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Rapid plasma clearance of albumin-acrolein adduct in rats

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Abstract

Protein adducts are used as markers of chemical exposure. Determination of the clearance rate of these adducts from the blood circulation will provide the time frame for their measurement. Radioactive albumin was prepared biosynthetically by repeated intraperitoneal injections of L-[4,5-^{3}H]lysine to a rat. After an affinity purification, an aliquot of this native [^{3}H-lysine]albumin was adducted with 5 mM acrolein. Both the native albumin (A-treated group) and the albumin-acrolein adduct (AAA-treated group) were intravenously injected to separate groups of rats, and the clearance of radioactivity from the plasma was measured as a function of time. At the end of the experiment (33 h after the injection), radioactivity in the whole plasma, and in homogenates of liver, kidney and spleen and their trichloroacetic acid(TCA)-soluble and -insoluble fractions in both A- and AAA-treated groups, was measured. The results, at the initial 11 h after the injection, showed that the radioactivity was cleared from the circulating plasma more rapidly in the AAA-treated group (32% of the injected radioactivity remained) than the A-treated group (52%). At 33 h after the injection, 22% of the injected radioactivity remained in the plasma in the AAA-treated group as compared to 32% in the A-treated group. The whole homogenates of liver and kidney and their corresponding TCA-soluble fractions showed higher radioactivity in the AAA-treated group as compared to the A-treated group. However, the TCA-insoluble fractions from livers and kidneys of the AAA-treated group showed lower radioactivity as compared to the A-treated group. These results indicated that the albumin-acrolein adduct was removed more rapidly from the circulation than the native albumin, and degraded more rapidly by the liver and kidney. There was no preferential removal or degradation of the adducted albumin by the spleen.

Key words: Acrolein; Albumin adduct; Plasma clearance; Protein degradation

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1. Introduction

Acrolein, one of the hazardous aldehydes, is an ubiquitous environmental pollutant due to its formation during incomplete combustion of gasoline, coal, wood and plastic material (for review see Refs. 1–3). It is also present in tobacco smoke and is formed during charcoal broiling of meat [3]. Moreover, acrolein is a toxic metabolite of several xenobiotics, such as allyl alcohol, allylamine, allyl-formate and the widely used anticancer drug, cyclophosphamide [3]. Acrolein may also be formed intracellularly by enzymatic oxidation of polyamines and during lipid peroxidation [3]. It is a mutagen [4,5] and a potential human carcinogen [6,7], although some recent studies suggest lack of neoplastic response to acrolein [8,9]. Acrolein is very toxic to mucosa of respiratory tract, eye, etc. and to skin and liver of mammalian species [3] due to its strong electrophilic nature to react with cellular nucleophiles containing thiol or amino groups.

Because of the potential of exposure and resulting toxicity of acrolein to humans, there is a need for a reliable method for biomonitoring of populations at risk [10]. Acrolein forms DNA adducts [5,11] and it binds covalently to hemoglobin [12] and albumin [13]. Studies from this laboratory have shown that acrolein modifies lysine and histidine of plasma α_1-proteinase inhibitor causing its inactivation [14–16]. Acrolein modified amino and imidazole functional groups of proteins mainly through the Michael addition reaction [13,14,16,17]. Recently, we described a method to quantitate blood protein adducts of acrolein using tritiated borohydride reduction reaction [18]. This method was found to be more rapid and sensitive as compared to the previously developed methods for the estimation of acrolein adducts of blood proteins [13]. However, it is very essential to have the information about the rate of plasma clearance of the albumin-acrolein adduct as compared to the native albumin to determine the optimum time frame for its measurement. In the present study we described the plasma clearance of the albumin-acrolein adduct as compared to that of the native albumin. The study further compares the cumulative uptake and degradation of the adducted albumin versus the native albumin by liver, kidney and spleen.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all chemicals used in the present study were purchased from Sigma Chemical Co. (St. Louis, MO). The radiochemical L-[4,5-^{3}H]lysine with specific activity of 87 Ci/mmol was purchased from Amersham Corporation (Arlington Heights, IL). The universal liquid scintillation counting solution (Trucount) was obtained from Tru-Lab Supply Co. (Libertyville, IL). Albumin removal affinity-filter cartridges were purchased from Affinity Technology, Inc. (Leonia, N.J.).

