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METHYL ALCOHOL INGESTION AS A MODEL ETIOLGIC AGENT IN MULTIPLE SCLEROSIS. W. Monte, D. Glanzman, and C. Johnston (SPON: S. Hoffman). Arizona State University, Tempe, AZ 85287

Human catalase, unlike that of all other species, does not metabolize methyl alcohol (methanol). This unfortunate evolutionary deficiency makes methanol a poison only to humans. Methanol is known to be a demyelinating toxin in humans, producing symptoms markedly similar to those in multiple sclerosis, including bizarre and inconsistent visual field disruptions. Human alcohol dehydrogenase metabolizes methanol directly to formaldehyde, which actively cross-links native proteins in-situ. Such formaldehyde-modified proteins have been shown to induce macrophage scaveging at a rate 100 times that of unmodified protein. What better method to elicit an autoimmune response than to react endogenous proteins with formaldehyde consistently and intermittently over a long period of time?

In our model, the neurotoxic effect of orally-administered methanol was visualized in the rat central nervous system using reduced silver degeneration staining techniques. Following chronic administration for 18-33 days, all experimental animals demonstrated massive cellular, axonal and terminal degeneration in numerous regions of brain, including cerebellum, hippocampus, brainstem nuclei, internal capsule and optic chiasm. These results show for the first time that by using sufficiently sensitive histological techniques, the neurotoxicity of methanol is revealed in the mammalian central nervous system.

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Dietary Methanol as a Cause of Multiple Sclerosis

Human Catalase, unlike that of <u>all</u> other species, cannot detoxify methanol. This unfortunate evolutionary deficiency makes methanol a "poison" only to humans, contradicting Richardson's Rule which successfully predicts ethanol as consistently more toxic than Methanol in all other species.

Methanol is known to be a demyelinating toxin in humans. The symptoms of chronic methanol poisoning in humans are identical to the symptoms of Multiple Sclerosis. Even to the bazaar nature and inconsistency of the visual field disruptions, thought to be the toxicological marker that sets methanol poisoning apart from all other intoxications.

Human alcohol dehydrogenase metabolizes methanol directly to Formaldehyde. Location of Alcohol dehydrogenase activity in the human brain, though individually variable, is generally consistent with MS Plaque distribution. The Liver also has ADH activity with concomitant high aldehyde dehydrogenase activity. Aldehyde dehydrogenase facilitates detoxification of Formaldehyde via 1-carbon metabolism to CO_2. Without ready availability of Aldehyde Dehydrogenase, Formaldehyde will "immediately" complex with any available protein.

Formaldehyde treatment of antigens is known to stimulate the immune response and is, in fact, the requisite proprietary mechanism normally utilized by pharmaceutical companies in the preparation of virus proteins for vaccine production.

Recently sites on macrophages specific to "Formaldehyde Modified Protein" have been elucidated. Protein modified by formaldehyde are scavenged by macrophages at a rate 100 times that of unmodified protein. What better method to elicit an auto-immune response than to react endogenous proteins with Formaldehyde consistently and intermittently over a long period of time.

Although differences in distribution and density of alcohol dehydrogenase sites in the brain may account for the great individual variability in symptoms and severity of MS and methanol poisoning, it is more likely that variability of ethanol levels in the blood may be an even more important factor. Alcohol Dehydrogenase(ADH) metabolizes ethanol preferentially to Methanol by a ratio greater than 9. For this reason ethanol is the only known antidote to methanol poisoning, its ingestion prevents the conversion to formaldehyde and allows methanol to be removed by the kidneys and the lungs.

There is some indication that endogenous ethanol produced by gut fermentation, can be found in the human bloodstream. Sobriety testing indicates that there is great variability in these residual levels of ethanol, perhaps due to the variation of the population of gut flora.

1

Small amounts of methanol are produced as a result of gut fermentation. There are sources of dietary methanol that are substantial enough to cause concern. Canned fruits and vegetables have been exposed to enough heat to liberate methanol from the pectin in the plant cell walls. This methanol would normally not be available to the digestive process of humans. Certain alcoholic beverages are so high in methanol as to not be exportable to the United States. It is worth noting that countries in which they are produced have the highest, per capita incidence of MS.

