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Immune response to acetaldehyde-human serum albumin adduct among healthy subjects related to alcohol intake

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ABSTRACT

Acetaldehyde (AA) is the main metabolic product in ethanol metabolism, although it can also derive from sources of airborne pollution. As a typical aldehyde, AA is able to react with a variety of molecular targets, including DNA and protein. This property justifies the hypothesis of a immune reaction against this kind of adduct, to be studied by a seroprevalence screening approach. In this study, the correlation between drinking habits and the amount of circulating AA-human serum albumin adduct (AA-HSA) was evaluated in a group of healthy subjects, non alcohol-addicted. Daily ethanol intake (grams) was inferred for each subject using the information collected through a questionnaire, and AA-HSA antibodies (AA-HSA ab) analyses were performed using the Displacement Assay on whole blood samples. The findings showed a correlation between ethanol intake and immune response to molecular adduct. These results underscore the evaluation of AA-HSA ab amount as a suitable molecular marker for alcohol intake that can be applied in future investigations on a large scale for prevention screening.

1. Introduction

Acetaldehyde (AA) is the first and principal product in the metabolism of ethanol. AA forms in the liver through detoxification reactions that are mainly catalysed by alcohol dehydrogenase and aldehyde dehydrogenase (Bosron and Li, 1986). However, AA also occurs in indoor environments as a member of the carbonyl chemical species, and it is an important factor in indoor air quality evaluations (Sarigiannis et al., 2011). Together with formaldehyde, AA represents one of the most abundant airborne aldehydes, which may be related to traffic emissions, smoking, combustion appliances, cosmetic products, and some hobby supplies such as photographic chemicals and special adhesives (Jurvelin et al., 2001; Wilke et al., 2004).

In general, aldehydes exhibit high reactivity, as they have a double bond that renders them a strong electrophile chemical species. Thus, they can easily partake in substitution reactions with aromatic compounds and in addition reaction with alkenes, which is typical for aldehydes. An example of this high reactivity is the formation of molecular adducts with DNA, which may be a critical initiating event in the multistage process of chemical carcinogenesis (Yokoyama and Omori, 2003). Evidence of this type of reactivity is observed in reactions between formaldehyde, which has the simplest structure among aldehydes, and DNA. This reaction forms malondialdehyde–deoxyguanosine adducts, which are found in pathologists who are occupationally exposed to formaldehyde (Bono et al., 2010). Moreover, AA has been demonstrated to form adducts in reactions involving a number of proteins, such as albumin (Donohue et al., 1983) and haemoglobin

Abbreviations: AA, acetaldehyde; HSA, human serum albumin; AA-HSA ab, AA-HSA antibodies.

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(Stevens et al., 1981), and also with DNA: the resulting DNA adduct in humans is a Schiff base N^2-ethylidene-2′-deoxyguanosine (N^2-ethylidene-dG) adduct (Vaca et al., 1995).

Concerning the development of alcoholic liver disease, multiple factors have been suggested. There has been increased interest in focusing on the possible role of immune mechanisms in the pathogenesis and perpetuation of this type of disease. In fact, many features of alcoholic liver disease suggest that immune mechanisms may contribute to liver tissue damage, as evidenced by the detection of circulating auto-antibodies and the presence of CD4+ and CD8+ lymphoid cells in the livers of diseased patients (Cook et al., 1996; Szabo, 1998). In particular, AA derived from the metabolism of ethanol has been shown to form stable adducts with plasma-circulating proteins, such as albumin, which are recognised by the immune system as foreign in both animals and in humans. Indeed, studies by Israel et al. have shown a type I hypersensitivity reaction yielding IgE antibodies against AA adducts when mice were immunised with plasma proteins binded with AA (Israel et al., 1992). Human studies showed an increase in anti-AA-albumin IgE antibodies among individuals who consume alcoholic beverages (Israel et al., 1992).

Humans naturally exhibit basal antibody titres against AA-protein adducts, which is likely due to variation in the amount of alcohol that is typically consumed throughout an individual's lifetime. Some alcoholics present significantly higher levels of anti-AA proteins and adduct antibodies (Hoerner et al., 1986; Niemela et al., 1987; Worrall et al., 1989), which have been proposed as factors that perpetuate alcohol-induced liver injury (Niemela et al., 1987). Other findings also show the existence in alcoholics of antibodies that specifically react with hepatocytes of animals pre-treated with alcohol (MacSween et al., 1981; Neuberger et al., 1984), thus highlighting a cytotoxic and auto-immune effect due to the existence of AA-protein adducts.