2.2. Animals

Male Sprague–Dawley rats (Harlan Sprague–Dawley, Houston Breeding Laboratories, Houston, TX), weighing 240–260 g, were used for the study. They were

acclimated for 7 days before treatment in our animal facilities, which have environmentally controlled rooms with an alternating light (06.00–18.00 h) - dark (18.00–06.00 h) cycle and an ambient temperature of 22–25°C. They were housed over untreated corn cob bedding and were given food (commercial rat chow No. 5001, Ralston Purina Co., St. Louis, MO) and water ad libitum.

2.3. Preparation of the ^{3}H-lysine-labelled albumin

L-[4,5-^{3}H]lysine was diluted in saline (0.9% NaCl) to achieve radioactivity of 1 μCi/10 μl. This solution was repeatedly injected, intraperitoneally, to a single rat at a dose of 20, 10, 10, 10, 40, and 20 μCi at 0, 3, 6, 9, 12 and 22 h respectively. Two hours after the last injection, the rat was anesthetized using diethyl ether and blood was drawn from the dorsal aorta and plasma was separated from the red cells by centrifugation at 5000 rev./min. The plasma was dialyzed against 50 mM phosphate buffer, pH 7.2. An aliquot of plasma was taken and albumin was separated using affinity filter column as described by Thakore et al. [18]. The albumin fraction obtained was dialyzed against distilled water and lyophilyzed. The lyophilized albumin was then dissolved in 5 ml of saline and radioactivity was measured. This [^{3}H-lysine]native albumin (~20 000 dpm/mg albumin) was used as a control and for the synthesis of [^{3}H-lysine]albumin-acrolein adduct.

An aliquot of this [^{3}H-lysine]native albumin (5 mg/ml) was adducted with acrolein (5 mM) using the procedure described by Thakore et al. [18]. At this concentration of acrolein 5 residues of lysine and 4 residues of histidine per mole of albumin were modified [13]. Acrolein, in 100 mM sodium phosphate buffer, pH 7.2, was added to the solution of albumin to obtain the 5 mM acrolein concentration. The reaction was performed in a screw-cap glass vial at 37°C for 2 h in a water bath. At the end of the incubation period the sample was dialyzed exhaustively against distilled water to remove unreacted acrolein followed by overnight dialysis against 100 mM phosphate buffer, pH 7.2. This sample of [^{3}H-lysine]albumin-acrolein adduct (~20 000 dpm/mg protein) was utilized to study plasma clearance in rats as described below.

2.4. In vivo plasma clearance of the [^{3}H-lysine]native albumin and its acrolein adduct

Six male rats were divided into two groups with three rats each. Control rats were injected with [^{3}H-lysine]native albumin (A-treated group), whereas experimental rats were injected with [^{3}H-lysine]albumin-acrolein adduct (AAA-treated group), through the tail vein. Each rat was injected with 10 mg of labelled albumin, containing approximately 200 000 dpm, in saline. After the injection, blood samples (25 μl) were collected from the tail vein of each rat at various time intervals up to 33 h and radioactivity in the plasma was measured at each time point and expressed as a percentage of injected radioactivity remaining per ml of plasma. The radioactivity injected per ml of plasma was calculated considering the total plasma volume as 10 ml [19] for each rat. The rate of clearance of radioactivity from the plasma was then compared between the A- and AAA-treated groups.

2.5. Distribution of the radioactivity in the tissues

At 33 h after the injection of either [^{3}H-lysine]native albumin (A-treated group)

or its acrolein adduct (AAA-treated group), rats were anesthetized using diethyl ether and blood was drawn in a heparinized tube from the dorsal aorta of each rat and the plasma was separated. Liver, kidney and spleen were collected, rinsed with saline and blotted. A portion of each tissue (approximately 1 g) was homogenized in 100 mM phosphate buffer, pH 7.2, to make a 10% homogenate.

(a) Measurement of radioactivity in the whole plasma, liver, kidney and spleen. Aliquots of the whole plasma (200 μl) or each tissue homogenate (1 ml, equivalent to 100 mg tissue, dissolved in 1.0 M NaOH) were added to scintillation vials, each containing 10 ml of liquid scintillation counting solution. The radioactivity was measured on a liquid scintillation counter (Model LS 6000 SE, Beckman, USA) and the counts (dpm) were expressed as per ml of plasma or g of tissue.

