THE EFFECTS OF ACETALDEHYDE IN VITRO ON PROTEASOME ACTIVITIES AND ITS POTENTIAL INVOLVEMENT AFTER ALCOHOLIZATION OF RATS BY INHALATION OF ETHANOL VAPOURS

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Abstract — Background/Aims: Some models of chronic ethanol administration resulted in decreased proteasome activities. The mechanisms still remain speculative. In the present study, we tested another model of alcoholization with high blood alcohol levels (BALs) and high acetaldehyde fluxes as well as the in vitro effect of acetaldehyde on proteasome. Methods/Results: Ethanol vapour chronically inhaled by adult Wistar rats up to a specific protocol, can reach high BALs (200 mg/dl) with significant circulating acetaldehyde levels. After 4 weeks of ethanol intoxication, although cytochrome CYP2E1 was increased, liver lipid peroxidation remained unchanged when protein carbonyls augmented selectively for high molecular weight with a decrease of the proteasome activities in ethanol rats. Several aldehydes inhibit proteasome function; we specifically explored the effects of acetaldehyde, the first alcohol metabolite. Adduct of acetaldehyde in vitro to cytosolic proteins inhibits proteasome in a dose-dependent manner. Acetaldehyde adducted to purified proteasome also exhibits a decrease in its activities. Furthermore, an acetaldehyde-adducted protein, i.e. bovine serum albumin (BSA) is less degraded than a native BSA by purified proteasome. These findings suggest that acetaldehyde, if overproduced, can inhibit proteasome activities and reduce the proteolysis of acetaldehyde-adducted proteins. Conclusions: Our study, for the first time, provided the evidence that acetaldehyde by itself inhibits proteasome activities. As the chronic inhalation model used in this study is not associated with an overt lipid peroxidation, one can suggest that high BALs and their subsequent high acetaldehyde fluxes contribute to impairment of proteasome function and accumulation of carbonylated proteins. This early phenomenon may have relevance in experimental alcohol liver disease.

INTRODUCTION

It has been shown that chronic ethanol consumption impairs hepatic protein catabolism (Ayuso et al., 1986; Poso and Hirsimaki, 1991; Donohue et al., 1994) and may cause protein accumulation in liver cells (Baraona et al., 1977). Current evidence indicates that proteasome represents a major extralysosomal proteolytic system and therefore, plays an essential role in the intracellular protein turnover. There is also evidence that oxidized proteins and abnormal proteins are degraded through the proteasome pathway (Pacifici et al., 1989; Donohue et al., 1994; Grune et al., 1997). The 20S proteasome, which is ubiquitin and ATP independent, is involved in 70–80% of the selective recognition and degradation of the mildly oxidized proteins in the cytosol (Davies, 1993, 2001). Studies using chronic ethanol administration by intragastric infusion to rats (Donohue et al., 1998; Fataccioli et al., 1999; French et al., 2001) or mice (Bardag-Grade et al., 2000) display decreased proteasome activities. However, by using other models of ethanol administration as the Lieber-De Carli ethanol diet (Donohue et al., 1998) or ethanol at 10% as the sole drinking fluid (Fataccioli et al., 1999), the authors failed to find significant changes in proteasome activities in liver of ethanol-treated rats. Although the mechanisms behind these effects remain unclear, this discrepancy could be related to different models of rat alcoholization leading to various acetaldehyde levels and/or oxidative stress.

Acetaldehyde is the highly reactive oxidation product of ethanol generated through alcohol dehydrogenase and cytochrome P4502E1 (CYP2E1) pathways. Oxidative metabolism of ethanol by the inducible CYP2E1 has been considered as a source of reactive oxygen species (ROS) and related free radical processes. Furthermore, acetaldehyde is considered to be largely responsible for cytotoxic effects that occur in alcoholism. Acetaldehyde exhibits variable adverse effects, such as interference with certain enzyme activities and stimulation of extracellular matrix protein genes expression (Eriksson, 2001). Many of these effects have been attributed to the modification of cellular proteins by acetaldehyde. This compound preferentially and easily reacts with L-amino groups and ε-amino groups of lysine in protein molecules to generate stable adducts (Donohue et al., 1983). Moreover, acetaldehyde–protein adducts have been detected in the liver of ethanol-treated rats (Niemela et al., 1994; Nakamura et al., 2000) and in liver biopsies from alcoholic patients (Niemela et al., 1991; Niemela, 2001) indicating that acetaldehyde adducts are physiologically relevant.

