



Perspectives

The uses of carcinogen-DNA adduct measurement in establishing mechanisms of mutagenesis and in chemoprevention

William M. Baird*, Brinda Mahadevan

*Department of Environmental and Molecular Toxicology, Oregon State University, 1007 ALS Building,
Oregon State University, Corvallis, OR 97331-7301, USA*

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Abstract

DNA adducts generated by carcinogenic chemicals reflects human exposure and DNA adducts are related to tumor formation. Most chemical carcinogens require activation to reactive intermediates that bind to nucleophilic centers in proteins and nucleic acids thereby forming covalent adducts. Also, many of the chemicals considered carcinogenic for humans form covalent DNA adducts. Therefore, such DNA damage is generally considered to be causative and linked to tumor formation. In this article we have summarized the work done for many years on the role of DNA adduct formation as an indicator of their carcinogenicity. We have also addressed the important role for measurement of DNA adducts in studies with potential chemopreventive agents for which it is central to have a marker that can be measured more rapidly than changes in cancer incidence. © 2004 Elsevier B.V. All rights reserved.

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The role of DNA interactions of carcinogens as an indicator of their carcinogenicity have been recognized for over 30 years since Brookes and Lawley [1] reported in *Nature* that there was a positive correlation between the relative carcinogenic potency of a series of six polycyclic aromatic hydrocarbons (PAH) ranging from the weak or non-carcinogenic dibenz[a,c]anthracene to the potent carcinogens dibenz[a,h]anthracene and 7,12-dimethylbenz[a]anthracene, with benzo[a]pyrene falling in the middle of the carcinogenicity scale and levels of binding [1].

In contrast, a plot of the level of binding of PAH to total proteins in mouse skin demonstrated that no correlation existed between protein binding and rela-

tive carcinogenic potency of the PAH [1]. Since this original observation much of the effort in the study of DNA-binding of carcinogens has been directed to use DNA adducts as an internal cellular trapping agent for reactive electrophilic carcinogen metabolites to determine the mechanisms of metabolic activation of the carcinogens to “ultimate carcinogenic metabolites” in cells and tissues [2].

Since DNA appears to be the target of the carcinogenic species in cells, logic indicates that if a “reactive ultimate carcinogenic” form can be synthesized and reacted with DNA *in vitro*, it should form the same DNA adducts as those formed in target tissues by the parent carcinogen. Boyland [3] had proposed that the most likely ultimate carcinogenic form of PAH would be an epoxide on the “K-region” of the molecule (see Fig. 1). Brookes and I recognized that in order to accomplish this for PAH it would be necessary to isolate the

* Corresponding author. Tel.: +1-541-737-1886;

fax: +1-541-737-0497.

E-mail address: william.baird@orst.edu (W.M. Baird).

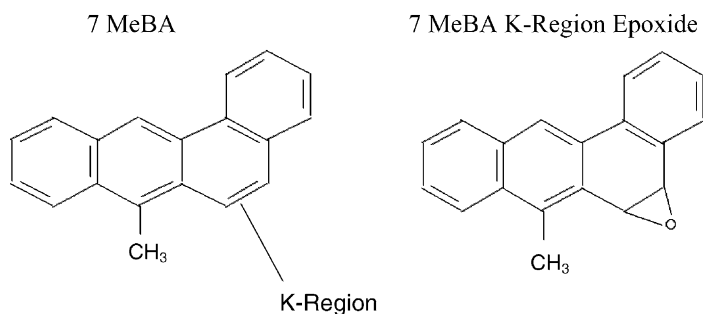


Fig. 1. Structure of 7-methylbenz[a]anthracene and 7-methylbenz[a]anthracene-5,6-oxide (K-region epoxide).

PAH-DNA adducts from the DNA. To do so we treated either mouse embryo cells in tissue culture or mouse skin with [³H]-7-methylbenz[a]anthracene (7-MeBA) and isolated the ³H-PAH-modified DNA and degraded it enzymatically to deoxyribonucleosides [4]. The 7-MeBA-deoxyribonucleoside adducts were then isolated from the unmodified deoxyribonucleosides by chromatographing on Sephadex LH20 columns eluted with a methanol–water gradient [4]. Dr. Philip Grover and the late Professor Peter Sims had succeeded in synthesizing the K-region epoxide of 7-MeBA [5]. This was reacted with DNA in aqueous solution to give 7-MeBA epoxide-DNA adducts [5]. After enzymatic digestion to deoxyribonucleosides this was co chromatographed with digested [³H]-7-MeBA DNA from mouse embryo cells. The cellular adducts were detected by counting the [tritium] eluted in each [fraction]. The 7-MeBA epoxide-DNA adducts were monitored by measuring the UV elution of the gradient at 246 nm. The 7-MeBA-DNA adducts eluted

well before the 7-MeBA epoxide adduct peaks indicating that this epoxide was not the reactive form of 7-MeBA formed by metabolism in cells, also suggesting that the “ultimate carcinogenic metabolite” of 7-MeBA was more polar than the K-region epoxide based upon the earlier elution of the cellular [³H]-7-MeBA-deoxyribonucleoside adduct peaks [5]. One possible explanation was that the methyl group of 7-MeBA might have been oxidized to a more polar group in cells which could account for the more polar adduct. Therefore we collaborated with Dr. Ronald G. Harvey who had synthesized the K-region epoxide of this carcinogenic hydrocarbon (see Fig. 2) benzo[a]pyrene (BP) [6]. Since BP has no methyl group, cellular metabolism of a methyl group would not affect the elution of the adducts formed in vivo. BP-K-region epoxide (BP-4,5-oxide) was reacted with DNA in aqueous solution, enzymatically digested to deoxyribonucleosides and the digest co chromatographed with a digest of DNA

