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From: Rich Murray <rmforall@gmail.com>
Sent: Sunday, October 23, 2016 8:50 PM
To: P65Public Comments
Cc: Woodrow C. Monte; Ralph G. Walton; Rong-Qiao He; Belpoggi Fiorella - Istituto Ramazzini; Yuri L. Dorokhov; Rich Murray
Subject: 2016 CIC Prioritization: recent studies that support WC Monte paradigm that methanol from aspartame is made by ADH1 enzyme into reactive formaldehyde within cells of 20 tissues, harmful only in humans: Rich Murray 2016.10.23

Follow Up Flag: Follow up
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2016 CIC Prioritization: recent studies that support WC Monte paradigm that methanol from aspartame is made by ADH1 enzyme into reactive formaldehyde within cells of 20 tissues, harmful only in humans: Rich Murray 2016.10.23

<http://rmforall.blogspot.com/2016/10/2016-cic-prioritization-recent-studies.html>

Comment Period - Prioritization 2016: Chemicals for Consultation by the Carcinogen Identification Committee

<http://oehha.ca.gov/proposition-65/events/comment-period-prioritization-2016-chemicals-consultation-carcinogen>

Friday, September 9, 2016 - 8:00 am to Monday, October 24, 2016 - 5:00 pm

The Office of Environmental Health Hazard Assessment (OEHHA) announces the beginning of a 45-day public comment period on the five chemicals or chemical groups listed below.

These chemicals will be discussed at the November 15, 2016 meeting of the Proposition 65 Carcinogen Identification Committee (CIC).

The CIC is the state's qualified experts on carcinogenicity for purposes of the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65).

The CIC will provide the Office of Environmental Health Hazard Assessment (OEHHA) with advice on the prioritization of these chemicals for possible preparation of hazard identification materials.

At a later date, OEHHA will select chemicals for preparation of hazard identification materials and announce those decisions in a separate notice.

No listing decisions will be made for these chemicals at the November 15 meeting.

OEHHA is the lead agency for the implementation of Proposition 65.

The evidence of hazard used in this current round of prioritization is an epidemiologic data screen and an animal data screen.

Chemicals or chemical groups passing either data screen were then subjected to a preliminary toxicological evaluation.

This screening follows the procedure described in the 2004 OEHHA document, Process for Prioritizing Chemicals for Consideration under Proposition 65 by the States Qualified Experts.

The five chemicals or chemical groups are:

Aspartame
Asphalt and Asphalt Emissions Associated with Road Paving and Asphalt and
Asphalt Emissions Associated with Roofing
Methyl Chloride
Type I Pyrethroids
Vinyl Acetate

The CIC will consider these chemicals at its meeting on Tuesday, November 15, 2016.

The meeting will be held in the Sierra Hearing Room at the CalEPA Headquarters building, 1001 I Street, Sacramento, California.

The meeting will begin at 10:00 a.m. and will last until all business is conducted or until 5:00 p.m.

The agenda for the meeting will be provided in an upcoming public notice published in advance of the meeting. OEHHA will send comments received on the prioritization documents for these chemicals to CIC members prior to the meeting.

Copies of the summaries of available scientific information on the chemicals and related attachments are available on OEHHA's web site or may be requested by calling [\(916\) 445-6900](tel:9164456900).

Interested parties may provide comment on the extent of the scientific evidence pertaining to the selection of any of these chemicals for possible preparation of hazard identification materials.

OEHHA must receive comments and any supporting documentation by 5:00 p.m. on Monday, October 24, 2016.

We encourage you to submit comments in electronic form, rather than in paper form.

Comments transmitted by e-mail should be addressed to

P65Public.Comments@oehha.ca.gov (link sends e-mail).

Please include 2016 CIC Prioritization in the subject line.

Comments submitted in paper form may be mailed, faxed, or delivered in person to the addresses below:

Mailing Address: Michelle Ramirez
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Sacramento, California 95814

severe methanol toxicity in humans only, made into uncontrolled formaldehyde within cells of 20 tissues by ADH1 enzyme, leading to random spots of inflammation, WC Monte paradigm: Rich Murray 2016.10.23

Ethanol is the potent antidote, as it preferentially binds to ADH1 enzyme.

