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Biochemical Pharmacology, Vol. 40, No. 11, pp. 2560–2562, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

The *in vivo* site of formation of a carcinogen–serum albumin adduct

(*Received* 17 *July* 1990; *accepted* 10 *August* 1990)

The macromolecular binding of xenobiotics in humans is of interest, because of its possible involvement in the production of a toxic response and also as a means of monitoring exposure to potentially harmful substances. Such interaction may take place by the direct covalent binding of a chemically reactive compound to circulating serum albumin, but many environmental toxins and carcinogens will react only after metabolic activation (usually mediated by cytochrome P450 mixed function oxidases). Aflatoxin B_1 (AFB_1) is an hepatotoxic and carcinogenic mycotoxin [1] which, after activation by liver parenchymal cells to AFB_1-8,9-epoxide [2, 3] (or its dihydrodiol derivative [4]), reacts with albumin. The AFB_1-albumin adduct thus formed would appear to offer a useful index of exposure to the toxin [5, 6] in those developing countries where it is believed to be associated with a high level of primary liver cancer [7]. The release of the highly reactive electrophilic AFB_1-8,9-epoxide from the cells in any significant amount appears unlikely and it is possible a large proportion of the reaction with albumin could occur (intracellulary) within the hepatocytes, near the endoplasmic reticulum, at sites adjacent to both the activation of the toxin and synthesis of proalbumin or hepatocyte albumin (formed by cleavage of an N-terminal hexapeptide from proalbumin [8, 9]). We have examined this possibility using a rat model system.

The time courses of the synthesis of albumin and AFB_1-adduct formation in the various albumin fractions were determined (Fig. 1a and b). The time course of synthesis of proalbumin, hepatocyte albumin and serum albumin is in close agreement with that reported by Dorling *et al.* [10]. The proalbumin plus hepatocyte albumin fraction was rapidly labelled with ^{14}C, the level of labelling increasing over the 20 min period following [^{14}C]leucine injection, and with little ^{14}C label being detected in the serum albumin at this time. Subsequently, ^{14}C label declined in the proalbumin plus hepatocyte albumin fraction and entered the serum albumin fraction as the newly-synthesized protein was released from the hepatocytes. The time course of ^3H labelling of the fractions demonstrated a rapid labelling of the serum albumin fraction, over the period (0–20 min following injection) when little newly synthesized ^{14}C labelled albumin had entered this fraction. Extraction of these fractions with chloroform demonstrated that >90% of the ^3H label was soluble in this solvent, and HPLC analysis [4] showed that 80–85% of the label was associated with AFB_1. This result is in agreement with the findings of Dirr and Schabort [11] who reported a tight binding of AFB_1 to rat serum albumin *in vivo*. There was a close parallel between the ^3H and ^{14}C labelling of the proalbumin plus hepatocyte albumin in all samples, the ratio being approximately 1. In the serum albumin sample the $^3H/^{14}C$

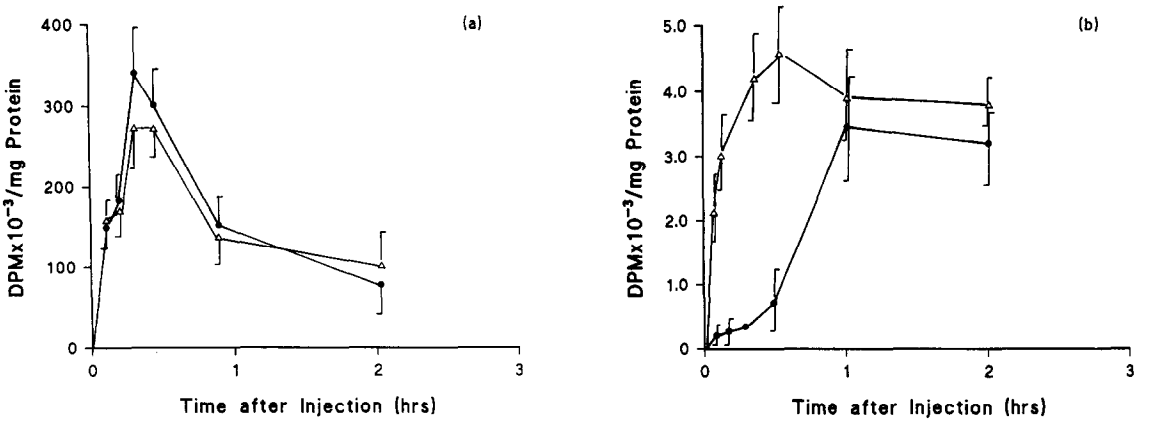

Fig. 1. Time course of labelling of rat albumin fractions following the injection of [^{3}H]AFB$_1$ and L-[^{14}C]leucine. Male Fischer F344 rats (190–210 g body wt) were injected simultaneously i.p. with 5 μCi/16 nmol L-U[^{14}C]leucine (Radiochemical Centre, Amersham, U.K.) and 1 μCi/0.25 μmol [^{3}H]AFB$_1$ (Moravek Biochemicals, Brea, CA, U.S.A.) in 100 μL dimethyl sulphoxide. Groups of three rats were killed by ether inhalation at intervals and blood samples collected by cardiac puncture. Serum samples were prepared. Liver samples (5 g) were used for the preparation of microsomal fractions. Proalbumin, hepatocyte albumin and serum albumin were precipitated from aliquots of solubilized protein obtained from the microsomal fractions and from serum [10, 14] using a sheep anti-rat albumin antibody (a generous gift from Prof. J. D. Judah), and pelleted by centrifuging. Following washing of the precipitates (by resuspension) and centrifugation (2×) aliquots of the resuspended fractions were used for dual-label liquid scintillation counting. Albumin–antibody complexes were dissociated using the method of Judah and Nicholls [14]. Aliquots of the solubilized albumin fractions were used for the determination of protein [15], and were also used for SDS–PAGE followed by transfer to membranes and detection by western blotting using the sheep anti-rat albumin antibody followed by a goat anti-sheep IgG peroxidase-conjugated second antibody. Positive reactions were obtained from the experimental samples corresponding with the molecular weight of the albumins [8, 9]. (a) Proalbumin + hepatocyte albumin (microsomal). (b) Serum albumin. (△) [^{3}H]AFB$_1$ (●) L-[^{14}C]leucine. Results are the means of three rats ± SD.

