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Authors

Bondy, SC
Lam, HR
Østergaard, G
[et al.](#)

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ORIGINAL INVESTIGATION

S.C. Bondy · H.R. Lam · G. Østergaard · S.X. Guo
Ø. Ladefoged

Changes in markers of oxidative status in brain, liver and kidney of young and aged rats following exposure to aromatic white spirit

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Abstract Levels of glutathione and activity of glutamine synthetase were assayed in organs of rats following inhalation of a heterogeneous solvent mixture containing both aliphatic and aromatic hydrocarbons. This mixture was administered for 3 weeks (6 h daily) at two levels in the inhaled air (400 and 800 ppm) to young adult (5-month-old) and aged (14-month-old) rats. Depression of levels of glutamine synthetase in the P2 fraction of kidney was observed, which was more severe in aged than young adult rats. Glutamine synthetase is a cytosolic enzyme especially susceptible to oxidative damage. A parallel depression of this enzyme was also seen in the corresponding hepatic fractions. However, levels of glutamine synthetase in the hippocampus were elevated by this exposure. Glutathione levels were depressed in P2 fractions of livers of exposed rats, and also in the corresponding renal fraction. Glutathione concentration was unchanged in cerebral fractions. Overall results were interpreted to imply that pro-oxidant events were elevated in kidney and liver following prolonged inhalation of the solvent mixture. The changes found in brain tissue did not reveal evidence of oxidative stress but, however, suggested that glial activation was taking place.

Key words Solvents · Aromatics · Free radicals · Reactive oxygen · Oxidative stress · Synaptosomes

S.C. Bondy (✉) · S.X. Guo
Center for Occupational and Environmental Health,
Department of Community and Environmental Medicine, University of California,
Irvine, CA 92717-1825, USA

H.R. Lam · G. Østergaard · Ø. Ladefoged
Institute of Toxicology, National Food Agency of Denmark,
DK-2860 Søborg, Denmark

Introduction

White spirits are widely used solvents, comprising different complex mixtures of both aliphatic (straight or branched chain) and aromatic hydrocarbons with a boiling point range of 150–220°C. The effect of prolonged, low level exposures to these solvents is unresolved, but there is increasing evidence that such exposure can be deleterious to health. More specifically, there is evidence that chronic exposures incurred in certain occupations can be neurotoxic. Such studies have reported both behavioral and physiological changes in specific worker populations (Mikkelsen et al. 1988; Arlien-Søborg et al. 1992; Trieberg et al. 1992).

The focus of the current study was upon a white spirit blend that contained a range of aromatic compounds. These have been suspected to be especially toxic, and therefore dearomatization is widely used, despite the fact that no firm evidence of a distinctive aromatic solvent-related neurotoxicity has emerged from human studies. However, using animal models, both pure aromatics such as xylene and toluene and aromatic mixtures have been implicated as distinctly neurotoxic (LeBel and Schatz 1988; Ameno et al. 1992; Mattia et al. 1993a,b). These studies have involved relatively high level dosing for rather short times. The current investigation was intended to study effects of a complex solvent mixture under conditions more closely resembling the exposures likely to be encountered in occupations where such exposures are possible. Under these conditions, solvents have also been found to modulate biochemical and behavioral indices (Slomianka et al. 1990; Ladefoged et al. 1991; Pryor 1991; Lam et al. 1992; Østergaard et al. 1993; Rasmussen and Jeppesen 1993). The present maximally allowed industrial exposure level for this kind of solvent is presently 100 ppm (OSHA). The dose levels used in this study (0, 400 and 800 ppm) were the same as those used in our previous study on dearomatized white spirit (Lam et al. 1994).