Although MS occurrence in populations varies with geographical and climatological consistency, a very believable case can be made for direct correlation to preformed dietary methanol.

METHANOL INDUCED NEUROPATHOLOGY IN

THE MAMMALIAN CENTRAL NERVOUS SYSTEM

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SUMMARY

The neurotoxic effect of methyl alcohol (methanol) was visualized in the rat central nervous system using reduced silver staining techniques. Following chronic administration of methanol (intubation with 0.95gm/kg for 18,25 or 33 days) all experimental animals showed massive axonal degeneration in multiple regions of brain, regardless of the duration of exposure. Histological processing yielded degeneration by-products of fibers with cells of origin lying in cerebellar cortex, deep cerebellar nuclei, cranial nerve nuclei and the red nucleus. Additional regions of axonal degeneration were found in the hippocampus, the flocculus, dorsal raphe nucleus, ventral cochlear nuclei, retrosplenium, the internal capsule of the corpus striatum and the optic chiasm. These results show that by using sufficiently sensitive neurohistological techniques, the neurotoxicity of methyl alcohol is revealed in the vertebrate central nervous system.

INTRODUCTION

Methanol has been widely suggested as a neurotoxin in humans (9,7), yet the demonstration of such purported toxicity has been difficult to achieve with consistency. "Surprisingly low levels" of methanol (14) are known to cause various and nonspecific neurological complaints, including headache, vertigo, chills, gastric pain, insomnia (23), tinnitus (4), shooting pains in the lower extremities, and a form of multiple neuritis characterized by paresthesia, numbness, prickling and shooting pain in the back of the hands and forearms as well as edema of the arms. Bilateral blindness, nystagmus (20,10), bladder paresis (7) and permanent motor dysfunction (9) are long term neurotoxic sequelae following acute poisonings (18). The most characteristic signs and symptoms of chronic methyl alcohol exposure in humans are diverse visual disturbances with progressive contraction of visual fields (23). Acute exposure to methanol can also lead to blindness. These data are inconsistent on two grounds: Reports of both transient and permanent blindness, as well as unilateral and bilateral disturbances, have appeared in the clinical literature (20,25). Methanol is generally considered to be a cumulative toxin, both due to its unusually long half life (estimated to be over thirty five hours in humans; 2), and to the progressive damage reported in test animals chronically exposed to methanol in early studies (10). Methanol poisoning of humans is the only known exception to "Richardsons' Rule," by which the toxicity of alcohols increases directly with the length of the carbon chain (18). Unfortunately, little is yet known of the mechanism by which methanol exerts its apparently selective cellular toxicity (22).

There are considerable differences methanol toxicity across species (19). For example, the minimum acute lethal dose (MLD) in rat is 9.5 g/kg, rabbit 7.0 g/kg and dog is 8 g/kg (19). Primates also vary considerably across species and strains, with lethality reported to occur in the range of 3-9 g/kg (24). Humans have succumbed to doses as low as 100 mg/kg (1); blood levels above 115 mg/dl (milligram percent) are generally considered lethal (3). {{See footnote 1}} Several early studies of chronic methanol exposure have reported, although with little substantiation, the occurrence of extensive peripheral "nerve damage" (12,21) and "destruction of the parenchyma [sic] cells of the cerebrum" (6) with long term inhalation of methanol both in monkeys and in dogs. Both the ingestion and the inhalation of methanol have been reported to induce behavioral abnormalities (11) and gross neurological teratology in rat pups whose dams had been exposed to methanol during gestation (15). Similarly, rabbits acutely exposed to methanol showed "thinning and focal loss" of myelin, though the nature and extent of the damage was not fully described (20).

Heretofore laboratory animals have not been considered as appropriate model systems for the study of methanol toxicity in humans, due to the increased methanol tolerance among all lower species thus far examined (19). The present experiments addressed the question of whether an adequate dose and treatment regimen could provide a reliable animal model of methanol neurotoxicity.