Methanol mainly comes from wood and cigarette smoke, aspartame, and unfreshed fruits juices and vegetables, sealed wet in containers.

methanol from cigarette smoke, aspartame, many foods, is made into free formaldehyde in cells of 20 tissues by ADH1 enzyme, leading to chronic diseases in humans only, WC Monte paradigm: Rich Murray 2016.10.06
<http://rmforall.blogspot.com/2016/10/methanol-from-cigarette-smoke-aspartame.html>

tiny doses methanol put formaldehyde into monkey brain cells and CSF -- March 2016 expert China study cites WC Monte book, While Science Sleeps 2011: Rich Murray 2016.08.30
<http://rmforall.blogspot.com/2016/08/tiny-doses-methanol-put-formaldehyde.html>

[Rich Murray: Yet another normal mainstream research paper adds to interest in the promising new WC Monte paradigm, that in humans only, methanol toxicity results from ADH1 enzyme in 20 tissues making uncontrolled formaldehyde right inside cells, notably the cells of the tissue side walls of brain blood vessels.

The potent antidote is ethanol, which ties up ADH1 enzyme, preventing formation of formaldehyde.

This team probably can run the experiment again with the same three monkeys, adding ethanol, to show that then no formaldehyde is formed, confirming a key feature of the paradigm.]

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4811046/> Epub. March 15, 2016

Correspondence should be addressed to: Xintian Hu xthu@mail.kiz.ac.cn

References 19 and 20:

H. Henzi, "Chronic methanol poisoning with the clinical and pathologic-anatomical features of multiple sclerosis," *Medical Hypotheses*, vol. 13, no. 1, pp. 63–75, 1984. View at Publisher · View at Google Scholar · View at Scopus

W. C. Monte, *While Science Sleeps: A Sweetener Kills*, Create Space Publishing, San Francisco, Calif, USA, 2011.

"Elevated FA has been implicated in some neurodegenerative diseases.

For example, elevated FA levels have been found in brains of patients suffering from neurodegenerative diseases like Alzheimer's disease (AD) or multiple sclerosis (MS) [22–24], where FA is known to cross-link proteins like tau (in AD) or myelin basic protein (MBP, in MS), which in turn results in the proteins losing their normal function and elicits an immune response that is characteristic of the diseases [20,24].

It is noteworthy that some human subjects suffering from methanol poisoning develop symptoms of MS, which may be related to methanol oxidation to FA in brain that leads to MBP structure and function modification by the reactive FA [19]."

"Moreover, the toxic actions of methanol have also been reported in the brain of primates [2–4, 11, 19]."

"Methanol is a natural chemical that poses dangers to human health.

It can be found in cigarette smoke, canned fruits and vegetables, and aspartame-sweetened food products [20], as well as in beverages where drinking alcohol is inadvertently or criminally substituted with methanol [8].

Although methanol has been found to induce central nervous system dysfunction [4, 11] and neurodegenerative conditions [2, 3, 19], the mechanisms underlying its toxicity to the brain remain inexplicit in primates.

The present study demonstrated that methanol could be oxidized to FA in primate brain and that a portion of the FA generated leaked out of the cells in which it was produced.

This suggests that FA produced from methanol not only affects the cell in which methanol is metabolized but also may affect the surrounding tissue.

It is noteworthy that formaldehyde levels in CSF present a gradual increasing trend which began at 3 hours following direct i.c.v. injection of methanol, although a significant elevation in FA levels only occurred after 18 hours."

<https://www.hindawi.com/journals/acp/2016/4598454/> free full text

375 views, 173 pdfs

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<http://dx.doi.org/10.1155/2016/4598454>

Research Article

Evidence for Conversion of Methanol to Formaldehyde in Nonhuman Primate Brain

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Academic Editor: Nady Braidy

methanol as the stealth agent for much harm in humans from formaldehyde -- toxic action inside cells via ADH1 enzyme in 20 tissues, including brain side walls of blood vessels -- WC Monte paradigm: Rich Murray 2016.08.27

Prof. WC Monte, retired (2004) since 2007 gives evidence that methanol (wood alcohol) is made by ADH1 enzyme into uncontrolled formaldehyde inside cells of 20 tissues, leading to cumulative harm from myriad random spots of inflammation, slowly leading to many modern chronic diseases, later cancers, and birth defects, including autism and Aspergers.

Only humans of all creatures lack an essential biochemical defense.

Ethanol (ordinary drinking alcohol) is the potent antidote.