The parameters that were assayed in the current study are both indices of oxidative stress. Glutathione is the major water soluble cytosolic antioxidant, and its intracellular concentration has often been found responsive to changes in pro-oxidant status (Mattia et al. 1992b; Shivakumar and Ravindranath 1992; Adams et al. 1993). Glutamine synthetase is an enzyme that is especially susceptible to site specific oxidative inactivation (Stadtman 1990; Carney et al. 1991). Its activity can be used as an index of rates of generation of reactive oxygen species (ROS), summated over a prolonged period. Previous work on aromatic solvents including toluene, benzene, xylenes, and styrene suggest that enhancement of rates of free radical generation may underlie some of the neurotoxicity of these compounds (Khan et al. 1990; Trenga et al. 1991; Mattia et al. 1993a,b). Furthermore, we have found evidence of excess pro-oxidant activity in an earlier study involving extended inhalation of aliphatic white spirit (Lam et al. 1994).

Materials and methods

Materials

Aromatic white spirit (Mineralsk Terpentin K-30) was purchased from Shell Chemical, Denmark. This contained 14–21% aromatic hydrocarbons (by volume) which included cumene, ethyl benzene and ethyl toluene. Nonane, decane and undecane were the most prevalent aliphatics. The boiling range of this mixture was 150–220°C and its density was 0.78. Monochlorobimane was purchased from Molecular Probes (Eugene, Ore.) All other chemicals were obtained from Sigma (St Louis, Mo.).

Animals and exposure procedure

Male Wistar rats aged either 5 months (young) or 14 months (aged) (Møllegaard Breeding Center, LeSkensved, Denmark), were housed two animals/cage at $22 \pm 1^\circ$, $55 \pm 5\%$ humidity on a 12-h light/dark cycle, and were allowed free access to rat diet and tap water. Food was removed during the inhalation exposure period.

Three exposure levels were used, sham exposure, 400 ppm (2290 mg/m^3) and 800 ppm (4580 mg/m^3) of aromatic white spirit in inhaled air for 6 h/day, 7 days/week for 3 weeks. The exposure conditions and equipment used have been previously described (Ladefoged et al. 1990).

One day after the last exposure, animals were killed by decapitation in carbon dioxide anesthesia. Part of the liver and the whole left kidney were excised. Brains were dissected into frontal cerebral cortex and hippocampus (Glowinski and Iversen 1966). Tissues were transferred to ice-cold 0.32 M sucrose and a 10% (w/v) homogenate was made (Lam et al. 1994).

Glutamine synthetase

This enzyme was assayed as γ -glutamyl transferase activity by incubation (30 min, 37°) of 0.1 ml P2 preparation together with (mM) L-glutamine (50) (Rowe et al. 1970), hydroxylamine (75), NaADP (0.5), MnCl_2 (0.2), imidazole-HCl, (50), sodium arsenate (25), in a final volume of 1 ml. γ -Glutamyl hydroxylamate formed could

then be quantitated after centrifugation (5000 g, 5 min.), by spectrophotometric assay of the colored product formed with acidified FeCl_3 (Rowe et al. 1970). A standard curve was concurrently generated, with γ -glutamyl hydroxylamate. The iron complex of 1 mmol/ml of this compound gave an absorbance of 0.340 at 535 nm.

Determination of reduced glutathione

Glutathione (GSH) levels were determined using a modification of the method of Shrieve et al. (1988). The principle behind the assay is that monochlorobimane (mBCl), a nonfluorescent compound, reacts with glutathione to form a fluorescent adduct. It has been shown that there is very little reaction between mBCl and protein sulfhydryl groups (Rice et al. 1986). mBCl was dissolved in ethanol to a concentration of 5 mM and stored at -10°C in the dark. mBCl was added to 2 ml of the P2 resuspension to a final concentration of 20 μM , after which the suspension was incubated for 15 min at 37° in 1% (w/v) Triton and then centrifuged for 10 min at 31 500 g. The fluorescence of the supernatant was read on a Perkin-Elmer Spectrofluorometer at excitation wavelength 395 nm and emission wavelength 470 nm. The tissue GSH concentration was determined using a GSH standard curve. This determination was performed in the presence of 0.1 unit/ml of liver glutathione transferase, in order to accelerate the formation of the fluorescent adduct derivative.

Protein determination

Protein concentration was assayed using the method of Bradford (1976).

