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REVIEW

Post-radiolabelling for detecting DNA damage

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Introduction

The biochemical and molecular basis of cancer continues to be an expanding area of research. Much of the driving force behind this important multi-disciplinary area is due to concerns about increasing human health risks caused by exposure to toxic chemicals of both industrial and natural origin. In some instances the aetiological agents, for example vinyl chloride, as a cause of angiosarcomas in human liver, and tobacco smoke as the major cause of lung cancer, have been identified by clinical and epidemiological studies involving high-exposure populations. There is, however, an increasing need for reliable prospective methods for predicting human cancer risks as a result of chemical exposures. The somatic mutation theory of cancer suggests that cancer is caused by genetic damage induced by chemicals or viruses. Such mutations resulting from covalent interactions of chemicals with DNA may be an important initial stage in chemical carcinogenesis. There is now considerable evidence which has established that many carcinogens or their metabolites covalently bind to DNA *in vivo* with the formation of DNA adducts. The formation of DNA adducts by indirect mechanisms can also occur with some carcinogens (Barrows and Shank, 1981). Other alterations in DNA such as strand breakage (Petzold and Swenberg, 1978), gene re-arrangement (Schimke, 1982; Lavi, 1982) or gene amplification (Cairns, 1981; Klein, 1981; Feinberg and Coffey, 1982) may also be important stages in carcinogenesis. However, the covalent binding of chemicals to DNA appears to be the significant initiating event which can lead to secondary genomic alterations unless the carcinogen adduct is repaired. Hence it is widely accepted that any chemical which forms covalent bonds with DNA of somatic and reproductive cells *in vivo* should be viewed as a potential mutagen, carcinogen and teratogen. Studies on the binding of chemicals to DNA and the mechanisms of formation of DNA adducts are therefore important in the identification of potential carcinogens. In terms of risk assessment, a major research objective is to determine the qualitative and quantitative relationships between the formation of DNA adducts and the resulting lesions in target tissues.

Much of our present knowledge on the interaction between carcinogens and cellular macromolecules has been obtained by the use of isotopically labelled compounds. Thus, the detection and quantitation of the covalent binding of different classes of carcinogens to nucleic acids and proteins both *in vitro* and *in vivo* has been possible. From such studies Lutz (1979, 1982) developed the concept of the covalent binding index which is defined as μmol of chemical bound per mole of DNA nucleotide/mmol of chemical per kg body wt of animal. However, this concept does not take qualitative features into account and it is now generally accepted that covalent binding should be determined at the level of DNA adducts. Thus in most studies radiolabelled carcinogens have been administered to rodents and the presence of reaction products with DNA has been determined after isolation of DNA from various organs, usually liver, followed by hydrolysis of the DNA. The identity of DNA adducts in the hydrolysate is usually determined by co-chromatography of the hydrolysate on h.p.l.c. with appropriate synthetic reference compounds. In this way DNA adducts of a variety of carcinogens including alkylating agents, polycyclic aromatic hydrocarbons, aromatic amines and mycotoxins have been identified. Modified DNA is usually enzymically digested to constituent deoxyribonucleosides which are then analysed for adducts. Some limitations can arise due to incomplete digestion or instability of adducts.

The application of these techniques is limited to those compounds which may be synthesized readily in radiolabelled form and the specific radioactivity achievable determines the limit of detection of adducts *in vivo*. Studies in humans using these methods are not possible. As a result, alternative methods for studying interactions of carcinogens with DNA which do not rely on radiolabelled carcinogens have been developed. At the forefront of such methods for detection of DNA adducts formed from non-radiolabelled compounds are immunochemical assays and post-radiolabelling. The incorporation of high-specific radioactivity in the form of ^{32}P into DNA adducts has thus far found widest application of the latter technique.

Post-labelling analysis of DNA

^{32}P-Post-labelling

Several years ago, Randerath and his co-workers reported a new ^{32}P-post-labelling method for the analysis of carcinogen–DNA adducts (K.Randerath *et al.*, 1981, 1984a, 1985a; Gupta *et al.*, 1982). The procedure is essentially a development of Randerath's methodology for base composition (K.Randerath *et al.*, 1980) and sequence analysis (K.Randerath *et al.*, 1980; Maxam and Gilbert, 1981; Sanger, 1981) of nucleic acids. The basic procedure involves the incorporation of ^{32}P into non-radioactive nucleic acid constituents by an enzyme-catalysed derivatization followed by chromatographic separation of radioactive products. The most widely used chromatographic method has been multidimensional thin layer chromatography (t.l.c.); though the use of h.p.l.c. has also been reported (Haseltine *et al.*, 1983). Although several modifications have now been incorporated into the method, and these are described below (and summarized in Figure 1), the essential procedure is as follows. DNA is isolated from tissues of animals exposed to suspect chemical carcinogens and then digested enzymically to deoxyribonucleoside 3′-monophosphates with micrococcal nuclease and spleen exonuclease. The deoxyribonucleoside 3′-monophosphates are then converted to their corresponding 5′-^{32}P-labelled 3′,5′-bisphosphates by enzymic derivatization involving [^{32}P]phosphate transfer from [γ-^{32}P]ATP using T4 polynucleotide kinase. T4 polynucleotide kinase exhibits absolute specificity for the transfer of [^{32}P]-phosphate from [γ-^{32}P]ATP to 5′-hydroxyl groups of ribo or deoxyribonucleoside 3′-monophosphates and their phosphodiester