(b) Measurement of radioactivity in the albumin and globulin fractions of the plasma. Albumin was separated from 200 μl of each plasma using an affinity chromatography column. The unabsorbed proteins (detected spectrophotometrically at 280 nm) were washed thoroughly with 50 mM phosphate buffer, pH 7.2. The wash was the globulin fraction. The bound albumin was then eluted with 10 ml of 500 mM NaCl in 50 mM phosphate buffer, pH 7.2. Both the albumin and globulin fractions were dialyzed overnight against distilled water, lyophilized, and dissolved in 0.5 ml of distilled water and radioactivity was measured (dpm/mg protein) in each fraction.

(c) Measurement of radioactivity in the TCA-soluble and -insoluble fractions of the whole plasma, liver, kidney and spleen. Aliquots of the whole plasma (200 μl) or each tissue homogenate (1 ml, equivalent to 100 mg tissue, dissolved in 1.0 M NaOH) were precipitated with equal volumes of 20% TCA, vortexed and centrifuged to separate the TCA-soluble and -insoluble fractions. The TCA-insoluble fractions were dissolved in 1.0 M NaOH (200 μl for the plasma and 1 ml for the tissue) and the radioactivity was measured and expressed as described above.

3. Results

3.1. In vivo plasma clearance of the [^{3}H-lysine]albumin and its acrolein adduct

As shown in Fig. 1, the radioactivity in plasma decreased with time after injection of either the albumin or the albumin-acrolein adduct to rats through tail vein. The sharp decline in radioactivity, in both the groups, at initial period up to about 1–2 h after injection may be due to the distribution of the injected albumin into the plasma and different tissue compartments of the body. Later, between 3 and 11 h after injection, the decline in the radioactivity in the plasma may be due mainly to the uptake as well as degradation of the albumin by the liver and other tissues. During this second phase, between 3–11 h, the significantly lower radioactivity was observed in the plasma of the AAA-treated group (32% of the injected radioactivity remained) as compared to the A-treated group (52%). This lower radioactivity in the plasma of the AAA-treated group suggested the higher rate of uptake and degradation of the albumin-acrolein adduct by the tissues. At later time points (26 and 33 h), there was a gradual decline in radioactivity in both the groups with higher decline in the case of the AAA-treated group (22% of the injected radioactivity remained vs. 3% in the A-treated group). We were unable to measure the half-life of injected albumin in the A- and AAA-treated groups, since the radioactive clearance was not measured

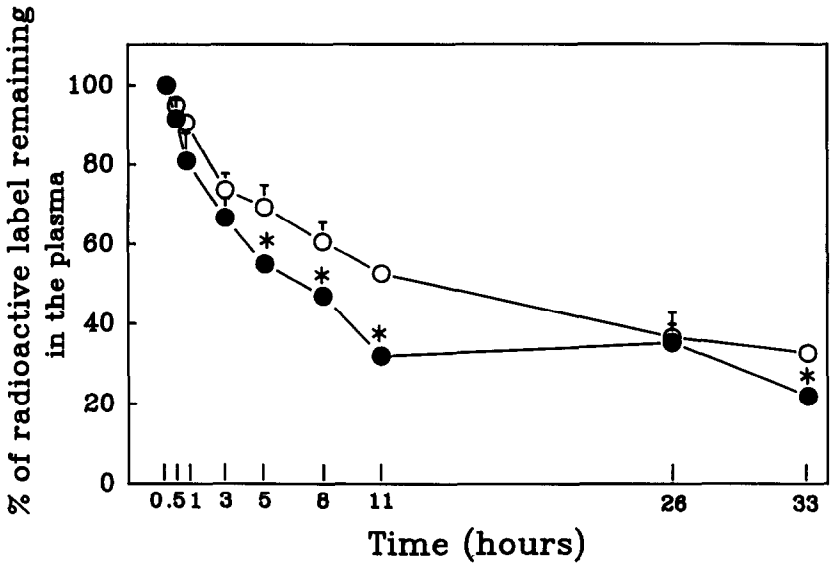

Fig. 1. Effect of albumin-acrolein adduct (5 mM) on its in vivo plasma clearance in rat. Percent of injected radioactivity remaining in the plasma at various time points after the intravenous injection of either the [^{3}H-lysine]albumin (A-treated group, O) or its acrolein adduct (AAA-treated group, ●) to rats, is shown. The values are mean ± S.E. for $n = 3$. The asterisk indicates significant difference from the A-treated group at the corresponding time points ($P < 0.05$).

for a longer period of time beyond 33 h. The half-life of plasma albumin in rat, as reported in the literature [20], is 3.7 days.