Methanol comes from wood, peat and cigarette smoke, common in winter;
aspartame (E951);

dark wines, liquors, and fruit brandies:

smoked foods, such as fish in Sweden;

fresh tomatoes;

fruits, juices, and vegetables, cut up, cooked, preserved wet at room temperatures in sealed cans, jars, plastics (common in winter);

portable stoves and heaters using methanol (very dangerous in tents and boats in extreme climates, undoubtedly causing many mysterious deaths...).

His 2012 book "While Science Sleeps" is a crisp scientific mystery epic, backed by a free online archive of 782 full text medical research pdfs at WhileScienceSleeps.com ...

11% of aspartame, methanol is made by ADH1 enzyme into uncontrolled formaldehyde within cells of 20 human tissues, harmful in humans only -- WC Monte paradigm: Rich Murray 2016.08.05

Moreno Paolini moreno.paolini@unibo.it

Carcinogenesis. 2016 Feb 24. pii: bgw025. [Epub ahead of print]

Aspartame, a bittersweet pill.

Pauline M 1, Vivarelli F 2 Soap 2, Canistro D 2.

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Abstract

For the first time, the aspartame case shows how a corporation decided to ban an artificial ingredient in the wake of public opinion notwithstanding the regulatory assurance claims that it is safe.

PepsiCo Inc. made an unprecedented decision most likely based on life-span carcinogenicity bioassay studies from the Cesare Maltoni Cancer Research Center of the Ramazzini Institute (CMCRC/RI), which provide consistent evidence of aspartame's carcinogenicity in rodents.

Although CMCRC/RI experiments have been criticized for not complying with Organisation for Economic Co-operation and Development (OECD) guidelines, the newly launched aspartame-free soft drink may not be an isolated case.

In the light of vinyl chloride-, formaldehyde- or benzene-associated carcinogenicity discovered for the first time by CMCRC/RI in the same way, it seems the guidelines need to be re-evaluated to avoid the credibility of international regulatory agencies being compromised by consumer opinion.

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PMID: 26912665 DOI: 10.1093 / Carco / bgw025
[PubMed - as supplied by publisher]

methanol, made into formaldehyde by ADH1 enzyme in humans, inside cells of brain blood vessel walls, initiates overactive immune system in 20 tissues -- WC Monte paradigm: Rich Murray 2016.06.28
<http://rmforall.blogspot.com/2016/06/methanol-made-into-formaldehyde-by-adh1.html>

Only in humans does methanol lead to harmed brain cell components that activate an immune system response -
- ethanol is the potent antidote -- WC Monte paradigm since fall 2007.

Research is now gingerly edging close to the new paradigm since fall 2007 by WC Monte. that ADH1 enzyme, notably inside the cells of the brain tissue side of blood vessels, swiftly makes all methanol, seeping easily in from the bloodstream into the cytosol, into free floating, highly reactive acidic formaldehyde hydrate, which binds durably to the nearest basic sites on DNA, RNA, and proteins, gradually harming and killing the cells, while formaldehyde labelled tissues strongly attract many kinds of white blood cells, leading to cumulative random small spots of inflamed tissue -- Monte gives a free archive of 782 full text pdfs of mainstream research references at his site WhileScienceSleeps.com .

Note that ethanol is the potent antidote, as it binds preferentially to ADH1 enzyme, preventing the formation of formaldehyde inside the cells of 20 tissues -- only humans lack a functioning biochemical defense.

Methanol comes from wood and cigarette smoke, aspartame, dark wines and liquors, fresh tomatoes, and unfresh fruits juices vegetables, cut up, heated, preserved wet at room temperature in sealed containers, as well as vehicle fuels in Iran and China.

leaky blood-brain barrier in early Alzheimers and multiple sclerosis -- fits methanol, made into active formaldehyde inside cells of tissue side walls of blood vessels by ADH1 enzyme in humans only -- WC Monte paradigm: Rich Murray 2016.06.01
<http://rmforall.blogspot.com/2016/06/leaky-blood-brain-barrier-in-early.html>

part A: new paradigm for Alzheimers type diseases, Tao Su, WC Monte, RQ He et al, review, 177 references, full plain text -- chronic methanol formed into loose formaldehyde inside cells by ADH1 enzyme in 20 human tissues: Rich Murray 2015.08.29
<http://rmforall.blogspot.com/2015/08/part-new-paradigm-for-alzheimers-type.html>

part B: new paradigm for Alzheimers type diseases, Tao Su, WC Monte, RQ He et al, review, 177 references, full plain text -- chronic methanol formed into loose formaldehyde inside cells by ADH1 enzyme in 20 human tissues: Rich Murray 2015.08.29
<http://rmforall.blogspot.com/2015/08/part-b-new-paradigm-for-alzheimers-type.html>

