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Diet composition exacerbates or attenuates soman toxicity in rats: Implied metabolic control of nerve agent toxicity

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ABSTRACT

To evaluate the role of diet composition on nerve agent toxicity, rats were fed four distinct diets *ad libitum* for 28 d prior to challenge with 110 μg/kg (1.0 LD_{50}, sc) soman. The four diets used were a standard rodent diet, a choline-enriched diet, a glucose-enriched diet, and a ketogenic diet. Body weight was recorded throughout the study. Toxic signs and survival were evaluated at key times for up to 72 h following soman exposure. Additionally, acquisition of discriminated shuttlebox avoidance performance was characterized beginning 24 h after soman challenge and across the next 8 d (six behavioral sessions). Prior to exposure, body weight was highest in the standard diet group and lowest in the ketogenic diet group. Upon exposure, differences in soman toxicity as a function of diet became apparent within the first hour, with mortality in the glucose-enriched diet group reaching 80% and exceeding all other groups (in which mortality ranged from 0 to 6%). At 72 h after exposure, mortality was 100% in the glucose-enriched diet group, and survival approximated 50% in the standard and choline-enriched diet groups, but equaled 87% in the ketogenic diet group. Body weight loss was significantly reduced in the ketogenic and choline-enriched diet groups, relative to the standard diet group. At 1 and 4 h after exposure, rats in the ketogenic diet group had significantly lower toxic sign scores than all other groups. The ketogenic diet group performed significantly better than the standard diet group on two measures of active avoidance performance. The exacerbated soman toxicity observed in the glucose-enriched diet group coupled with the attenuated soman toxicity observed in the ketogenic diet group implicates glucose availability in the toxic effects of soman. This increased glucose availability may enhance acetylcholine synthesis and/or utilization, thereby exacerbating peripheral and central soman toxicity.

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1. Introduction

Diet composition and nutritional status have been shown to influence the progression of diseases induced by toxic substances (Hennig et al., 2007; Shakman, 1974). Specific nutrient deficiencies can exacerbate the toxicity of many substances (Shakman, 1974). Furthermore, dietary supplementation with specific nutrients has been shown to mitigate the toxicity produced by a number of substances (Hennig et al., 2007; Shakman, 1974). Diet composition and nutrient levels can modify the physiological and neurobehavioral effects of pharmacological and toxicological compounds by altering neurological chemistry, metabolic processes, and pharmacokinetics (Fenech, 2005; Keenan et al., 1999; Nold et al., 2001). Diet content, as an independent variable in the effects of chemical warfare nerve agents (CWNA), has received little attention. However, the manipulation of diet content has received considerable attention in recent years, in both clinical and research applications, from those investigating seizure control in epilepsy disorders (Bough and Rho, 2007; Hartman et al., 2007).

Fasting has been used since antiquity as a treatment for the control of seizures (DeVivo et al., 1975; Hartman et al., 2007); however, the mechanism(s) through which fasting functions as an effective anticonvulsant is unknown. Fasting is known to induce metabolic changes, including decreased blood glucose and increased fatty acid metabolism, resulting in the production of ketone bodies (i.e., β-hydroxybutyrate, acetoacetate, and acetone) (DeVivo et al., 1975; Sokoloff, 1973) via the metabolic process known as ketosis. The ketogenic diet (KD) was introduced in the 1920s as an alternative to fasting (to produce the effects of fasting without starvation) for the control of seizures (DeVivo et al., 1975; Hartman et al., 2007). The KD, in its typical formulation, has a high percentage (by weight) of fats (≥70%), a moderate percentage of proteins (∼20%), and a low percentage of carbohydrates (<10%). Similar to prolonged fasting, the KD has been shown to function as an effective treatment for epilepsy in clinical applications (for review see Bough and Rho, 2007). Furthermore, using a variety of

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animal models of epileptic disorders (6 Hz audiogenic seizures, pentylenetetrazole [PTZ], kainic acid [KA], pilocarpine, etc.), the KD has been shown to elevate seizure thresholds and/or have anticonvulsant properties (Stafstrom, 1999). The mechanism(s) by which the KD exerts its effects is unclear (Rho and Sankar, 2008). Under normal dietary conditions the brain derives most of its energy from the metabolism of glucose (glycolysis). However, during prolonged fasting or protracted consumption of a KD, blood glucose levels decrease due to reduced glycolysis, blood ketone body levels increase due to enhanced lipid metabolism, and the brain uses ketone bodies as an alternative energy source (Sokoloff, 1973). The beneficial effects of the KD on seizure control may be due to reduced glucose levels, increased ketone body production, increased free fatty acids, and/or altered neurotransmitter synthesis (Bough and Rho, 2007).

Support for a role of glucose in epileptic disorders comes from studies indicating that seizure control produced by the KD is rapidly reversed upon ingestion of carbohydrates (Bough and Rho, 2007). Similarly, other means of regulating glucose levels (e.g., fasting, caloric restriction) have been shown to have beneficial effects on the regulation of seizures (Greene et al., 2003; Maalouf et al., 2009). A few recent reports indicate that elevated sugar intake (glucose or high fructose corn syrup in drinking water) exacerbates the toxicity of parathion poisoning, an organophosphorus (OP) insecticide (Liu et al., 2005, 2007; Olivier et al., 2001). In Liu et al. (2005), adult rats that had consumed high fructose corn syrup in drinking water for 7 d prior to parathion exposure showed decreased weight gain, increased salivation, lacrimation, urination, and defecation (SLUD) signs and increased involuntary movements over a 7 d period following exposure. The study also demonstrated reduced toxicity in a group of rats maintained under caloric restriction compared to *ad libitum* fed rats and systematically replicated the increased toxicity of parathion in rats consuming sweetened water, providing further support for the role of glucose in OP toxicity.