3.2. Radioactivity in the plasma

As shown in Fig. 2, at 33 h after the injection of either the native albumin or its acrolein adduct, radioactivity in the whole plasma was significantly lower in the AAA-treated group as compared to the A-treated group. The radioactivity in the TCA-soluble fractions of the plasma of both the groups was very low and did not differ significantly from each other. However, the radioactivity in the TCA-insoluble fractions of plasma of both the groups was relatively high, but it was significantly lower in the AAA-treated group than the A-treated group.

Fig. 3 shows the distribution of the radioactivity in the albumin and globulin fractions after affinity column separation of the plasma of the A- and AAA-treated groups. As shown, the majority of the radioactivity (~90%) in the plasma was associated with the albumin fraction and a relatively small amount (~10%) with the globulin fraction in both the groups.

3.3. Radioactivity in tissues

Liver: Fig. 4a shows the radioactivity in whole liver and its distribution in the TCA-soluble and -insoluble fractions of the A- and AAA-treated groups. The

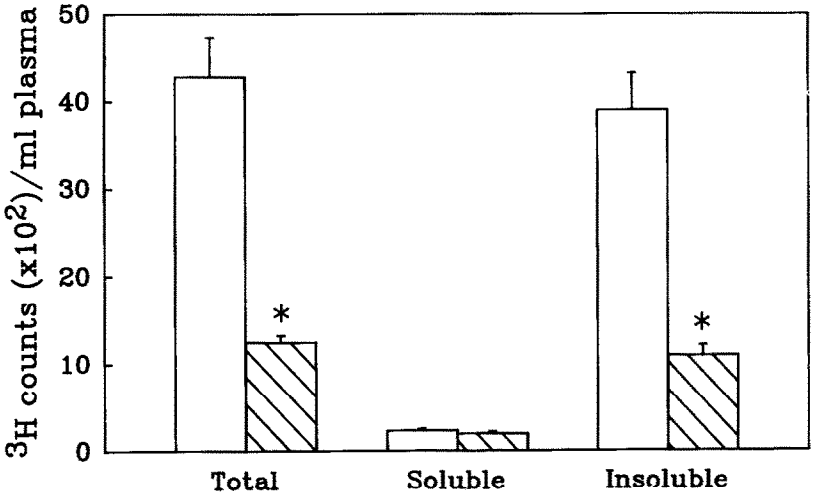

Fig. 2. Effect of albumin-acrolein adduct (5 mM) on its in vivo plasma clearance in rat. Radioactivity/ml of plasma in the whole plasma and its distribution in the TCA-soluble and -insoluble fractions, collected at 33 h after the injection of either the [^{3}H-lysine]albumin (A-treated group, □) or its acrolein adduct (AAA-treated group, ▨) to rats, is shown. The values are mean ± S.E. for $n = 3$. The asterisk indicates significant difference from the A-treated group ($P < 0.05$).

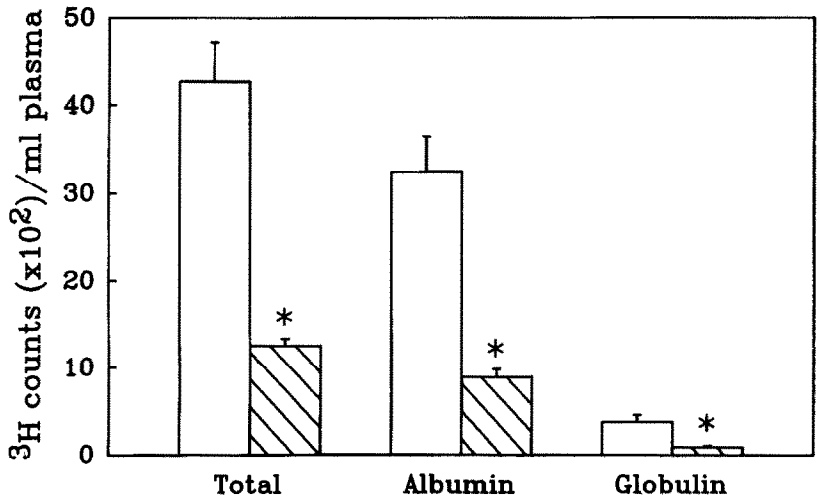

Fig. 3. Effect of albumin-acrolein adduct (5 mM) on its in vivo plasma clearance in rat. Radioactivity/ml of plasma in the whole plasma and its distribution in the albumin and globulin fractions, collected at 33 h after the injection of either the [^{3}H lysine]albumin (A-treated group, □) or its acrolein adduct (AAA-treated group, ▨) to rats, is shown. The values are mean ± S.E. for $n = 3$. The asterisk indicates significant difference from the A-treated group ($P < 0.05$).

