Nutrition 32 (2016) 877–883

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

The effects of diet on the severity of central nervous system disease: One part of lab-to-lab variability

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ARTICLE INFO

Article history:
Received 12 May 2015
Accepted 10 January 2016

Keywords:
Experimental autoimmune encephalomyelitis
Theiler's murine encephalomyelitis virus
Seizures
Diet

ABSTRACT

Objective: Many things can impact the reproducibility of results from laboratory to laboratory. For example, food from various sources can vary markedly in composition. We examined the effects of two different food sources, the Teklad Global Soy Protein-Free Extruded Rodent Diet (irradiated diet) and the Teklad Sterilizable Rodent Diet (autoclaved diet), on central nervous system disease.
Methods: Three preclinical models for human disease: Two different experimental autoimmune encephalomyelitis models (multiple sclerosis) and the Theiler's murine encephalomyelitis virus-induced seizure model (epilepsy), were examined for the effects of two different food sources on disease.
Results: We found that mice fed the irradiated diet had more severe clinical disease and enhanced seizures compared with animals provided the autoclaved diet in both experimental autoimmune encephalomyelitis models examined and in the Theiler's murine encephalomyelitis virus-induced seizure model, respectively.
Conclusions: Therefore, just altering the source of food (lab chow) can have marked effects on disease severity and outcome.

Introduction

The effects of food and nutrition on the initiation and pathogenesis of neurologic diseases of the central nervous system (CNS) have not been extensively examined. Such variables likely contribute to differences observed among laboratories using similar preclinical models of human disease. Here we examine the effects of two different food sources on Theiler's murine encephalomyelitis virus (TMEV)-induced seizures and two different experimental autoimmune encephalomyelitis (EAE) models.

TMEV, a non-enveloped, positive-sense, single-stranded RNA picornavirus, is a naturally occurring enteric pathogen of the mouse [1,2]. Intracerebral infection of C57 BL/6 mice with the Daniels (DA) strain of TMEV induces acute behavioral seizures in about 50 to 80% of the mice [3,4]. The seizures occur at a frequency of one per mouse per 2 h observation period and typically last 1 to 2 min [3,5]. Seizures start on day 3 post infection (p.i.), peak on day 6 p.i., and resolve by day 10 p.i. The severity of the seizures are typically observed to be a Racine scale seizure score 3 (forelimb clonus) and above (score 4, rearing; score 5, rearing and falling) [3,6]. Following a latent period many of these animals go on to develop epilepsy [7]. The TMEV-induced seizure model is a new animal model [3] that is currently being examined by just a few labs [8–11]. Therefore, lab-to-lab variability has not been examined. However, we noticed variability in seizure susceptibility upon change of animal facilities in conjunction with a change in diet.

EAE is a commonly used animal model of multiple sclerosis [12]. EAE can be induced in SJL/J or C57 BL/6 mice through inoculation with CNS myelin peptides, from myelin proteolipid protein (PLP) or myelin oligodendrocyte glycoprotein (MOG),

We would like to thank Mitchell A. Wilson, Kelley M. Ingram and Samantha P. Duzy, for excellent technical assistance, F. Lynn Sonderegger, PhD, for many helpful discussions and Daniel J. Harper for the outstanding preparation of the manuscript. This work was supported by NIH T32 AI055434 (MFC), 5 R01 NS065714 (RSF), and 1 R01 NS082102 (RSF). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Conception and design: MFC, JLR, RSF. Generation, collection, and analysis of data: JEL, DJD, JTS. Manuscript drafting: JEL, MFC, RSF. Manuscript approval: JEL, DJD, JTS, MFC, JLR, RSF.

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http://dx.doi.org/10.1016/j.nut.2016.01.009
0899-9007/