ratio was initially >20, but in the 1 and 2 hr samples this ratio declined to between 1.1 and 1.2; values little higher than those in the proalbumin plus hepatocyte albumin fractions. Extractions of the 2 hr serum albumin samples with chloroform showed only 15–20% of the ^{3}H label was soluble in this solvent. HPLC again demonstrated that >80% of the label was associated with AFB$_1$.

The results are consistent with the following series of events. Initially there is a tight binding of the administered [^{3}H]AFB$_1$ to serum albumin, following its absorption from the peritoneal cavity, which permits distribution of the highly lipophilic toxin; the polar [^{14}C]leucine is not associated with proteins. During the 1 hr period following injection, the non-covalently bound AFB$_1$ is removed from the albumin, redistributed in the tissues and is largely metabolized in the hepatocytes. The AFB$_1$-8,9-epoxide reacts with adjacent nucleophiles, two of these being nuclear DNA [12] and newly synthesized albumin. This could account for the reported dose-independent parallel between the levels of adduct formation in these fractions following AFB$_1$ administration [13]. Such a relationship could permit extrapolation of levels of AFB$_1$–albumin adduct in humans to probable levels of hepatic DNA binding and hence assessment of possible carcinogenic risk. The release of AFB$_1$-adducted, newly synthesized albumin into the serum (1 and 2 hr samples) resulted in ^{3}H/^{14}C ratios reflecting those in the proalbumin plus hepatocyte albumin fractions at all stages. The slightly higher ratios in the serum albumin was due in part to a persistence of some tightly-bound non-metabolized AFB$_1$ in this fraction, but there could also have been a small contribution to circulating albumin adduct formed by the release of activated AFB$_1$ from hepatocytes (or other AFB$_1$-metabolizing cells).

In summary, the results obtained are consistent with the majority (>90%) of the AFB$_1$-adducted rat serum albumin being formed by a modification of the protein at the time of its synthesis in the hepatocyte. This finding could have implications in terms of the interpretation of human exposure data based on the level of AFB$_1$–serum albumin adducts, and also the possible site of modification of serum albumin by other carcinogens and xenobiotics requiring metabolic activation.

Acknowledgement—Z. S. C. Okoye was a recipient of a Yamagiwa-Yoshida award from the UICC.

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Biochemical Pharmacology, Vol. 40, No. 11, pp. 2562–2564, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Monoamine oxidase substrates in Parkinson's disease

(*Received* 30 *April* 1990; *accepted* 13 *August* 1990)

Platelet monoamine oxidase (MAO, EC 1.4.3.4) is a membrane bound mitochondrial enzyme that is involved in the metabolism of both endogenous and exogenous biogenic amines [1]. Human platelet MAO is classified as type B on its inhibitor and substrate specificity [2]. It was originally reported that MAO-B in platelets from Parkinsonian patients was slightly reduced compared with controls [3], when 4-hydroxy-phenylethylamine was used as substrate, and that on treatment of the patients with L-dopa, a further reduction of platelet MAO-B activity was observed. However, a more recent report suggested that platelet MAO-B activity using phenylethylamine, a different substrate, was elevated in treated Parkinson's disease (PD) patients compared to controls [4]. This work was recently repeated with untreated Parkinson's disease patients and the results were found to be in agreement [5]. The aim of this study was to investigate whether substrate specificities of platelet MAO-B activity are different in control and Parkinson's disease patients.

Methods

Patients with clinically defined untreated idiopathic Parkinsonism were selected as previously reported [5]. Healthy volunteers were used as controls. Platelets were isolated and assayed for MAO-B activity as described [6] but using 50 μM dopamine instead of 20 μM phenylethylamine as substrate. Deprenyl was used as an inhibitor of MAO-B activity [7] to provide blank results for the assay. 3,4-Dihydroxyphenylacetic acid (DOPAC) was determined by HPLC with electrochemical detection [8]. Protein concentration of platelets was determined by a colorimetric method [9]. Determinations were carried out in duplicate.

Results and Discussion

The result of the investigation can be seen in Table 1d. There was no age correlation between the control and PD population with regard to MAO-B activity nor was there a sex correlation in the PD population with MAO-B activity. There was a difference in MAO-B activity between the sexes in the control population but this was not significant ($P < 0.2$, two-tailed Student's t-test). It can be seen, though, that the MAO-B activity was clearly different in the two populations. A mean control platelet MAO-B activity of 476.2 nmol DOPAC/mg/hr was seen compared with a value of 230.6 nmol DOPAC/mg/hr in the PD population ($P < 0.005$, two-tailed Student's t-test).

These values show that the pattern of MAO-B activity in platelets of PD patients differs depending on which substrate is used; this can be seen when results from other workers are tabulated and compared with the data from this investigation (Table 1). When phenylethylamine was used as the substrate both treated PD (Table 1a) and untreated PD (Table 1b) platelets had a significantly higher