METHOD

Eight adult male and female Long Evans derived rats weighing between 200-250 grams were intubated once a day with 20 percent (v/v) spectral grade methanol (Sigma Chemical Company, M3641) in glass distilled water sufficient to provide 0.95 g/kg body weight (10 percent of the MLD). Six control animals received intubation with an equivalent volume of glass distilled water. Animals were randomly selected for histological examination on day 18, 25 or 33 of treatment. For histology, animals were deeply anesthetized with sodium pentobarbital (100 mg/kg), and perfused transcardially with normal saline followed by 4% paraformaldehyde, pH adjusted to 7.4. Brains were removed from the calvaria and postfixed in the perfusate for 7-48 days awaiting further analysis. On the day before sectioning brains were transferred to 10% sucrose to facilitate sectioning. Frozen sections were cut at 40 microns and processed for degenerating neuronal byproducts using the reduced silver method of Giolli and Pope (8). Sections were then mounted on gelatin coated slides, counter stained with thionin, cleared and cover slipped. Tissue was analyzed and regions of degenerating neuronal byproducts were photographed using conventional bright field light microscopic techniques.

RESULTS

Analyses of degenerating neuronal tissue were performed by three investigators. All experimental animals showed massive axonal degeneration in numerous and widely distributed regions. Microscopic analyses indicated degeneration of fibers whose cells of origin lay in cerebellar cortex, deep cerebellar nuclei, several cranial nerve nuclei and in the red nucleus. Of particular interest was the surprising absence of neuronal cell body involvement: All observable damage was restricted to axons and axon terminals.

All experimental animals showed massive degeneration throughout the medullary layer of the cerebellum. The spinocerebellar tracts were so heavily stained with degeneration byproducts as to preclude tracing the course of individual fibers. The corticospinal tract, rubrospinal tract, the trapezoid body, the trigeminal nerve, trigeminal nucleus and particularly the NTST nerve) were virtually filled with degenerating fibers. Exceptionally heavy degeneration was observed in the flocculus and in the ventral-most aspect of the periventricular gray (dorsal raphe nucleus). There was also extensive damage to the dorsal and ventral cochlear nuclei.

The optic chiasm showed patchy areas of degeneration. The neocortex was mostly free of degeneration except for the retrosplenium. The corpus striatum showed damage only in the isolated fibers of the internal capsule. The hippocampus exhibited degeneration scattered throughout regions CA 1 thru CA 4, with some involvement of the dentate leaf. The locus and extent of the axonal damage was independent of the duration of methanol exposure and of the sex of the experimental animals. The thalamus, hypothalamus, cingulate cortex, substantia nigra and the reticular formation showed no signs of degeneration in any animal.

DISCUSSION

Our present findings indicate that chronic high doses of methanol are capable of inducing severe axonal damage in many brain loci of the rat. There are surprisingly few published reports of the effect of long term methanol exposure in any species, due, in part, to the relatively high resistance to methanol found in virtually all lower animals. Many of the symptoms of acute and chronic methanol toxicity in humans are indicative of neurological damage (perhaps via demyelination). There is virtually no literature addressing the long term exposure of humans to this ever-increasing environmental contaminant (16) and food toxicant (13) which has a particularly high, and as yet unexplained, potency toward man. It is highly unlikely that this neurological damage is caused by the direct effect of methanol itself, but rather by one or more of its metabolic products. Both formaldehyde and formic acid are far more potent neurotoxins.

LITERATURE CITED

(1) Bennett, I.L., Cary , F.H., Michell , G.L. and Cooper, M.N. (1953) Acute Methyl Alcohol Poisoning: A Review Based on Experience in an Outbreak of 323 Cases. *Medicine* **35**, 431-463.

(2) Bergeron, R., Cardinal, J. and Geadah, D. (1982) Prevention of Methanol Toxicity by Ethanol Therapy. *N Engl J Med* **304(24)**, 1528.

(3) Berkow, R. (1982) *Merck Manual.* p. 2186 vol. 14. Merck and Co Inc., New Jersey.

(4) Browing, E. (115) *Methanol Toxicology: In Toxicity and Metabolism of Industrial Solvents.* p. 315-323. Elsevier Publishing Co., Amsterdam.