Table 1
Nutrient composition of irradiated and autoclaved diets

Ingredient	Irradiated	Autoclaved	Unit of measure
Macronutrients			
Crude protein	19.1	24.5	%
Fat	6.5 (acid hydrolysis)	4.3 (ether extract)	%
Carbohydrate (available)	47.0	38.2	%
Crude fiber	2.7	3.7	%
Neutral detergent fiber	12.3	14.0	%
Ash	5.1	8.0	%
Energy density	3.1 (13.0)	2.9 (12.1)	kcal/g (kJ/g)
Calories from protein	24	34	%
Calories from fat	16	13	%
Calories from carbohydrates	60	53	%
Minerals			
Calcium	0.9	1.2	%
Phosphorus	0.7	1.0	%
Non-phytate Phosphorus	0.4	0.7	%
Sodium	0.1	0.3	%
Potassium	0.4	0.9	%
Chloride	**0.4**	**0.4**	%
Magnesium	**0.2**	**0.2**	%
Zinc	60	84	mg/kg
Manganese	80	106	mg/kg
Copper	15	23	mg/kg
Iodine	6	2	mg/kg
Iron	200	300	mg/kg
Selenium	0.23	0.35	mg/kg
Amino Acids			
Aspartic acid	1.1	2.3	%
Glutamic acid	3.5	4.2	%
Alanine	1.2	1.4	%
Glycine	0.7	1.3	%
Threonine	0.6	0.9	%
Proline	1.9	1.7	%
Serine	0.9	1.6	%
Leucine	2.3	1.9	%
Isoleucine	0.7	1.0	%
Valine	0.9	1.2	%
Phenylalanine	1.0	1.1	%
Tyrosine	0.5	0.9	%
Methionine	0.5	0.4	%
Cystine	0.3	0.4	%
Lysine	0.9	1.4	%
Histidine	0.4	0.6	%
Arginine	0.8	1.6	%
Tryptophan	0.2	0.3	%
Vitamins			
Vitamin A	15.0	37.0	IU/g
Vitamin D_3	1.5	3.0	IU/g
Vitamin E	110	170	IU/kg
Vitamin K_3 (menadione)	50	100	mg/kg
Vitamin B_1 (thiamin)	17	120	mg/kg
Vitamin B_2 (riboflavin)	15	17	mg/kg
Niacin (nicotinic acid)	75	129	mg/kg
Vitamin B_6 (pyridoxine)	18	21	mg/kg
Pantothenic acid	33	107	mg/kg
Vitamin B_{12} (cyanocobalamin)	0.08	0.13	mg/kg
Biotin	0.40	0.93	mg/kg
Folate	4	8	mg/kg
Choline	1200	2380	mg/kg
Fatty Acids			
C 16:0 palmitic	0.6	0.7	%
C 18:0 stearic	**0.1**	**0.1**	%
C 18:1 ω9 oleic	1.1	0.8	%
C 18:2 ω6 linoleic	2.6	1.8	%
C 18:3 ω3 linolenic	0.3	0.1	%
Total saturated	0.8	0.9	%
Total monounsaturated	1.1	1.0	%
Total polyunsaturated	2.9	1.9	%
Other			
Cholesterol	–	50	mg/kg

Bold indicates ingredients found in both diets at the exact same concentrations.

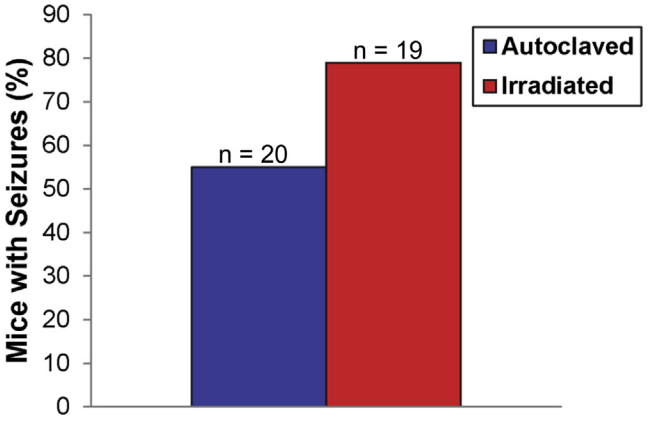

Fig. 1. Seizure frequency (Racine scale stages 3–5) in mice maintained on either irradiated or autoclaved diet. DA-infected C57 BL/6 mice were monitored for seizures through day 21 p.i. The total number of mice infected (n) is shown. The percentages of mice with seizures were calculated: (number of mice with seizures/total number of mice infected) × 100.

emulsified in complete Freund's adjuvant. The clinical course of EAE varies depending on the particular myelin peptide and strain of mouse [12]. Weight loss, ataxia, incontinence, and flaccid or spastic hind limb paralysis are common clinical signs of EAE [13]. Researchers have noticed that variability in day of onset, severity, and incidence of EAE can occur within an experimental group, from experiment to experiment, and from lab-to-lab, even when using the same peptide and reagents in genetically identical mice [14–16]. The reasons for the variability are unknown, but differences in diet may be one factor accounting for some of the lab-to-lab variability.

We performed a TMEV-induced seizure experiment and two EAE experiments, where disease was induced with peptides from either PLP or MOG, to examine the effects of two different food sources, the Teklad Global Soy Protein-Free Extruded Rodent Diet (irradiated diet) and the Teklad Sterilizable Rodent Diet (autoclaved diet) (Envigo, Cambridgeshire, UK), on CNS disease.

Methods and materials

Animals

Four-week-old, male C57 BL/6 and female SJL/J mice were obtained from the Jackson Laboratory. All animal experiments were reviewed and approved by the University of Utah Institutional Animal Care and Use Committee and conducted in accordance with the guidelines prepared by the Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animals Resources, and National Research Council. Animals were maintained on a 12 h light/12 h dark cycle at 72°F. All efforts were made to minimize suffering. For each of the three experiments, mice were randomly divided into two groups: One received Teklad Global Soy Protein-Free Extruded Rodent Diet (irradiated diet) (cat. #2920 X; Harlan Laboratories) and the other received Teklad Sterilizable Rodent Diet (autoclaved diet) (cat. #8656; Harlan Laboratories). Food and water was available ad libitum. A comparison of the nutrient composition of the two varieties

Table 2
Seizures, Racine Scale–maximum severity of disease

Diet	Number of mice	% Of mice with maximum clinical stages				
		1	2	3	4	5
Autoclaved	11	0	0	0	9	91
Irradiated	15	0	0	0	0	100