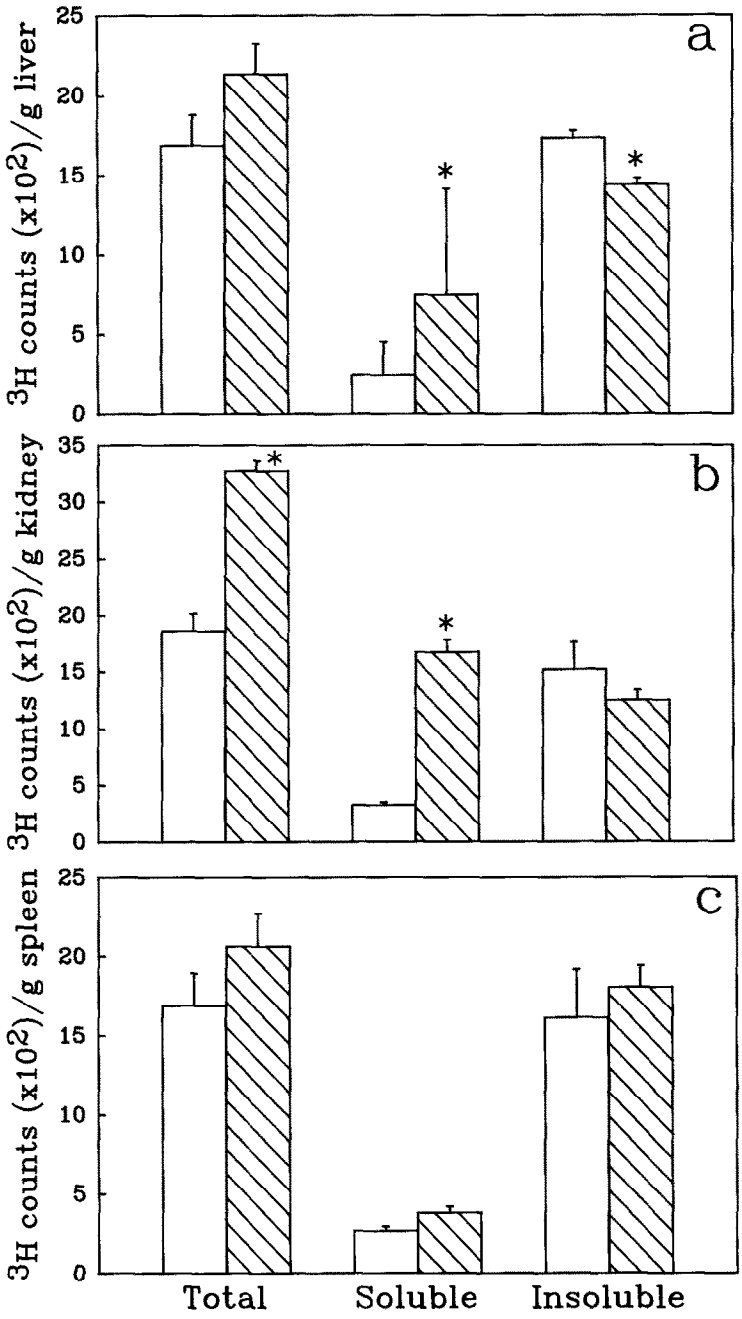

Fig. 4. Effect of albumin-acrolein adduct (5 mM) on its in vivo degradation by the liver (a), kidney (b) and spleen (c) of rat. Radioactivity/g of tissue in the whole tissue and its distribution in the TCA-soluble and -insoluble fractions, collected at 33 h after the injection of either the [^{3}H-lysine]albumin (A-treated group, □) or its acrolein adduct (AAA-treated group, ▨) to rats, is shown. The values are mean ± SE for $n = 3$. The asterisk indicates significant difference from the A-treated group ($P < 0.05$).

radioactivity in the whole liver was higher in the AAA-treated group as compared to the A-treated group; however, it was not statistically significant. The radioactivity was also higher in the TCA-soluble fraction and lower in the TCA-insoluble fraction for the AAA-treated group as compared to the A-treated group.

