EtAde suggest that there may be some increase with smoking, although the source of ethylating agent(s) is as yet unknown.

FIGURE 2. Selected ion monitoring traces of four 3-alkyladenines and their corresponding deuterated internal standards.

Specific Mercapturic Acids

Measures of alkylating agent exposure based on total urinary mercapturic acids are often confounded by the presence of natural background levels. In contrast, the determination of individual mercapturic acids should be characteristic of a particular exposure.

In view of interest in the role of N-nitroso-N-methylbenzylamine (NMBzA) as a possible etiological agent in esophageal cancer in China, the utility of S-benzylmercapturic acid (SBzMA) as a marker of benzylation was investigated. The excretion of SBzMA in the urine of rats treated with NMBzA was determined by GC-MS using d1-SBzMA as an internal standard. The amount of urinary SBzMA varied with the dose of NMBzA (up to 5 mg/kg) and with rat strain. For the three strains investigated, most of a 2.5 mg/kg dose of SBzMA was excreted within 24 hr. Comparison of the levels of SBzMA excreted by rats treated with equivalent doses of either NMBzA or benzaldehyde indicates that urinary SBzMA is derived mainly from benzylating species resulting from the hydroxylation of the methyl group of NMBzA (30).

In view of the success of immunochemical methods, in particular, immunoaffinity purification, in the area of urinary DNA adducts, current work is aimed at preparing antibodies against mercapturic acids either individually (e.g., SBzMA) or as a group.

Endogenous Nitrosation

3-Nitrotyrosine (NTyr) in tissue or blood proteins was evaluated as a possible exposure marker for exogenous and endogenous nitrosating or nitrating agents. A sensitive and selective method for analyzing NTyr by gas chromatography with a thermal energy analyzer (GC-TEA) was developed. Using this method, kinetic studies were carried out. It was found that free and protein-bound tyrosine residues easily react with nitrating/nitrosating agents to yield NTyr. NTyr formation in vivo showed a dose dependent increase in NTyr in both plasma proteins and hemoglobin obtained from rats 24 hr after IP injection of various doses (0.5-2.5 µmole/rat) of tetranitromethane. Major urinary metabolites of NTyr, given orally to rats, were isolated and identified by GC-MS as 3-nitro-4-hydroxyphenylacetamide (NHPA) and 3-nitro-4-hydroxyphenylactic acid (NHPL). About 44 and 5% of the oral dose of NTyr (100 µg/rat) was excreted as NHPA and NHPL, respectively. Eleven
24-hr human urine samples were analyzed for NHPA by GC-TEA after ethyl acetate extraction and HPLC purification: quantities ranging from 0 to 7.9 μg/24 hr (mean ± SD, 2.8 ± 2.3, n = 11) were detected (detection limit 0.2 μg/L). Thus, NTyr in proteins or its metabolites in urine can be readily analyzed by GC-TEA as a marker for endogenous nitrosation and nitrination (31).

Initial attempts to prepare antibodies to NTyr in order to prepare immunoaffinity columns were not particularly successful, and an approach using novel haptenic forms of NTyr is currently being pursued.

Discussion

The methods described here are undergoing continuous refinement, particularly in the area of specificity, as well as being thoroughly validated. A criticism that may be leveled against the use of urinary markers such as excreted adducts is that they may be derived from total nucleic acid alkylation (i.e., DNA and RNA) because only the modified purine base is excreted. Although an experimental approach involving the use of stable isotope labeled DNA in vivo is being developed to directly address this question (Shuker, unpublished data), it is nonetheless interesting to consider the published information about RNA alkylation and repair and compare it to that on DNA alkylation.

N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea (MNU) react in vitro with various forms of RNA to give primarily 7-methylguanine (7-MeGua; 80–90%) along with small amounts of other adducts [O6-MeGua, 3–4%; 1-MeAde, 2–4%; 3-MeAde, ~1% (32,33)]. Similar patterns of adduct formation were obtained in liver ribosomal RNA (which makes up >80% of total RNA) of rats treated with NDMA (34).

Interestingly, there is apparently no repair of methyl adducts in liver RNA in either rats or Syrian golden hamsters. 7-Methylguanine in rat liver RNA, as a result of methylating agent exposure, disappears with the same kinetics as labeled RNA [t1/2 = 5 days (35)]. Adduct ratios did not alter over a 4-day period in hamster liver RNA after treatment with NDMA, suggesting that even minor products such as O6-MeGua and 3-MeGua were not repaired (36). In comparison, various adducts in rat liver DNA after treatment with NDMA underwent active and sometimes rapid repair (adducts t1/2: 7-MeGua, 29 hr; O6-MeGua, 21 hr; 3-MeAde, 6.5 hr), with similar results being obtained forethyl adducts after treatment with N-ethyl-N-nitrosourea (37). Overall, these results suggest that increases in urinary alkylatedpurine bases at short time periods following alkylating agent exposure are likely to be mainly of DNA origin.