Since reactive aldehydes, among them 4-hydroxy-2-nonenal (4-HNE) (Okada et al., 1999) and isoketals (Davies et al., 2002), alter the proteasome activity, the first formed reactive ethanol metabolite, acetaldehyde could also play a role in proteasome inhibition.

In an effort to elucidate the mechanisms underlying ethanol-decreased proteasome activities, the effects of another model of chronic ethanol administration especially by inhalation of ethanol vapour as well as the in vitro effects of acetaldehyde on proteasome activities were examined and analysed.

Although alcoholization by chronic ethanol vapour inhalation has been generally used for behaviour and addiction studies and to render animals dependent on alcohol (Le Bourhis, 1975; Aufrère et al., 1997), this paradigm has not been used often to investigate liver pathology. Nevertheless, it was not considered as clinically relevant when compared with other
models of chronic ethanol administration since it was not a common habit in humans to inhale alcohol. Recently, a tentative to promote alcohol without liquid (AWOL), which consists of inhaling vaporized alcoholic beverages, was made in Great Britain and USA. The selling company argued that this was a way to consume alcohol (www.awolusa.com). In fact, this way of consuming alcohol is quite deleterious for the brain (Di Luzio and Stege, 1979) and according to the exact paradigm also for other organs, such as the liver.

If the exact model used in this study with rats inhaling ethanol vapour for 4 weeks, seems to give a low hepatic dysfunction, it is probably owing to the standard diet concomitantly consumed and partly because this administration shunts the passage by the intestine and limits the endotoxemia that is now known to play a part in the alcoholic hepatic attack (Rao et al., 2004). However, manipulating the environment, the duration of the treatment (Rouach et al., 1983), the animal species (Goldin et al., 1995) and/or the lipid content of the diet permit to obtain clear pathological liver (data not shown).

Rikans and Gonzalez (1990) have reported that long-term intoxication by inhalation can give rise to high blood alcohol levels (BALs) without inducing overt lipid peroxidation process in the liver of animals fed a standard diet. We, therefore, explored the effects of such a specific model of alcoholization on rat liver proteasome in order to distinguish the effects of high BALs and its subsequent high acetaldehyde fluxes from those of lipid peroxidation by-products on proteasome activities.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA; fatty acid free) was obtained from Sigma, acetaldehyde from Merck, protein reagent from Bio-Rad, Oxy Blot™ from Oncor, ECL detection kit from Amersham and Bioxytech LPO-586™ kit from Oxis International Inc. Malondialdehyde (MDA) was prepared from tetramethoxypropane (Sigma) with H2SO4 (1%). All other chemicals were purchased from Merck or Sigma.

Animal experiments

Adult male rats were used (Wistar Ico: WI AF/Han, supplied by Ifa-Credo, France) for the studies. All animals received human care according to the guidelines from ‘Ministère de l’Agriculture, France’.

The animals (180–200 g) were housed individually at 22–24°C, 40–60% humidity and under 12 h light and 12 h dark cycle. They had free access to water and to a standard laboratory diet; proteins 27% calories, carbohydrates 61% and lipids 12% during the whole experiment. Animals were chronically intoxicated by inhalation of ethanol vapour, 24 h/day, for 4 weeks in well-ventilated chambers (Le Bourhis, 1975; Aufrère et al., 1997). The present protocol for ethanol intoxication has already been previously described in detail (Le Bourhis, 1975). Briefly, ethanol concentration was increased from 15 to 24 mg/l of air in successive steps of 1 mg/l every 2–4 days, so that the average BAL was continuously increasing. Animals remained in rather good healthy conditions with an increase in body weight. In these conditions, ethanol represents along the alcoholization ~25–30% of the total consumed calories (Aufrère and Le Bourhis, 1987). BALs were determined every 2–4 days with an ethanol dehydrogenase enzymatic kit (Behring) to permit the adjustment of ethanol vapour concentrations also determined on the air samples within individual chambers.