Kidney: As seen with liver, radioactivity in the whole kidney (Fig. 4b) was also significantly higher in the AAA-treated group as compared to the A-treated group. The TCA-soluble and -insoluble fractions also showed the pattern, similar to that seen with liver, for both the groups. The radioactivity was higher in the TCA-soluble fraction for the AAA-treated group as compared to the A-treated group.

Spleen: The radioactivity in the whole spleen and its distribution in the TCA-soluble and -insoluble fractions for the A- and AAA-treated groups are shown in Fig. 4c. The radioactivity in the whole spleen and its distribution in the TCA-soluble and -insoluble fractions for the AAA-treated group was consistently higher as compared to the A-treated group but it was not statistically significant.

4. Discussion

The present study compares the plasma clearance rate of the albumin-acrolein adduct with that of the albumin in rat. The cumulative uptake and degradation of the adducted albumin in the liver, kidney and spleen at the end of the experiment (33 h after the injection) were also compared with that of the native albumin. Generally, turnover rate of the protein in vivo is increased when a normal amino acid is substituted by an analog [21,22]. Several such modifications have been identified by phosphorylation, carbomylation, deamidation, oxidation and formation of mixed disulfide derivatives of amino acid residues [23–27]. The covalent modification of an amino acid residue in the protein (such as in albumin-acrolein adduct) may be equivalent to the replacement of that residue with an analog.

Acrolein, an α,β-unsaturated aldehyde, is a strong electrophile and therefore shows a high reactivity with nucleophiles such as sulfhydryl, amino, and hydroxyl functional groups [28]. Recent studies from our laboratory have shown that acrolein covalently binds to amino and imidazole functional groups of proteins mainly through the Michael addition reaction [13,14,16]. Therefore, it would be possible that the covalent adducts of acrolein with amino groups of proteins results in transformation of these proteins into the forms which could be recognized by the body as 'foreign bodies' and hence cleared rapidly from the circulation.

In the present study also, the results showed that the albumin-acrolein adduct disappeared from the circulation more rapidly than the native albumin during the initial 11 h after injection. At the end of the experiment (33 h after the injection) 22% of the injected radioactivity remained in the plasma of the AAA-treated group as compared to 32% in the A-treated group. This shows that the rate of in vivo plasma clearance of the injected albumin-acrolein adduct was significantly higher as compared to the rate observed for the native albumin. Moreover, the radioactivity was present mainly in the TCA-insoluble fraction with only a small amount in the TCA-soluble fraction for both the groups, indicating that the radioactivity released by the catabolism of albumin is not recirculated into the plasma. Further, the radioactivity was mainly found in the albumin fraction of the plasma with a relatively small

amount in the globulin fraction, suggesting that the radioactivity which was released from the metabolism of albumin in the liver is not recycled for the protein biosynthesis.

The present data support the earlier observation that albumin, modified in vitro with formaldehyde, is rapidly cleared from the circulation and it is taken up rapidly by the sinusoidal liver cells, the major scavenger cells in vivo, via receptor-modified endocytosis [29]. Scavenger function of macrophages or macrophage-derived cells for chemically modified proteins has been known for formaldehyde-treated bovine serum albumin [29,30], maleylated albumin [31], malondialdehyde-modified [32] and acetylated low-density lipoproteins [33,34].

We also observed higher levels of radioactivity in the whole liver homogenate of the AAA-treated group as compared to the A-treated group, indicating the increased rate of uptake by the liver of the adducted albumin as compared to the native albumin. The radioactivity was higher in the TCA-soluble fraction and lower in the TCA-insoluble fraction of the liver in the AAA-treated group as compared to the A-treated group, which further suggested the higher rate of metabolism of the adducted albumin in the liver as compared to the native albumin. The radioactivity in the whole kidney, as well as in its soluble fraction, was also higher in the AAA-treated group as compared to the A-treated group, which suggested the increased rate of uptake and metabolism of the adducted albumin by the kidney. The radioactivity in the whole spleen and in its TCA-soluble and -insoluble fractions, though statistically nonsignificant, was also higher in the AAA-treated group as compared to the A-treated group, which might be indicative of the response of the spleen to this acrolein-modified protein as 'foreign bodies' for its rapid uptake and removal from the body.

The results of the present study suggested that the albumin-acrolein adduct was cleared from the blood circulation more rapidly as compared to the native albumin. Therefore, the rate of plasma clearance of the protein adducts should also be considered to establish their efficacy as biological markers of exposure.

5. Acknowledgements

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