142 mg methanol weekly is provided by 6.5 cans aspartame diet drink, about 1 can daily, the amount used by 161 moms, whose kids became autistic, over twice the methanol taken by 550 moms who had no autistic kids.

dietary methanol and autism, Ralph G. Walton, Woodrow C. Monte, in press, Medical Hypotheses (now peer reviewed), free full rich text, 38 references: Rich Murray 2015.07.06

<http://rmforall.blogspot.com/2015/07/dietary-methanol-and-autism-ralph-g.html>

Download a free copy of Chapter 9 and have a read

[99 pages, including list of all 782 full text online references for book -- Chapter 9 has 146 references]:

Chapter 9 Multiple Sclerosis

<http://www.whilesciencesleeps.com/files/While%20Science%20Sleeps%20-%20Chapter%209%20%28Prepublication%20copy%29%20Website%203-15-2012.pdf>

The references are located on his website:

<http://www.whilesciencesleeps.com/references/> ,

as are the simple dietary changes that you must follow because your life may depend on it:

<http://www.whilesciencesleeps.com/monte-diet/> .

California OEHHA sets methanol ingestion level 23 mg daily, same as from 1 can aspartame diet soda, 10 cigarettes, 3 tomatoes, or 4 cans green beans: Rich Murray 2013.07.03

<http://rmforall.blogspot.com/2013/07/california-oeaha-sets-methanol.html>

diet, mostly aspartame, fruit and soda drinks correlate with depression NIH-AARP Diet and Health Study 11,311 cases depression after 2000 in 263,923 seniors 50-71 who gave beverage use in 1995-6, Honglei Chen team 2014 April: Rich Murray 2014.12.23

<http://rmforall.blogspot.com/2014/12/diet-mostly-aspartame-fruit-and-soda.html>

careful expert China team finds formaldehyde harm in retinas of old people -- methanol may be a source -- relevant to Alzheimers disease: Rich Murray 2016.05.19

<http://rmforall.blogspot.com/2016/05/careful-expert-china-team-finds.html>

aspartame dose same as 14 cans diet drink raised methanol 4 to 7 times individual levels for next 2 hours in exhaled breath, David Smith and 3 colleagues: Rich Murray 2015.12.09

<http://rmforall.blogspot.com/2015/12/aspartame-dose-same-as-14-cans-diet.html>

neurobehavioral effects of aspartame, GN Lindseth et al 2014, funded by Army, free full plain text -- 25% of 28 healthy young university students had obvious harm from a dose same as 9 cans daily for just 8 days: Rich Murray 2015.07.05

<http://rmforall.blogspot.com/2015/07/neurobehavioral-effects-of-aspartame-gn.html>

"While Science Sleeps" textbook, 2012 January:

"Methanol travels easily to breast tissue and has been found in human milk. 219

The cells that produce milk within the breast, cells prone to develop the most common of breast cancers, adenocarcinoma 358, contain high levels of ADH1 enzyme, 358 allowing methanol's conversion to formaldehyde [inside the cells as free floating acidic hydrated formaldehyde, highly reactive on both sides of molecule].

Mammary epithelial cells have no way to protect themselves from formaldehyde 216 -- no means to render it harmless.

They, unlike other breast tissue, contain no aldehyde dehydrogenase enzyme (ADH 3) that could transform formaldehyde into the non-carcinogenic formic acid. 216

Of particular interest are recent findings implicating ADH as playing a pivotal role in the formation of breast cancer, documenting a greater incidence of the disease in women with higher levels of ADH1 activity in their breasts. 357"

[see also, for value of vegan diet,

DrMcDougall.com

VegSource.com

ForksOverKnives.com

TrueHealthInitiative.org/#!/the-solution

<http://proteinaholic.com>

in 2013 Kaiser Permanente, 10 million members, notified physicians to prescribe whole foods plant based only diet to prevent and cure most major diseases: Rich Murray 2016.05.26