Control animals were kept in an alcohol-free chamber and submitted to the same procedures. On day 28 of the experiment, the rats were killed by decapitation and the livers immediately removed.

Determination of acetaldehyde levels

Acetaldehyde levels were determined several times during the inhalation period according to the sensitive high performance liquid chromatography (HPLC) method with dinitrophenylhydrazine reagent, developed by Lucas et al. (1986) which minimizes the non-enzymatic production of acetaldehyde from ethanol in denatured blood.

Homogenization and subcellular fractionation

Liver samples (5% w/v) were homogenized in Tris–HCl (50 mmol/l, pH 8.0) containing ethylenediaminetetraacetic acid (0.1 mmol/l) and 2-β-mercaptoethanol (1 mmol/l). The total liver homogenate was centrifuged at 10 000 g for 15 min then at 100 000 g for 1 h at 4°C and the supernatants (cytosolic fractions) were used for the determination of peptidase activities or preparation of adducted proteins. The 100 000 g pellets were used for the determination of CYP2E1 activity. The supernatants used for the determination of protein carbonyl content were recovered after centrifugation of liver homogenates at 27 000 g for 30 min at 4°C.

Adduction of proteins with aldehydes

Cytosolic proteins and proteasome adducts. Established methods were followed to prepare acetaldehyde condenses with proteins (Israel et al., 1986). Cytosolic proteins (0.5 mg/ml) were combined with 10–48 mM of acetaldehyde in 50 mM of Tris buffer, pH 7.4 for 15 min at 20°C, then 1 h at 37°C followed by a further 30 min incubation in the presence of sodium cyanoborohydride (10 mmol/l). Subsequently, the mixture was dialysed at 4°C for 24 h against buffer containing HEPES (50 mmol/l, pH 8.0), KCl (20 mmol/l), Mg acetate (1 mmol/l), DTT (0.5 mmol/l) and 10% glycerol. Similarly, proteasome adducts were prepared by exposing ~30 μg of purified proteasome in 50 mM Tris buffer pH 7.4 to acetaldehyde under reducing conditions. Unmodified proteins are submitted to the same experimental protocol in the absence of acetaldehyde. After addition, proteasome peptidases activities were determined.

BSA adducts. Aliquots of 4 mg BSA dissolved in 1 ml of PBS, pH 7.8 in the presence of 100 mmol/l of acetaldehyde were incubated at 37°C for 2 h and then 30 min in the presence of sodium cyanoborohydride (10 mmol/l). Unbound acetaldehyde and cyanoborohydride were removed by 24 h dialysis against HEPES (50 mmol/l, pH 8.0), KCl (100 mmol/l) and MgCl2 (1 mmol/l). By using the same experimental protocol malondialdehyde adducts (MA) were generated by the reaction of BSA with 40 mmol/l MDA and malondialdehyde–acetaldehyde adducts (MAA) by the reaction of BSA with 1 mmol/l acetaldehyde and 1 mmol/l MDA.

Proteasome peptidase activities

Chymotrypsine-like activity (ChT-L) and peptidylglutamyl-peptide hydrolase (PGPH) activities were determined with
fluorogenic synthetic peptides such as N-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (LLVY-AMC) and N-benzoylcarbonyl-Leu-Leu-Glu-2β-naphthamide (LLE-2NA), respectively as described previously (Fataccioli et al., 1999).

Typical assays (final volume: 200 μl) contained cytosolic fractions (75 μg of protein), fluorogenic substrates (80 μmol/l) and 0.06% SDS in Tris–HCl (150 mmol/l; pH 8.0). Fluorogenic substrates were dissolved in dimethyl sulfoxide (<4%). The mixture was incubated for 30 min at 37°C. The reaction was stopped by adding 1 ml of 1% SDS and 2 ml of sodium borate (0.1 mol/l, pH 9.1).