Statistical analyses

Differences between groups were assessed by one-way Analysis of Variance followed by Fisher's Least Significant Difference Test. The acceptance level of significance was $p < 0.05$ using a two-tailed distribution. Each value presented was obtained from six to eight individual rats.

Results

During the 1st week of exposure, the white spirit-exposed groups of rats showed signs of mucous membrane irritation and were somewhat sedated. These effects gradually diminished during the 2nd week. The body weights of animals did not differ between groups after treatment. Brain, liver and kidney weights were also unaffected by solvent exposure.

Glutamine synthetase activity

Levels of glutamine synthetase were significantly depressed in the hepatic P2 fractions from both young and aged rats, and this effect was significant at both solvent levels used. In young rats, a clear dose-response relation was apparent (Fig. 1). Glutamine synthetase activity was depressed in the kidney of aged but not young rats by treatment with both the high and low

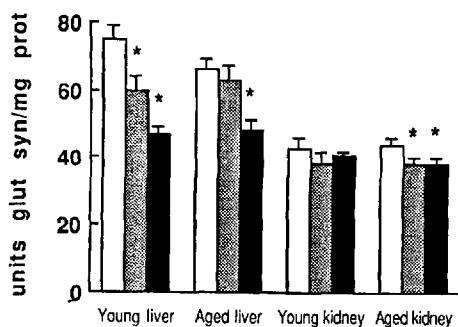


Fig. 1 Activity of glutamine synthetase in P2 fractions of liver and kidney of rats following 3 weeks of aromatized solvent inhalation. Each data point represents the mean of values derived from 6–8 individual animals \pm SEM. *Level differs significantly from the corresponding control ($p < 0.05$, Students *t*-test after analysis of variance and Fisher's Least Significant Difference). \square 0, \blacksquare 400, \blacksquare 800 ppm

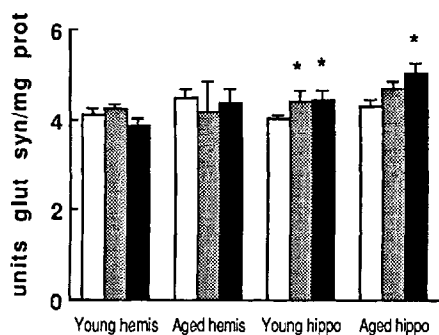


Fig. 2 Levels of glutamine synthetase in P2 fractions of frontal cortex and hippocampus of rats following 3 weeks of aromatized solvent inhalation. Each data point represents the mean of values derived from 6–8 individual animals \pm SEM. * Level differs significantly from the corresponding control ($p < 0.05$, Students *t*-test after analysis of variance and Fisher's Least Significant Difference). \square 0, \blacksquare 400, \blacksquare 800 ppm

levels of solvent (Fig. 1). Solvent inhalation significantly increased the level of glutamine synthetase within the P2 fraction of hippocampus from both young and aged rats, but cortical levels of glutamine synthetase were unaffected by treatment (Fig. 2).

Glutathione content

Hepatic glutathione content of P2 fractions was depressed by treatment in both young and aged animals. Levels of GSH were also depressed in kidney P2 fractions of aged rats but not young adults, following inhalation of the solvent mixture (Fig. 3). Hepatic effects appeared to be dose-related but in kidney, no significant differences were noted between the two concentrations of solvent used. GSH levels in the P2 fractions of cerebral cortex or hippocampus were not significantly altered (data not shown).

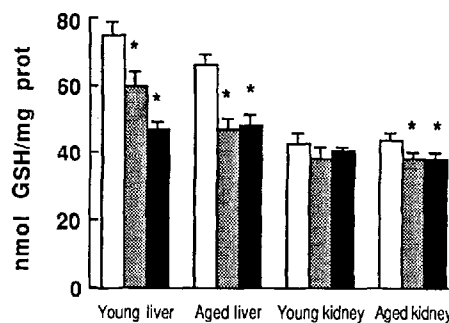


Fig. 3 Glutathione content in P2 fractions from liver and kidney of rats following 3 weeks of aromatized solvent inhalation. Each data point represents the mean of values derived from 6–8 individual animals \pm SEM. * Level differs significantly from the corresponding control ($p < 0.05$, Students *t*-test after analysis of variance and Fisher's Least Significant Difference). \square 0, \blacksquare 400, \blacksquare 800 ppm