<http://rmforall.blogspot.com/2016/05/in-2013-kaiser-permanente-10-million.html>

American College of Lifestyle Medicine -- global professional association started 2004: Rich Murray 2016.05.21

<http://rmforall.blogspot.com/2016/05/american-college-of-lifestyle-medicine.html>

<http://lifestylemedicine.org/>]

"As a matter of course, every soul citizen of Earth has a priority to quickly find and positively share evidence for healthy and safe food, drink, environment, and society."

within the fellowship of service,

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Research Article

Evidence for Conversion of Methanol to Formaldehyde in Nonhuman Primate Brain

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Abstract

Many studies have reported that methanol toxicity to primates is mainly associated with its metabolites, formaldehyde (FA) and formic acid. While methanol metabolism and toxicology have been best studied in peripheral organs, little study has focused on the brain and no study has reported experimental evidence that demonstrates transformation of methanol into FA in the primate brain. In this study, three rhesus macaques were given a single intracerebroventricular injection of methanol to investigate whether a metabolic process of methanol to FA occurs in nonhuman primate brain. Levels of FA in cerebrospinal fluid (CSF) were then assessed at different time points. A significant increase of FA levels was found at the 18th hour following a methanol injection. Moreover, the FA level returned to a normal physiological level at the 30th hour after the injection. These findings provide direct evidence that methanol is oxidized to FA in nonhuman primate brain and that a portion of the FA generated is released out of the brain cells. This study suggests that FA is produced from methanol metabolic processes in the nonhuman primate brain and that FA may play a significant role in methanol neurotoxicology.

1. Introduction

Methanol, a single carbon alcohol, is an important public health and environmental concern because it leads to metabolic acidosis, visual impairment, central nervous system dysfunction, neurodegenerative conditions, and death [1–5]. Elevated methanol in the body can occur after accidental or intentional consumption and/or exposure to other exogenous methanol sources. Elevated methanol levels can also occur as a result of increased endogenous methanol production, such as in the generation of methanol through the hydrolysis of protein carboxymethyl esters, catalyzed either by methylesterases or through spontaneous nonenzymatic reactions [6].

Methanol metabolism and mechanisms responsible for its toxic actions in primates have been extensively investigated in the periphery. Typically, with respect to methanol metabolism in primates, there are three steps involved. The first step in the metabolic pathway is oxidation of methanol to formaldehyde (FA). An alcohol dehydrogenase (ADH) is primarily responsible for the initial step [7, 8]. The second step is the oxidation of FA to formic acid. A glutathione-dependent formaldehyde dehydrogenase specific for FA catalyzes the conversion of FA to formic acid [1]. Another formaldehyde dehydrogenase, which is NAD dependent, catalyzes this conversion in human erythrocytes [7, 8] and a high-activity aldehyde dehydrogenase is responsible for this conversion in liver mitochondria [9]. The third step is the oxidation of formic acid to carbon dioxide. 10-formyl-THF dehydrogenase, a ubiquitous enzyme in mammalian tissues, catalyzes this step [1, 10]. Notably, the rate of the final step is far lower in primates than it is in rodents [1, 11]. With respect to methanol toxicity, many studies have demonstrated that formic acid is primarily responsible for methanol's toxicity. For example, formic acid has been found to be responsible for the metabolic acidosis witnessed in methanol-intoxicated humans [12, 13] and nonhuman primates [14, 15] and the ocular toxicity observed in methanol-poisoned humans [12, 16] and nonhuman primates [17, 18].

Moreover, the toxic actions of methanol have also been reported in the brain of primates [2–4, 11, 19]. Given the fact that methanol is nonreactive [20] and less toxic than its metabolites [21], FA, the metabolic intermediate of methanol, was considered responsible for these effects because there is compelling evidence that suggests FA is related to AD pathology, both in vivo and in vitro [22–26].

While methanol metabolic processes in the brain of primates remains inexplicit, it is likely that the brain will use similar enzymatic pathways to metabolize methanol, as found in liver. While catalase has been reported to be expressed in human brain [27], the expression of ADH1 in primate brain has been controversial [7]. The expression of catalase provides a potential for the oxidation of methanol to FA in the primate brain, but no study has demonstrated this metabolic process through the direct evaluation of intracranial FA levels after injection of methanol into the brain of primates.