Additional measurements were carried out including a powerful proteasome inhibitor (lactacystin) to ensure that the activity observed was actually because of the proteasome activity. The peptidase activity was determined fluorometrically, the excitation and emission wavelengths were 355/455 nm in the case of amino-4-methylcoumarin and 336/424 nm in that of β-naphthylamine. A standard curve of fluorescence for amino-4-methylcoumarin and β-naphthylamine was used to calculate the concentration of liberated products in the assays.

Measurement of protein degradation

Purification of 20S proteasome from rat liver was done according to the procedure described (Conconi et al., 1996) and provided by Professor B. Friguet. Proteolysis of various forms of BSA was achieved in the presence of 20S-purified proteasome and determined by a gel-electrophoretic method or by fluorescamine assay. Proteolysis of unmodified BSA and adducted BSA were performed by incubation of 60 μg protein substrate with 2 μg of purified proteasome at 37°C for 2 h in 200 μl total volume of HEPES buffer (50 mmol/l, pH 8.0) containing KCl (100 mmol/l), MgCl₂ (1 mmol/l) and DL-dithiotreitol (5 mmol/l). Aliquots of the reaction mixture were subjected to 12% SDS–PAGE and the gels were stained with silver. As an alternative, the fluorescamine assay was performed. The reaction was stopped by adding TCA (16%), with silver. As an alternative, the fluorescamine assay was performed by incubation of 60 μg protein substrate with 2 μg of purified proteasome at 37°C for 2 h in 200 μl total volume of HEPES buffer (50 mmol/l, pH 8.0) containing KCl (100 mmol/l), MgCl₂ (1 mmol/l) and DL-dithiotreitol (5 mmol/l). Aliquots of the reaction mixture were subjected to 12% SDS–PAGE and the gels were stained with silver. As an alternative, the fluorescamine assay was performed. The reaction was stopped by adding TCA (16%), with silver. As an alternative, the fluorescamine assay was performed by incubation of 60 μg protein substrate with 2 μg of purified proteasome at 37°C for 2 h in 200 μl total volume of HEPES buffer (50 mmol/l, pH 8.0) containing KCl (100 mmol/l), MgCl₂ (1 mmol/l) and DL-dithiotreitol (5 mmol/l). Aliquots of the reaction mixture were subjected to 12% SDS–PAGE and the gels were stained with silver. As an alternative, the fluorescamine assay was performed.

CYP2E1 activity and lipid peroxide assay

CYP2E1 activity was assessed using chlorozoxazine metabolism in liver microsomes (Lucas et al., 1996). The assay for measuring lipid peroxide levels was performed according to Mihara (Mihara and Uchiyama, 1978) and with the LPO-586™ kit (Oxis International Inc.) which monitors more specifically both MDA and 4HNE.

Protein carbonyl assay

The protein carbonyl content was either measured according to the 2,4-dinitrophenyhydrazone (DNPH) derivatization method described by Levine et al. (1990) or evaluated by immunoblot analysis. An Oxyblot kit was used to detect derivative carbonyl groups with DNPH prior to gel electrophoresis. Proteins (15 μg) for detection of DNPH derivatized carbonyls were separated by SDS gel electrophoresis (7.5% polyacrylamide) and transferred to polyvinylidene difluoride membranes. After a brief incubation with 1% BSA, the membranes were immunolabelled using rabbit anti-DNP antibody at a dilution of 1:150. The membranes were then exposed to a goat anti-rabbit horseradish peroxidase-coupled secondary antibody (1:300 dilution) and antigens were visualized by enhanced chemiluminescence.

Protein assay

Protein concentrations were determined by the Bradford method (Bradford, 1976) with BSA as the standard.

Data analysis

Data are expressed as mean ± SEM, with the number of experiments indicated in the figure legends. Statistical evaluation was performed using Student’s t-test.