Discussion

Aromatic white spirit is a very complex mixture composed of numerous different aliphatic and aromatic hydrocarbon components. The content of aromatics in the batch employed was 14–21% by volume. The use of such a heterogeneous, occupationally relevant mixture of solvents necessarily complicates mechanistic interpretation and understanding of results obtained. However, catabolism of hydrocarbons is generally by way of mixed function oxidase systems, and involves oxidation to alcohols, aldehydes and ultimately organic acids which can undergo phase II conversion to excretable glucuronide or sulfatide conjugates. These oxidative metabolic pathways involve several possibilities for the transient appearance of reactive oxidative species. Both oxidases which are responsible for the direct scission of molecular oxygen, and to a lesser degree, dehydrogenases acting upon solvents such as ethanol have the ability to promote ROS formation (Dicker and Cederbaum 1990; McCay et al. 1992).

The data obtained highlight the potential susceptibility of the liver and kidney to experience elevated ROS production in the course of catabolism and excretion of solvents. Results also raise the possibility that the kidney of aged animals may have greater vulnerability than that of young adults. This greater sensitivity of the aged kidney to solvent-induced changes is illustrated by the magnitude of the responses to solvent inhalation of both levels of glutathione and glutamine synthetase. This may relate to reports suggesting that normal cellular antioxidant defenses are compromised during aging and thus the aged animal is more susceptible to harmful ROS-inducing metabolic changes (Ku et al. 1993).

Depression of hepatic glutamine synthetase activity revealed that the liver is also a potentially vulnerable target organ following extended inhalation of solvents, and parallel changes were apparent in the hepatic

glutathione content of treated rats. Since the course of GSH responses can be bi-directional in response to pro-oxidant conditions (Adams et al. 1993), the potential exists for a zero-effect of an oxidative stressor upon GSH concentration at a critical time point. This can complicate dose-response studies.

The elevation of glutamine synthetase in both young and aged hippocampi after inhalation of the 800 ppm concentration of solvent mixture was unexpected. This enzyme is predominantly glial and this increase may reflect glial activation within the hippocampus. Such changes may precede more frank morphological evidence of gliosis. The hippocampus is more vulnerable than most brain regions to many neurotoxic agents, including trimethyltin, where gliosis is involved (Brown et al. 1979). Furthermore, developmental abnormalities within the hippocampus have been described following toluene exposure (Slomianka et al. 1990).

When the results from the current study are compared with our previous experiments using dearomatized white spirit, the data obtained from liver are more pronounced with the aromatized solvent used in the current study. The kidney also appears to respond more dramatically to the aromatized solvent mixture, perhaps due the concentration of aromatic metabolic intermediates in the renal tubules prior to excretion.

The response of cerebral tissues to the solvent used did not give clear evidence of pro-oxidant events. This is in contrast to our previous study of dearomatized white spirit (Lam et al. 1994). It is also in contrast to our earlier findings of the induction of cerebral pro-oxidant events by acute intraperitoneal administration of a single aromatic solvent, toluene (Mattia et al. 1993b). However such high-dose, short-term studies are very different from the prolonged low level exposure used here. The expression of neurotoxicity by solvent mixtures may then relate critically to their precise composition. A disadvantage of the white spirit used is its extreme heterogeneity and indeterminate composition. This obviously complicates mechanistic interpretation of our findings. However, the widespread utilization of this solvent, and the prolonged low level exposures used in the current study, make the results obtained most relevant to the potential hazards for humans.

The use of two groups of animals of differing ages is obviously of utility in relating effects detected to normal aging processes. In addition, the close parallels found with two separate groups of animals also help to strengthen the significance of all the changes found. The dose-response trends detected in this study further validate the meaningfulness of the findings.

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