In this study, direct injections of methanol into the lateral ventricles of rhesus monkeys were carried out to directly investigate whether metabolic process of methanol to FA occurs in the brain of rhesus macaques. This approach allowed the direct investigation of methanol metabolic processes under precise control of dose to the animals' brain. The FA levels in CSF were then assessed at the different time points following a single methanol injection.

2. Materials and Methods

2.1. Animals and Treatment

All animal care and treatment in this study were performed in accordance with the guidelines for the national care and use of animals approved by the national animal research authority (China). All animal experiments were carried out after approval by the Institutional Animal Care and Use Committee (IACUC) of the Kunming Institute of Zoology.

Three 12-year-old male rhesus monkeys (*Macaca mulatta*) were recruited in this study. The body weights of the monkeys were as follows: Monkey #1 10.8 kg, Monkey #2 10.3 kg, and Monkey #3 11.4 kg. Each monkey was individually housed under standard laboratory conditions [28]. In order to provide the precise location of the right lateral ventricle and avoid interference caused by surgical operation in the results, as well as allowing animals to recover to stabilized FA levels, a surgical operation to implant a stainless steel tube into the right lateral ventricle was performed on each rhesus macaque prior to the experiment. Each animal was anesthetized with intramuscular atropine (20 mg/kg), ketamine (10 mg/kg), and sodium pentobarbital (20 mg/kg). The head of the animal was fixed in a stereotaxic instrument and the skull over the parietal lobe was exposed under aseptic conditions by a longitudinal skin incision followed by removal of the connective tissue. A small hole on the skull (<2 mm in diameter) was created with an electric drill at the following coordinates: anteroposterior (AP): interaural: 17 mm; mediolateral (ML): -2 mm. Then stainless steel tubing with a length of 40 mm (21-gauge, New England Small Tube Corporation, USA) was inserted into the right lateral ventricle (dorsoventral (DV) depth ranged from 18 to 22 mm). A successful puncture was judged by observing the cerebrospinal fluid (CSF) flowing out or CSF pulsations at the orifice. The outer portion of the stainless steel tube was then fixed on the skull with composite dental cement fixed to titanium nails screwed into the skull. After the operation, each monkey was intramuscularly injected with penicillin (1600 K Unit, Harbin Pharmaceutical Group Sixth Pharm Factory, Harbin, China) for at least seven days. All animals were allowed to recover after the surgery for more than two weeks.

Each monkey received a single injection of 200 μ L volumes of 5% (v/v) methanol in 0.9% (w/v) saline into lateral ventricle over a 15-minute period. After the injection, the needle was held in the place for 5 minutes. The methanol was purchased from Sigma (USA).

2.2. CSF Collection

In order to determine whether the level of FA in CSF was elevated following single intracerebroventricular (i.c.v.) methanol injections, the CSF from the methanol injected animals was collected at 0, 3, 6, 12, 18, 24, and 30 hours after the administration. The 0 hr refers to the point before methanol injection. Animals were anesthetized with ketamine (10 mg/kg) and approximately 0.5 mL of CSF was withdrawn through a lumbar puncture using a 22-gauge needle. Then the CSF samples were immediately frozen in liquid nitrogen and later transferred and stored in a -80°C freezer until analysis.

2.3. CSF Formaldehyde Measurements

Formaldehyde levels in the CSF following a single methanol injection were measured with the DFOR-100 formaldehyde detection kit as per the manufacturer's instructions (BioAssay Systems, Hayward, CA, USA). Briefly, CSF samples were deproteinated and neutralized prior to assaying. To deproteinate the CSF samples, 50 μ L of 10% TCA was added into each 100 μ L sample. Each sample was then vortexed and centrifuged at 14000 rpm for 5 min; 100 μ L of clear supernatant was transferred to a clean tube and mixed with 25 μ L of Neutralizer. Samples (50 μ L) were mixed with the DFOR reagent for 30 min and then assayed in a FlexStation 3 Multi-Mode Microplate Reader: = 370 nm; = 470 nm.

2.4. Statistics

All statistical analyses were carried out with the GraphPad Prism 5 software. The levels of formaldehyde in the CSF were analyzed by analysis of variance with repeated measures followed by Tukey's test for intergroup difference. The level of significance was set at .