By chemical analogy, Thrasher et al. (Thrasher et al., 1987, 1988) demonstrated that formaldehyde binds human serum albumin (HSA) covalently, giving rise to a molecular adduct having formaldehyde as hapten. They found antibodies to the formaldehyde-HSA adduct in symptomatic subjects with long-term inhalation exposure to formaldehyde (Thrasher et al., 1990). Haemoglobin (Hb) and HSA are preferred for monitoring molecules because they are accessible in large amounts, are chemically stable and are not prone to repair mechanisms such as DNA-adducts. Because of the long life span of Hb (approximately 120 days) and the long half-life of HSA (approximately 19 days (Kratz, 2008)), these adducts accumulate in the human body, making their concentrations very sensitive parameters for human biomonitoring (Angerer et al., 2007). Similar results were found among smoker subjects who inhaled formaldehyde through smoke. In that analysis, the Displacement Assay was confirmed to be a useful method for evaluating formaldehyde exposure (Carraro et al., 1997).

There were two primary aims of this work. First, the correlation between AA-HSA antibodies (AA-HSA ab) and the amount of alcohol intake was investigated. Second, to investigate a possible role for the AA-HSA adduct as an early indicator of the biological effect of alcohol intake, it was necessary to quantify the immune activity of this adduct in typical alcohol consumers.

2. Materials and methods

2.1. *Epidemiological sampling and questionnaire*

The sample population was chosen among blood donors of A.V.I.S. Association (Associazione Volontari Italiani Sangue – Torino), without any *a priori* selection concerning drinking habits. Subjects were informed about the objective of the study, and after asking for their voluntary participation, a written consent form was signed and collected. Through this procedure, 76 healthy subjects overall were recruited during a sampling period of four months.

A questionnaire was administered to all 76 of the subjects, who were all healthy and regular alcohol consumers or teetotalers, without any subjects alcohol-addicted. Information about individual and clinical features, smoking habits, and profession were acquired through specific questions about type, quantity and frequency of alcoholic drinks were asked.

The quantity of ethanol assumed varies among different type of alcoholic beverages; specifically, ethanol intake was calculated taking into account its specific weight (=0.8 gr/cm^3), the percentage in volume (vol.%) of ethanol quantity of beverages, and the volume ingested. Thus, a number of grams of ethanol was attributed to each subject, taking into account the self-reported average daily alcohol consumption in the questionnaire and the values suggested by the literature reported in Table 1.

2.2. *Blood sampling and Displacement Assay*

Taking into account possible cumulative effects of the target molecule, subjects had not undergone to any restriction concerning the drinking habits before the biological sampling day.

After completing the questionnaire, a whole blood sample (10 mL) was collected from each subject to perform the IgG anti-AA-HSA analysis, with the Displacement Assay, considering the analogous reactivity among aldehydes. The Displacement Assay was the analytical method chosen for anti-AA-HSA ab quantification, as it assured high sensitivity for the seroprevalence screening of FA-HSA IgG in an healthy population study (Carraro et al., 1997, 1999); through this method an indirect measurement of AA-HSA adduct can be provided. This assay was applied as described by Gilli et al. (Gilli et al., 2008), with no other relevant modifications. This test is based on a competitive reaction of human IgG antibodies against AA-HSA with rabbit IgG anti-AA-HSA antibodies. Briefly, human serum (1:8 titre, 200 μL) (Thrasher et al., 1990) obtained from heparinized blood was incubated with the antigens (2 h at 37 °C), and then purified rabbit IgG (1:1000 titre, 200 μl) were added (2 h incubation at 37 °C). The reliability of the immunological response to AA-HSA was evaluated by comparison with the response against HSA. Positive responses in the human sera were revealed by a decrease of the optical density (OD) compared with the OD value obtained with rabbit IgG (positive control). The subject response was considered positive (presence of AA-HSA adduct antibodies) when the sample OD was lower than the positive control OD (rabbit serum). Negative responses (absence of antibodies to AA-HSA)