RESULTS

Characteristics of ethanol inhaled-rats

After 1 month of ethanol inhalation, the alcohol-treated rats presented a slower and smaller progressive increase in their body weight than the control rats (Fig. 1A). BALs of rats are shown in Fig. 1B. BALs increased gradually and reached values of ~43 mM (200 mg/dl) on day 25 of the inhalation period. Acetaldehyde levels also augmented along the alcoholization and even reached the value of ~0.1 mM (98 μM) at the time of killing.

Ethanol inhalation and cellular free radicals targets and proteasomal activities

Chronic ethanol inhalation (Table 1) was associated with a 3.3-fold increase in CYP2E1 activity (P ≤ 0.001). In spite of the increase of CYP2E1, the hepatic lipid peroxidation was not modified following this chronic ethanol paradigm irrespective of the technique of determination used. MDA levels determined according to Mihara were 39.32 ± 2.89 and 42.03 ± 0.70 nmol MDA/g of liver for five inhaled and five control animals, respectively. The results obtained with the Oxis kit are presented in Table 1.

However, cytosolic proteins were oxidatively modified, as evidenced by the increased carbonyl content in the ethanol-treated animals (+25%). To identify specific proteins exhibiting carbonyl modifications, anti-DNPH immunoblotting was performed. The western blots (Fig. 2) showed increased carbonyl content of higher molecular weight proteins (~150 kDa, +107%, P ≤ 0.05) derived from hepatic cytosol of ethanol-treated rats compared with the controls.

Our data (Table 1) show that both ChT-L and PGPH activities of the proteasome were significantly decreased (~21%) in the liver of ethanol-inhaled rats. Such a decrease in ChT-L was still apparent 24 h after ethanol withdrawal (data not shown).

Acetaldehyde in vitro and proteasome activities

To test whether acetaldehyde may be implicated in the decline of proteasome activities, the effect of this compound on the proteasome was investigated in vitro.

We first designed an experiment to test the proteolytic capacity of acetaldehyde-adducted cytosolic proteins.
Cytosolic proteins were exposed to various efficient concentrations of acetaldehyde under reducing conditions and after dialysis we measured the peptidase activities with the fluorogenic peptides. A dose-dependent inhibition of the two activities of proteasome occurs on exposure to acetaldehyde (Fig. 3). Such an inhibition is more pronounced for ChT-L than for PGPH activity. To obtain a loss of ~50% of their initial activities in the exposed cytosolic proteins, 20 mM of acetaldehyde are required for ChT-L and 48 mM for PGPH, respectively. However, since several kinds of adducted proteins could be generated, it was necessary to determine whether the decrease in the proteolytic activities of adducted cytosolic proteins was because of direct inactivation of proteasome by acetaldehyde, alterations of cytosolic proteins or both.

To test the direct effect of the acetaldehyde adduction on the activities of the proteasome, acetaldehyde was added to the purified proteasome. The ChT-L and PGPH activities declined after exposure of the proteasome to acetaldehyde under reducing conditions (Fig. 4). Here again the inactivation of ChT-L is higher than that of PGPH.

Proteasome and aldehyde-modified protein degradation

We then determined the effect of acetaldehyde adduction on degradation of a model protein, BSA. As shown in Fig. 5, degradation of acetaldehyde-adducted BSA was reduced significantly compared with native BSA. In fact, BSA was degraded 3.6-fold more than acetaldehyde-adducted BSA by the purified proteasome.

The degradation of unmodified and aldehyde-adducted BSA was measured by monitoring the disappearance of those proteins after SDS–PAGE of the incubation mixture. The results of a representative experiment are shown in Fig. 6. Aldehyde-adducted BSA, such as those formed on reaction of BSA with acetaldehyde (AA), malondialdehyde (MA) or with acetaldehyde and malondialdehyde (MAA) were degraded to a lesser extent than native BSA. Aldehyde-adducted proteins did not appear to be good proteolytic substrates for proteasome (Fig. 6) as compared with unmodified BSA.