It is clear, therefore, that noninvasive methods for measuring DNA damage based on the urinary excretion of repaired adducts is both technologically feasible, based on recent advances in analytical methodology, and scientifically valid, based on the published literature. However, a direct experimental validation is underway. The measurement of specific urinary mercapturic acids offers the potential of sensitive detection of exposure, particularly for alkylating agents for which this is a major pathway of excretion, such as acrylamide.

Endogenous nitrosation can arise from endogenous sources of nitrosating agents such as macrophage-mediated synthesis of nitric oxide or exogenous sources such as nitrogen oxides (NOx) from cigarette smoke or urban pollution. Measurements of nitrotyrosine and its metabolites offer a possibility to quantitate this factor and are being used, particularly in studies involving urban pollution.

Each of the three approaches described in this paper have the potential to convey information about various aspects of human exposure to alkylating carcinogens. It is clear, however, that a combination of these approaches is more powerful than when they are applied individually. For example, DNA adducts arising from preformed tobacco-specific nitrosamines can be readily determined using urinary adducts, but it will be interesting, in addition, to assess the role of endogenous nitrosation of tobacco alkaloids, using 3-nitrotyrosine, as this may be a major contributing pathway.

This manuscript was presented at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

The technical assistance of Liliane Garren and Isabelle Brouet is gratefully acknowledged. Partial funding support from the U.S. National Cancer Institute is gratefully acknowledged (CA 48473).

REFERENCES

1. Marx, J. Zeroing in on individual cancer risk. Science 253: 612–616 (1991).
2. Shields, P. G., and Harris, C. C. Molecular epidemiology and the genetics of environmental cancer. J. Am. Med. Assoc. 266: 681–687 (1991).
3. Groopman, J. D., Sambioni, G., and Wild, C. P. Molecular dosimetry of human aflatoxin exposures. In: Molecular Dosimetry and Human Cancer (J. D. Groopman and P. L. Skipper, Eds.), CRC Press, Boca Raton, FL, 1991, pp. 303–324.
4. Phillips, D. H. DNA-adduct analysis by 32P-postlabeling in the study of human exposure to carcinogens. In: Molecular Dosimetry and Human Cancer (J. D. Groopman and P. L. Skipper, Eds.), CRC Press, Boca Raton, FL, 1991, pp. 151–170.
5. Hecht, S. S., Haley, N. J., and Hoffman, D. Monitoring exposure to tobacco products by measurement of nicotine metabolites and derived carcinogens. In: Molecular Dosimetry and Human Cancer (J. D. Groopman and P. L. Skipper, Eds.), CRC Press, Boca Raton, FL, 1991, pp. 325–361.
6. Craddock, V. M., and Magee, P. N. Effect of administration of the carcinogen dimethylnitrosamine on urinary 7-methylguanine. Biochem. J. 104: 435–440 (1967).
7. Gombar, C. T., Zubroff, J., Strahan, G. D., and Magee, P. N. Measurement of 7-methylguanine as an estimate of dimethylnitrosamine formed following administration of aminopyrine and nitrite to rats. Cancer Res. 43: 5077–5080 (1983).
8. Bennett, R. A., Essigmann, J. M., and Wogan, G. N. Excretion of an aflatoxin-guanine adduct in the urine of aflatoxin B1 treated rats. Cancer Res. 41: 650–654 (1981).
9. Karran, P., and Lindahl, T. Cellular defence mechanism against alkylating agents. Cancer Surv. 4: 585–599 (1985).
10. Chasseaud, L. F. The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv. Cancer Res. 29: 175–274 (1979).
11. Henderson, P. T., van Doorn, R., Leijdekkers, C.-M., and Bos, R. P. Excretion of thioethers in urine after exposure to electrophilic chemicals. In: Monitoring Human Exposure to Carcinogenic and Mutagenic Agents (A. Berlin, M. Draper, K. Hemminki, and H. Vainio, Eds.), IARC Scientific Publication No. 59, International Agency for Research on Cancer, Lyon, 1984, pp. 173–187.
12. Hemminki, K. Dimethylnitrosamine adducts excreted in rat urine. Chem.-Biol. Interact. 39: 139–148 (1982).
13. Suzuki, E., Osabe, M., Okada, M., Ishizaka, T., and Suzuki, T. Urinary metabolites of N-nitrosobutylamine and N-nitrobutylamine in the rat: identification of N-acetyl-S-alleryl-L-cysteines. Jpn. J. Cancer Res. 78: 382–385 (1987).
14. Aringer, L., and Lidums, V. Influence of diet and other factors on urinary levels of thioethers. Int. Arch. Occup. Environ. Health 61: 123–130 (1988).
15. Jakubowski, M., Linhart, I., Pielas, G., and Kopecky, J. 2-Cyanoethyl-mercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile. Br. J. Ind. Med. 44: 834–840 (1987).
URINARY MARKERS FOR ALKYLATING AGENTS AND PRECURSORS

16. Strommel, P., Muller, G., Stucker, W., Veroyen, C., Schobel, S., and Norpith, K. Determination of S-phenylmercapturic acid in the urine: an improvement in the biological monitoring of benzene exposure. Carcinogenesis 10: 279–282 (1989).