3. Results

In order to investigate elevated levels of intracranial FA following methanol injections, CSF samples from monkeys given a single methanol injection were taken before administration (noted as "0" point) and at 3, 6, 12, 18, 24, and 30 hours after the injection. FA levels were then measured using a formaldehyde measurement kit. FA levels in the CSF following methanol treatments displayed an increasing trend and reached prominent differences compared to the "0" time point as a baseline at 18 and 24 hrs after the injection, respectively (Figure 1). The elevated FA levels returned to normal physiological levels at 30 hours (Figure 1). These findings indicated that endogenous methanol metabolism led to elevated intracranial FA levels in the brain of monkeys treated with methanol.

Figure 1: Formaldehyde (FA) levels in CSF samples following an i.c.v. injection of methanol in rhesus monkeys. FA levels in the CSF were measured at different time points. "0 h" refers to the point prior to the methanol injection. There was no significant difference until 18th hour after the methanol injection, albeit an increasing trend began after the 3rd hour. Data points represent the average CSF formaldehyde levels of the monkeys at each time point. All values are represented as the mean \pm SEM. ; ; .

4. Discussion

Methanol is a natural chemical that poses dangers to human health. It can be found in cigarette smoke, canned fruits and vegetables, and aspartame-sweetened food products [20], as well as in beverages where drinking alcohol is inadvertently or criminally substituted with methanol [8]. Although methanol has been found to induce central nervous system dysfunction [4, 11] and neurodegenerative conditions [2, 3, 19], the mechanisms underlying its toxicity to the brain remain inexplicit in primates. The present study demonstrated that methanol could be oxidized to FA in primate brain and that a portion of the FA generated leaked out of the cells in which it was produced. This suggests that FA produced from methanol not only affects the cell in which methanol is metabolized but also may affect the surrounding tissue. It is noteworthy that formaldehyde levels in CSF present a gradual increasing trend which began at 3 hours following direct i.c.v. injection of methanol, although a significant elevation in FA levels only occurred after 18 hours. The time lapse of the first significant elevation in FA levels was dependent on (a) numbers of samples; (b) diffusion velocity of methanol into brain tissues; (c) metabolic capacity and speed of brain tissues to oxidize methanol to FA; (d) reactivity of produced FA to surrounding molecules; (e)

diffusion velocity of FA from produced sites to CSF. Moreover, these results are consistent with formic acid data measured in primates, which suggests that methanol metabolism in the primate brain undergoes oxidation from methanol, via FA, to formic acid and carbon dioxide. FA is a significant consideration for human health because its toxicity is due to its high reactivity. FA readily attaches to proteins forming adducts or causes protein cross-linking by forming methylene bridges between amino groups [29, 30] and has the ability to damage DNA [31]. Elevated FA has been implicated in some neurodegenerative diseases. For example, elevated FA levels have been found in brains of patients suffering from neurodegenerative diseases like Alzheimer's disease (AD) or multiple sclerosis (MS) [22–24], where FA is known to cross-link proteins like tau (in AD) or myelin basic protein (MBP, in MS), which in turn results in the proteins losing their normal function and elicits an immune response that is characteristic of the diseases [20,24]. It is noteworthy that some human subjects suffering from methanol poisoning develop symptoms of MS, which may be related to methanol oxidation to FA in brain that leads to MBP structure and function modification by the reactive FA [19].

Although FA is, without doubt, produced following methanol administration, it is not considered to be a toxic metabolite of methanol in the periphery. This is mainly due to FA being undetectable in the blood following methanol administration. This limited detection is likely due to its rapid metabolism to formic acid in the liver [32] and the blood [1] and because FA has a half-life of approximately 1.5 min in the blood of monkeys following its intravenous infusion [21]. This suggests that methanol metabolism through FA to formic acid in the periphery is rapid and that methanol toxicity might possess different mechanisms in the periphery and brain. The findings that methanol is converted to FA in the brain and found in the CSF after 18 hrs suggest that methanol toxicity may have deleterious effects in the CNS via FA.

5. Conclusion

In summary, elevated levels of intracranial FA were found in this study following a single methanol injection, which is the first demonstration of methanol oxidation to FA in the nonhuman primate brain. This study links the toxicity of methanol to its metabolites, FA and/or formic acid, in the brain.

Competing Interests

The authors declare no competing financial interests.

Authors' Contributions

Rongwei Zhai and Na Zheng contributed equally to this work.

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