PIOTR WIDLAK and JOANNA RZESZOWSKA-WOLNY

FORMATION AND REMOVAL OF DNA ADDUCTS IN LIVER OF RATS TREATED WITH HEPATOCARCINOGENS 2-AMINOFLUORENE OR 2-ACETYLAMINOFLUORENE

Department of Tumor Biology, Institute of Oncology, 44-100 Gliwice, Poland

The level of adducts in DNA of rats treated with 2-aminofluorene (2-AF) and 2-acetylaminofluorene (2-AAF) was compared at the times from 1 h till 28 days after injection. The highest amount of DNA adducts was observed 12 h after treatment with 2-AF and 24 h after treatment with 2-AAF, and reached values of about 18 and 21 fmol per μg DNA, respectively. Participation of the nonacetylated form, dG-C8-AF, in the total amount of DNA adducts was only slightly greater in rats treated with 2-AF then in those treated with 2-AAF.

2-AF and its acetylated derivative 2-AAF are potent mutagens and hepatocarcinogens. During their metabolic activation various reactive metabolites are formed which may bind covalently to macromolecules. After administration of either carcinogen three different adducts to deoxyguanosine are formed in hepatic DNA: the acetylated dG-C8-AAF and dG-N²-AAF, and nonacetylated dG-C8-AF [1]. The dG-C8-AAF adducts were found to be responsible for a major distortion of the DNA structure [2] and chromosomal aberrations [3], while the dG-C8-AF adducts were responsible mainly for point mutations [4]. It has been suggested that the two kinds of adducts play different roles in hepatocarcinogenesis [5, 6].

2-AF and 2-AAF are known to show similar hepatocarcinogenicity [5, 7]. It seemed of interest to study whether the animals treated with these carcinogens had a similar pattern of DNA adducts. In the present work we treated rats with both forms of aminofluorene and analysed the formation of hepatic DNA adducts using the ^32P postlabeling method.

MATERIALS AND METHODS

Male Wistar rats (200-250 g, 2-3 rats/group, fasted for 12 h before treatment) were injected with a single dose (50 mg/kg body weight) of 2-AF or 2-AAF (from Sigma Chemical Company) in 200-250 μl of dimethylsulfoxide
(DMSO). The animals were killed 1, 3, 6, 12, 24 hours and 3, 7, 28 days after injection. Livers were collected and immediately frozen. DNA was isolated by standard SDS/phenol treatment [8].

Adducts were analysed by the postlabeling method of Randerath et al. [9]. The $^32$P-labeled adduct nucleotides were separated by 4-directional PEI-cellulose thin-layer chromatography (t.l.c.). Total nucleotides were analysed by 2-directional PEI-cellulose t.l.c. after appropriate dilution. Adduct's level was calculated according to the formula given by Gupta et al. [10].

RESULTS AND DISCUSSION

We have studied the formation of adducts in rat liver DNA after administration of acetylated (2-AAF) and nonacetylated (2-AF) derivatives of aminofluorene. The quantitative and qualitative pattern of DNA adducts at different times after a single injection of carcinogen was estimated.

DNA adducts can be visualized by 4-directional chromatography of labeled nucleotides (Fig. 1). On both chromatograms (1A and 1B) three spots can be clearly distinguished. According to literature data [10] spot 1 should contain the major nonacetylated adduct dG-C8-AF, whereas spots 2 and 3 contain probably the acetylated forms of adducts.

Both carcinogens gave similar pattern of adducts in liver DNA. Early after the injection the contribution of the nonacetylated form dG-C8-AF in the total amount of DNA adducts was slightly higher after treatment with 2-AF than after treatment with 2-AAF (Fig. 2). Later, in the phase of the adduct removal, the contribution of dG-C8-AF in the total amount of adducts increased (Fig. 2). This effect was probably caused by the proportionally lower rate of removal of nonacetylated forms of adducts from DNA [11].

Figure 3 represents the kinetics of 2-AF and 2-AAF adduct formation and removal. Generally, the rates of formation and removal of hepatic DNA adducts and their maximal levels were similar in animals treated with 2-AF and 2-AAF, however within the first 12 h after the injection the rate of the 2-AF adduct formation was slightly higher.

$N$-Hydroxylation is the first step of metabolic activation of both 2-AF and 2-AAF, and produces $N$-hydroxy-2-AF and $N$-hydroxy-2-AAF. The $N$-hydroxylated derivatives of these carcinogen are also known to possess greater mutagenic and carcinogenic activity [12]. On comparing our results with literature data [13] we found that 2-AAF had a weaker capability to form DNA adducts than its hydroxylated derivative $N$-hydroxy-2-AAF. The maximal level of DNA adducts in liver of rats treated with $N$-hydroxy-2-AAF was observed 1.5 h after injection [13], i.e. about 20 h earlier than after injection of 2-AAF. This suggests that $N$-hydroxylation is time-limiting step for the adduct formation.
Fig. 1. Four directional chromatography of labelled nucleotides prepared from hepatic DNA of rats treated with a single dose of 2-AF (A), 2-AAF (B) or DMSO alone (C) and killed 24 h after treatment.
Fig. 2. Distribution of adducts in DNA of rats treated with 2-AF or 2-AAF and killed 24 h or 7 days after treatment. White area on each bar shows the contribution of the nonacetylated form dG-C8-AF.

Fig. 3. Rate of formation and removal of DNA adducts in rat liver following a single dose of 2-AF or 2-AAF. Each point represents the mean value from 2-3 analyses.

The formation of nonacetylated DNA adducts from 2-AF goes through the N-hydroxy-2-AF step and seems to be much simpler and faster than the ways leading to formation of adducts from 2-AAF (Scheme 1). This is probably the reason why, during the first period after the injection, 2-AF formed more DNA adducts than did 2-AAF. There is more than one way of obtaining the nonacetylated forms of DNA adducts from 2-AAF [14, 15]. These ways involve the deacetylation or transacetylation steps (Scheme 1). Acetylated adducts have been suggested to arise mainly by the reaction of DNA with N-sulfoxy-2-AAF [16]. Therefore the formation of acetylated
DNA adducts from 2-AF can be expected to involve the acetylation step (Scheme 1).

We expected that administration of acetylated derivatives of the carcinogen would result in a significantly higher level of acetylated forms of adducts.

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\begin{align*}
\text{2-AF} & \xrightarrow{N\text{-acetylase}} \text{N-acetylated adducts} \\
& \xrightarrow{\text{oxygenase}} \text{N-hydroxy-2-AF} \\
& \xrightarrow{N\text{-deacetylase}} \text{nonacetylated adducts} \\
\text{2-AAF} & \xrightarrow{\text{oxygenase}} \text{N-hydroxy-2-AAF} \\
& \xrightarrow{N\text{-deacetylase}} \text{N-acetoxy-2-AAF} \\
& \xrightarrow{\text{sulfotransferase}} \text{N-sulfooxy-2-AAF} \\
& \xrightarrow{O\text{-acetylase}} \text{N-acetoxy-2-AAF} \\
\end{align*}
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Scheme 1. Possible metabolic pathways for the activation of 2-AF and 2-AAF into reactive species. Prepared on the basis of ref.: 14-16

However, the results presented in Fig. 1 and 2 show that the differences between the two carcinogens were not very striking.

We have found a similar pattern of the DNA adducts formed by 2-AF and 2-AAF in rat liver. Among possible reasons for similar hepatocarcinogenic ability of the two carcinogens the similar pattern of different types of adducts may be of great importance.
REFERENCES