17. Gérin, M., Tardif, R., and Brodeur, J. Determination of specific urinary thiocarbethoxydes derived from acrylonitrile and ethylene oxide. In: Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention (H. Bartsch, K. Hemminki, and I. K. O’Neill, Eds.), IARC Scientific Publication No. 89, International Agency for Research on Cancer, Lyon, 1988, pp. 275–278.

18. Toyokawa, T., Suzuki, T., Saito, Y., and Takahashi, A. Electrochemical detection of mercapturic acid derivatives after separation by high performance liquid chromatography. J. Chromatogr. 475: 391–399 (1989).

19. Norstrom, A., Andersson, B., and Levin, J. O. A procedure for the analysis of S-benzyl-N-acetylcysteine and S-(o-methylbenzyl)-N-acetylcysteine in human urine. Xenobiotica 16: 525–529 (1986).

20. Ohshima, H., and Bartsch, H. Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosopropionic acid excreted in urine. Cancer Res. 41: 3658–3662 (1981).

21. Bartsch, H., Ohshima, H., Pignatelli, B., and Calmels, S. Human exposure to endogenous N-nitroso compounds: quantitative estimates in subjects at high risk for cancer of the oral cavity, esophagus, stomach and urinary bladder. Cancer Surv. 8: 335–362 (1989).

22. Iqbal, Z. H., Dahl, K., and Epstein, S. S. Role of nitrogen oxide in the biosynthesis of nitrosamines in mice. Science 208: 1475–1477 (1980).

23. Mirvish, S. S., Sams, J. P., and Issenberg, P. The nitrosating agent in mice exposed to nitrogen dioxide: improved extraction method and localization in the skin. Cancer Res. 43: 2550–2554 (1983).

24. Calmels, S., Ohshima, H., Vincent, P., Gounot, A. M., and Bartsch, H. Screening of microorganisms for nitrosation catalysis at pH 7 and kinetic studies on nitrosamine formation from secondary amines by E. coli strains. Carcinogenesis 6: 911–915 (1985).

25. Marletta, M. A. Mammalian synthesis of nitrite, nitrate, nitric oxide, and N-nitrosoating agents. Chem. Res. Toxicol. 1: 249–257 (1988).

26. Margison, G. P., and O'Connor, P. J. Nucleic acid modification by N-nitroso compounds. In: Chemical Carcinogens and DNA (P. L. Grover, Ed.), CRC Press, Boca Raton, FL, 1979, pp. III–159.

27. Prevost, V., Shuker, D. E. G., Bartsch, H., Pastorelli, R., Stillwell, W. G., Trudel, L. J., and Tannenbaum, S. R. The determination of urinary 3-methyladenine by immunoaffinity chromatography-monoclonal antibody-based ELISA: use in human biomonitoring studies. Carcinogenesis II: 1747–1751 (1990).

28. Eberle, G., Glüsenkamp, K. H., Drosdziek, W., and Rajewsky, M. F., Monoclonal antibodies for the specific detection of 3-alkyladenines in nucleic acids and body fluids. Carcinogenesis II: 1753–1759 (1990).

29. Lin, D. X., Friesen, M., Malaveille, C., Shuker, D. E. G., and Bartsch, H. Urinary excretion of S-benzylmercapturic acid as an indicator of N-nitroso-N-methylbenzylamine exposure. Cancer Lett. 57: 193–198 (1991).

30. Ohshima, H., Friesen, M., Brouet, I., and Bartsch, H. Nitrotroysteine as a new marker for endogenous nitrosation and nitration of proteins. Food Chem. Toxicol. 28: 647–652 (1990).

31. Lawley, P. D., and Shah, S. A. Methylation of ribonucleic acid by the carcinogens dimethyl sulphate, N-methyl-N-nitrosourea and N-methyl-N-nitro-N-nitrosoguanidine. Biochem. J. 128: 117–132 (1972).

32. Pegg, A. E. Alkylation of transfer RNA by N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea. Chem.-Biol. Interact. 6: 393–406 (1973).

33. O’Connor, P. J., Capps, M. J., Craig, A. W., Lawley, P. D., and Shah, S. A. Differences in the patterns of methylation in rat liver ribosomal ribonucleic acid after reaction in vivo with methyl methanesulphonate and NN-di-methylnitrosamine. Biochem. J. 129: 519–528 (1972).

34. McElhone, M. J., O’Connor, P. J., and Craig, A. W. The stability of rat liver ribonucleic acid in vivo after methylation with methanesulphonate or dimethylnitrosamine. Biochem. J. 125: 821–827 (1971).

35. Margison, G. P., Margison, J. M., and Montesano, R. Persistence of methylated bases in ribonucleic acid of Syrian golden hamster after administration of dimethylnitrosamine. Biochem. J. 177: 967–973 (1979).

36. Den Engelse, L., Menkveld, G. J., De Brij, R. J., and Tate, A. D. Formation and stability of alkylated pyrimidines and purines (including imidazole ring-opened 7-alkylguanine) and alkylphosphorriesters in liver DNA of adult rats treated with ethyl nitrosourea and dimethyl nitrosamine. Carcinogenesis 7: 393–403 (1986).