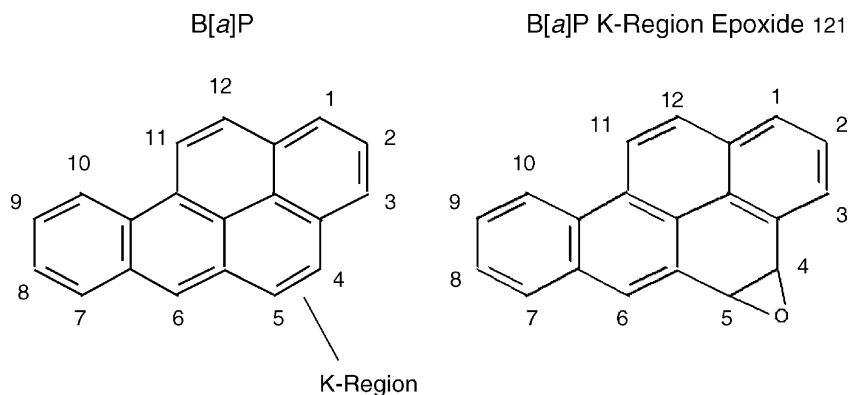


Fig. 2. Structure of benzo[a]pyrene and benzo[a]pyrene-4,5-oxide (K-region epoxide).

from [³H]-BP-treated cells in culture [6]. Again the ³H-BP-deoxyribonucleoside adduct peaks eluted earlier than the BP-4,5-epoxide-DNA adduct peaks [6] indicating that the activation of BP in cells resulted in a more polar “ultimate carcinogenic metabolite” than the K-region epoxide [6]. Studies by Rasmussen and his group [7] of the binding of various BP dihydrodiols to DNA in hamster microsomes demonstrated that the 7,8-dihydrodiol gave exceptionally high levels of DNA binding suggesting that this might be involved in the pathway of activation of BP to its “ultimate carcinogenic metabolite” [7]. Based upon this finding Drs. Grover and Sims hypothesized that the ultimate carcinogenic metabolite of BP might be an epoxide on the 9,10 region of the 7,8 diol or a diol epoxide. They prepared this BP-diol epoxide (BPDE) synthetically and reacted it with DNA in aqueous solution and digested it to deoxyribonucleosides [8]. When this was chromatographed with [³H]-BP-DNA-adducts prepared from hamster embryo cells in culture by treatment of the cells with [³H]-BP the [³H]-BP deoxyribonucleoside adducts co chromatographed with the BPDE-DNA adducts, indicating that this was indeed the “ultimate carcinogenic form” of BP formed in cells [8]. Further studies by Jerina et al. led to synthesis of the two stereo isomers of BPDE and the recognition that they differed greatly in their carcinogenic and mutagenic activity. Only one isomer, that with the epoxide on the opposite face of the molecule from the 7-hydroxyl exhibited high carcinogenic activity (reviewed in [9]).

Another important role for measurement of DNA adducts is in studies of the effect of potential cancer chemopreventive agents. For example, Dashwood et al. [10], demonstrated that chlorophyllin inhibited aflatoxin B₁ induced tumor formation in rainbow trout and showed that this decrease in tumorigenesis was correlated with a decrease in the formation of Aflatoxin B₁-DNA adducts [10]. These depurinated adducts were measured in human urine. Since chlorophyllin works by blocking uptake of the carcinogen through a complexation mechanism [11], if it is effective in humans one would expect the level of Aflatoxin B₁ adducts produced in urine to be reduced [12] whereas waiting for the appearance of more tumors to determine the efficacy of chlorophyllin treatment in chemoprevention would

require a number of years. This measurement of urinary adducts levels allowed rapid evaluation of the success of chlorophyllin treatment in reducing dietary aflatoxin effects on humans in a clinical trial conducted in Qidong, Tianjsu Province, People’s Republic of China, an area with a high incidence of hepatocellular carcinoma which is attributed to high aflatoxin ingestion by individuals [12]. This was demonstrated in a double-blind placebo-controlled [12] study that chlorophyllin treated individuals had a 50% reduction in the excreted Aflatoxin-DNA adduct marker compared to placebo-treated individuals demonstrating that chlorophyllin blocked the bioavailability of the dietary aflatoxin to which these individuals were exposed indicating that chlorophyllin is likely to be an effective chemopreventive agent for individuals unavoidably exposed to aflatoxins [12].

Another area where adduct analysis is already demonstrating an important role is in evaluating the risk posed by tamoxifen, a compound used in therapy for estrogen positive breast cancer [13], although the level of tamoxifen-DNA adducts formed in humans remains unresolved [13]. A recent conference on the genotoxicity of tamoxifen suggested that “if genotoxicity is relevant to tamoxifen induced endometrial cancer it may be possible to identify women at risk through detection of tamoxifen-DNA adducts” [13].

It is clear that in addition to their role in determining the mechanism of metabolic activation of carcinogens and mutagens, DNA adducts and their analysis will continue to be an important tool in establishing carcinogens that are likely to act through a genotoxic mechanism or as promoter type compounds that act by non-genotoxic mechanisms. This has important ramifications for the appropriate strategies for determining the regulation of human exposure to these compounds in order to minimize the risk they pose to humans.

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