Table 1. Effects of ethanol inhalation on cellular free radical targets and proteasome peptidase activities in rat liver

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<thead>
<tr>
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<th>Control rats</th>
<th>Ethanol rats</th>
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<tr>
<td>Lipid peroxidation</td>
<td>36.21 ± 2.40 (6)</td>
<td>35.42 ± 1.92 (6) NS</td>
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<tr>
<td>(nmol MDA + HNE/g liver)</td>
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<tr>
<td>Protein carbonyls</td>
<td>1.01 ± 0.04 (5)</td>
<td>1.27 ± 0.06 (5)**</td>
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<tr>
<td>(nmol/mg protein)</td>
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<tr>
<td>ChT-L activity</td>
<td>29.13 ± 1.71 (5)</td>
<td>22.85 ± 0.92 (5)**</td>
</tr>
<tr>
<td>(nmol/30 min/mg protein)</td>
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<tr>
<td>PGPH activity</td>
<td>12.18 ± 0.50 (5)</td>
<td>9.58 ± 0.21 (5)**</td>
</tr>
<tr>
<td>(nmol/30 min/mg protein)</td>
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<tr>
<td>CYP2E1</td>
<td>4.17 ± 0.52 (6)</td>
<td>13.70 ± 1.30 (6)***</td>
</tr>
<tr>
<td>(nmol/min/mg protein)</td>
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Values are mean ± SEM. Numbers of animals are stated within the parenthesis. Statistically significant differences between groups were determined by Student’s t-test. **P ≤ 0.02; ***P ≤ 0.001; NS: not significant.
DISCUSSION

The specific way of chronic 4 week progressive ethanol inhalation used in this study, produces high BALs and results in an enhanced CYP2E1 activity in the liver and an increase in protein carbonyl content, although without significant change in lipid peroxidation. One explanation for this lack of effect could be that the usual recommended rat diet contained low levels of lipids limiting the extent of lipid peroxidation. It has been emphasized that dietary fat, mainly polyunsaturated fatty acid (PUFA) content strongly affects the increased radical formation (Reinke et al., 2000). It has been often claimed and supported by numerous studies that PUFA levels of the diet play an important role in alcoholic liver disease (Nanji, 2004). Indeed, in preliminary results with ethanol inhaled rats at the same intensity but receiving concurrently diets rich in PUFAs, we obtained a clear-cut increase in lipid peroxidation.

What is relevant in this study is that even under the conditions of no overt lipid peroxidation, the activities of the two specific liver proteasome peptidases are clearly decreased. This decline in proteasome activities may constitute a contributing mechanism of accumulation of the carbonylated proteins.
proteins following chronic alcoholizations (Rouach et al., 1997; Colantoni et al., 2000). Furthermore, anti-DNP immunoassays have indicated a selective nature of the carbonylated proteins. Only high molecular weight proteins exhibited an increase in carbonylation, which might be the effect of an increased oxidation of specific targets and/or of a decreased turnover.

Previous studies have suggested that CYP2E1 could be responsible for the increase in oxidized proteins and the decline in proteasome activities. Indeed, it was shown (Bardag-Gorce et al., 2000) that a decrease in proteasome activity associated with an increase in carbonyl protein content was observed in wild-type but not in CYP2E1 knockout mice receiving ethanol chronically. Our studies (Fataccioli et al., 1999) with intragastrically ethanol-fed rats that were simultaneously given phenylisothiocyanate, an inhibitor of CYP2E1, show a reduced lipid peroxidation in these animals and an attenuated decrease of proteasome activity, suggesting that ROS related processes keep contributing events resulting in the decrease in proteasome activities.

In the present study, the absence of an overt lipid peroxidation actually allows us to rule out the predominant role of lipid peroxidation derivatives (among them, 4-HNE) in the inhibition of proteasome activities. However, such a chronic ethanol model is associated with high BALs and consequently with large acetaldehyde fluxes when CYP2E1 also contributes to ethanol metabolism. It can then be suggested that, in this situation, acetaldehyde could play a role in inhibition of proteasome activities.