As alluded to above, nutrient supplementation has been shown to either reverse or mitigate the effects of toxic insult (Hennig et al., 2007; Shakman, 1974). Administration of choline or choline analogues has been shown to influence the action of both anticholinesterases (Patterson et al., 1989; Stovner, 1956) and anticholinergics (Wecker et al., 1978). Furthermore, there appears to be a causal relationship between choline availability during development and cognitive function in adulthood (McCann et al., 2006). Dietary choline supplementation has been shown to reduce spatial memory impairments and hippocampal cell loss induced by KA seizures (Holmes et al., 2002) as well as to improve the behavioral, histological, and neurochemical outcomes associated with traumatic brain injury (Guseva et al., 2008).

The doctrinal treatment of CWNA poisoning for U.S. military personnel consists of the rapid administration of an anticholinergic (e.g., atropine sulfate) in conjunction with an oxime reactivator (e.g., pralidoxime, 2-PAM). Additionally, if the signs and symptoms warrant, a benzodiazepine (e.g., diazepam) is administered to control seizures induced by overstimulation of cholinergic synapses (Sidell et al., 2008). Ideally, these post-exposure treatments would be supplemented by a prophylactic regimen consisting of a reversible acetylcholinesterase inhibitor (e.g., pyridostigmine) that would sequester (reversibly inhibit) approximately 30% of the available acetylcholinesterase (Sidell et al., 2008) prior to nerve agent exposure. The current focus of research groups worldwide appears to be investigating compounds from these three pharmacological classes (i.e., anticholinergics, oxime reactivators, and benzodiazepines) that will have greater efficacy in preventing or reducing the sequelae associated with CWNA poisoning (Bajgar et al., 2009; McDonough et al., 2009; Shih et al., 2007; Wetherell et al., 2007). However, a few novel approaches to the management of CWNA poisoning include the use of exogenously administered enzymes that function as either stoichiometric or catalytic bioscavengers (Lenz et al., 2007; Saxena et al., 2006). Others are investigating the use of compounds that antagonize both cholinergic and glutamatergic synapses to reduce seizure activity and the resultant neuropathology associated with prolonged seizures (Myhrer et al., 2008, 2010). Others in the field are investigating the use of centrally active acetylcholinesterase inhibitors (i.e., galantamine, huperizine, and physostigmine) as alternatives to pre-treatment with pyridostigmine (Aracava et al., 2009; Haigh et al., 2008; Lallement et al., 2002; Wetherell et al., 2007). There have also been considerable efforts to develop a broad-spectrum oxime for the effective treatment of poisoning by CWNAs of different structural and chemical compositions (Kassa et al., 2010; Kuca et al., 2010).

There have been few studies to examine the influence of dietary variables on CWNA toxicity. Two relevant studies have examined the impact of short-term fasting (~18–24 h) (Fletcher et al., 1988b; Myers et al., 2005) and both found exacerbated toxicity of CWNA following fasting. However, as revealed in the epilepsy literature, prolonged (≥48 h), but not short-term (≤24 h), fasting has an anticonvulsant effect. In the present study, we examined the effects of the KD, a glucose-enriched diet, and a choline-supplemented diet on CWNA toxicity by evaluating toxic signs, body weight changes, and two-way active avoidance responding following an acute CWNA exposure. It was expected that the KD would produce metabolic effects similar to prolonged fasting (without starvation and weight loss) and have a protective effect against CWNA toxicity. Similarly, we expected the increased availability of monosaccharides in the glucose-enriched diet to exacerbate CWNA toxicity. Finally, given the encouraging results from choline supplementation studies, we predicted that choline supplementation would have beneficial effects on recovery of function following CWNA insult.

2. Method

2.1. Subjects

Sixty (60) male Sprague–Dawley rats (Crl:CD(SD)) were obtained from Charles River Laboratories (Wilmington, MA, USA). Rats weighed between 175 and 200 g at the time of arrival and were acclimated to our facilities and observed for evidence of disease for 5 d prior to initiating the study. Rats were implanted subcutaneously (sc) with sterile transponders (IPTT-300; BioMedic Data Systems Inc., Seaford, DE, USA) for animal identification and fed a standard rodent diet *ad libitum* until the dietary variable was implemented. Throughout the study, the rats were pair-housed in polycarbonate cages with *ad libitum* water in fully AAALAC accredited facilities under a 12-h light/dark cycle (lights on at 0600) with temperature ($21 \pm 2\ ^{\circ}C$) and relative humidity ($50 \pm 10\%$) controlled.

2.2. Apparatus

Active avoidance training was conducted in eight commercially available chambers (Gemini System, San Diego Instruments Inc., San Diego, CA, USA). Each avoidance chamber had exterior dimensions of 66 cm (W) × 33 cm (D) × 44 cm (H) and was composed of two compartments each measuring 24 cm (W) × 20 cm (D) × 20 cm (H). Each compartment was equipped with a cue light centered on the distal wall and 12.5 cm above the grid floor, a house light centered over the compartment, and a speaker located in the front distal corner of the ceiling. Each compartment was equipped with eight infrared emitter-receiver