(5) Clay, K.L., Murphy, W.C. and Watkins, W.D. (1975) Experimental Methanol Toxicity in the Primate: Analysis of Metabolic Acidosis. *Toxicology and Applied Pharmacology* **34**, 49-61.

(6) Eisenberg, A.A. (1917) Visceral Changes in Wood Alcohol Poisoning by Inhalation. *American Journal of Public Health* **7**, 765.

(7) Erlanson, P., Fritz, H., Hagstam, K. E., Liljenberg, B. (115) Tryding, N., Voigt, G., Severe Methanol Intoxication. *Acta Medica Scand.* **177(4)**, 393-408.

(8) Giolli, R.A. and Pope, J.E. (1973) The Mode of Innervation of the Dorsal Lateral Geniculate Nucleus and the Pulvinar of the Rabbit by Axons Arising from the Visual Cortex. *Journal of Comparative Neur.* **147**, 129-144.

(9) Guggenheim, M.A., Couch, R. and Weinberg, W. (1971) Motor Dysfunction as a Permanent Complication of Methanol Ingestion. *Archives of Neurology* **24**, 550-554.

(10) Hunt, R. (1902) The Toxicity of Methyl Alcohol. *John Hopkins Hospital Bulletin.* **13**, 213-225.

(11) Infurna, R., Schubin, W. and Weiss, B. (1981) Developmental Toxicology of Methanol, *Toxicologist* **1**, 32.

(12) McCord, C.P. (1931) Toxicity of Methyl Alcohol (Methanol) Following Skin Absorption and Inhalation. *Industrial and Engineering Chemistry* **23**, 931-936.

(13) Monte, W.C. (1984) Aspartame: Methanol and the Public Health. *Journal of Applied Nutrition.* **36(1)**, 42-54.

(14) *National Institute for Occupational Safety and Health.* Health Hazard Evaluation Report No HETA-81-177,178-988: NTIS Order No. 1982;PB82-194648:1-14.

(15) Nelson, B.K., Brightwell, W.S., MacKenzie, D.R, Khan, A., Burg, J.R., Weigel, W.W. and Goad, P.T. (1985) Teratological Assessment of Methanol and Ethanol at High Inhalation Levels in Rats. *Fundam Appl Toxicol* **5**, 727-736.

(16) Posner, H.S. (1975) Biohazards of Methanol in Proposed New Uses. *Journal of Toxicology and Environmental Health* **1**, 153-171.

(17) Rao, K.R., Aurora, A.L., Muthaiyan, S. and Ramakrishnan, S. (1977) Methanol toxicity - an experimental study. *Jawaharlal Inst. Post-Grad. Med. Educ. Res* 2, 1-11.

(18) Roe, O. (11955) The Metabolism and Toxicity of Methanol. *Parmacological Review* **7**, 399-412.

(19) Roe, O. (1982) Species Differences in Methanol Poisoning. I. Minimal Lethal Doses, Symptoms, and Toxic Sequelae of Methanol Poisoning in Humans and Experimental Animals. *CRC Critical Reviews in Toxicology*, 275-286.

(20) Roe, O.(1946) Methanol Poisoning: Its clinical course, pathogenesis and treatment. *Acta Medica Scandinavica* **126**(supplement 182), 1-253.

(21) Scott, E., Helz, M.K. and McCord, C.P. (1933) The Histopathology of Methyl Alcohol Poisoning. *American Journal of Clinical Pathology* **3**, 311-319.

(22) Smith, E.N. and Taylor, R.T. (1982) Acute Toxicity of Methanol in the Folate-Deficient Acatalasemic Mouse. *Toxicology* **25**, 271-287.

(23) *U. S. Department of Health, Education, and Welfare.* Occupational Exposure to Methyl Alcohol: HEW Pub. No. (NIOSH) 76-148, March 1976.

(24) Wimer, W.W., Russell, J.A. and Kaplan, H.L. (1983) *Alcohols Toxicology: Alcohols Toxicology.* p. 1-277. Noyes Data Corporation.

(25) Wood, C.A. and Buller, F. (1904) Poisoning by Wood Alcohol. *Journal of the American Medical Association* **43**, 972-977, 1058-1062, 1117-1123, 1213-91, 1289-1301.