The in vitro part of this study supported this hypothesis and provided evidence that the proteasomal system is impaired in acetaldehyde-treated cytosol. The loss of both ChT-L and PGPH activities could be explained, at least in part, by direct attachment of acetaldehyde to the proteasome. Alternatively, the inhibition of proteasome by acetaldehyde-adducted proteins may be also possible. The observation that cleavage of LLVY-AMC and LLE-2NA were significantly decreased in purified 20S proteasome treated with acetaldehyde suggests that acetaldehyde directly interferes with the proteolytic activity of 20S proteasome. For several enzymes, it has been established that, in the response to acetaldehyde, their activity is inhibited by covalent modification of amino groups. This appears to be a likely mechanism for acetaldehyde-induced proteasome inhibition since modification of amino or thiol groups of key aminoacids is considered to inhibit proteasome activity (Kisselev et al., 2000; Ferrington and Kapphahn,
proteins and the resulting adducts generated may interfere with the proteolytic process. The results showed that acetaldehyde–BSA adducts (AA) and adducts generated from action of MDA and acetaldehyde, separately or together (MA or MAA), are poor substrates of the proteasome. However, such adducts may be possible inhibitors of the proteasome acting as HNE (Friguet and Szweda, 1997) and isoketal-adducted proteins (Davies et al., 2002). Nevertheless, the finding that protein adducts degrade more slowly than the native protein suggests a potential mechanism whereby the modified proteins accumulate in the cells, increasing the opportunity for oxidation and aggregation.

The concentrations of acetaldehyde, which resulted in an inhibition of proteasome activities, ranged from 10 to 48 mM. These concentrations are higher than those reported at the tissue level (# 0.2 mM) (Erikkson, 1980) in chronic abusers. However, because acetaldehyde is highly volatile, the actual concentrations in the in vitro system are lower and probably closer to those observed in vivo. Moreover, it is not uncommon to use such concentrations in the in vitro assays (Blasiak et al., 2000). Furthermore, it is reasonable to assume that this kind of in vitro assay could represent an isolated acute treatment for a short time (Hard et al., 2001) as compared with the long-term inhalation in vivo giving raise to constant exposure to acetaldehyde. Although the in vitro data cannot be directly extrapolated to the in vivo situation, alterations of various functions by acetaldehyde adducts formation may be physiologically relevant.

Our chronic ethanol inhalation model that is characterized by high BALs and subsequent elevated acetaldehyde concentrations all along the alcoholization period could create conditions which favour acetaldehyde adducts and lead to a relative development of dependence. In this respect, in the studies of Niemela group (Niemela, 1999; Niemela et al., 1994) acetaldehyde adducts were found to be most abundant in those animals, which showed withdrawal symptoms during the course of the experiment indicating that individual BALs may be associated with increased amounts of acetaldehyde adducts in tissues. Acetaldehyde adducts are also found in human alcoholic liver (Niemela et al., 2001) and it is obvious that humans are relatively more sensitive to ethanol toxicity compared with rodents (Nakajima et al., 2004).

Our study provided evidence, at least in vitro, that acetaldehyde significantly inhibits proteasome activities in a manner quite similar to aldehyde by-products of lipid peroxidation. Such acetaldehyde effects may be relevant following the chronic alcoholizations that are associated with high BALs. In the intragastroellular model, the 40% inhibition in the catalytic activities of the proteasome (Rouach et al., 1997) may be linked in part to the lipid peroxidation and in part to acetaldehyde, whereas in the present inhalation model, the 20% inhibition of the proteasome activities is probably linked to acetaldehyde.

Since proteasome inhibition results in various altered processes (Jokelainen et al., 1998; Kawazoe et al., 1998), our data suggest that aldehyde-related inhibition of the proteasome could account for many of the effects of alcohol on liver and may be a contributing factor to the pathology of conformational disorders (Lindsten and Dantuma, 2003; Bardag-Gorce et al., 2004).

In summary, if the present study did not exclude a role for lipid peroxidation in the proteasome inhibition following alcoholization, it has permitted to reveal, for the first time, that acetaldehyde may act also as the other aldehyde by-products of peroxidation on proteasome functions. The increased oxidation of proteins and/or inadequate detoxification of altered proteins could be a prime event for alterations of different dysfunctioning of organs.

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REFERENCES


