Toxicology and Applied Pharmacology 243 (2010) 332–339

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Chronic ingestion of 2-deoxy-D-glucose induces cardiac vacuolization and increases mortality in rats

Robin K. Minor [a], Daniel L. Smith Jr. [b], Alex M. Sossong [a], Susmita Kaushik [c], Suresh Poosala [d], Edward L. Spangler [a], George S. Roth [e], Mark Lane [f], David B. Allison [g], Rafael de Cabo [a], Donald K. Ingram [h,*], Julie A. Mattison [a,*]

[a] Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA
[b] Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[c] Departments of Developmental and Molecular Biology and of Anatomy and Structural Biology, Marion Bessin Liver Research Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[d] Research Resources Branch, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA
[e] GeroScience, Inc., Pylesville, MD 21132, USA
[f] Medimmune, LLC, Gaithersburg, MD 20878, USA
[g] Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[h] Nutritional Neuroscience and Aging Laboratory, Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA 70808, USA

ARTICLE INFO

Article history:
Received 8 July 2009
Revised 14 November 2009
Accepted 30 November 2009
Available online 22 December 2009

Keywords:
Deoxyglucose
Calorie restriction
Lifespan
Mortality
Cardiac vacuolarization

ABSTRACT

Calorie restriction (CR), the purposeful reduction of energy intake with maintenance of adequate micronutrient intake, is well known to extend the lifespan of laboratory animals. Compounds like 2-deoxy-D-glucose (2DG) that can recapitulate the metabolic effects of CR are of great interest for their potential to extend lifespan. 2DG treatment has been shown to have potential therapeutic benefits for treating cancer and seizures. 2DG has also recapitulated some hallmarks of the CR phenotype including reduced body temperature and circulating insulin in short-term rodent trials, but one chronic feeding study in rats found toxic effects. The present studies were performed to further explore the long-term effects of 2DG *in vivo*. First we demonstrate that 2DG increases mortality of male Fischer-344 rats. Increased incidence of pheochromocytoma in the adrenal medulla was also noted in the 2DG treated rats. We reconfirm the cardiotoxicity of 2DG in a 6-week follow-up study evaluating male Brown Norway rats and a natural form of 2DG in addition to again examining effects in Fischer-344 rats and the original synthetic 2DG. High levels of both 2DG sources reduced weight gain secondary to reduced food intake in both strains. Histopathological analysis of the hearts revealed increasing vacuolarization of cardiac myocytes with dose, and tissue staining revealed the vacuoles were free of both glycogen and lipid. We did, however, observe higher expression of both cathepsin D and LC3 in the hearts of 2DG-treated rats which indicates an increase in autophagic flux. Although a remarkable CR-like phenotype can be reproduced with 2DG treatment, the ultimate toxicity of 2DG seriously challenges 2DG as a potential CR mimetic in mammals and also raises concerns about other therapeutic applications of the compound.

Published by Elsevier Inc.

Introduction

An expansive body of research has shown that calorie restriction (CR) can produce numerous beneficial health and functional effects in a diverse array of organisms (Masoro, 2002). CR involves long-term reduced intake of a nutritious diet about 20–50% below the *ad libitum*, or unrestricted, level of consumption and health benefits ranging from improved cardiovascular health to reduced tumorigenesis have been demonstrated in laboratory rodents on such regimens (Guo et al., 2002; Hursting et al., 2003). A few well-controlled clinical trials as well as studies of humans voluntarily practicing a CR lifestyle are beginning to indicate that CR could also have beneficial effects on human health (Weiss et al., 2006; Civitarese et al., 2007; Fontana et al., 2007; Larson-Meyer et al., 2008; Lefevre et al., 2009). In addition to its myriad health benefits, CR produces robust increases in lifespan in many species and has been predicted to be able to confer at least a modest effect on human lifespan (Ingram et al., 2006a; Everitt and Le Couteur, 2007). However, concerns about the practicality of lifelong

* Corresponding authors. J. Mattison is to be contacted at Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA. Fax: +1 301 480 0504. D.K. Ingram, Nutritional Neuroscience and Aging Laboratory, Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA 70808, USA. Fax: +1 225 763 0261.
E-mail addresses: donald.ingram@pbrc.edu (D.K. Ingram), mattisonj@mail.nih.gov (J.A. Mattison).

0041-008X/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.taap.2009.11.025

human CR including issues of compliance and potential negative impact to quality of life have prompted the search for CR mimetics (CRM). Whether dietary constituents or pharmacological products, CRM would be selected by their ability to stimulate signaling pathways involved in CR and reproduce the benefits of CR without necessitating a chronic reduction in food consumption (Ingram et al., 2006b).

One aspect of physiology that is profoundly altered by CR and therefore may be a target for CRM research is energy metabolism. CR exerts several metabolic effects indicating a shift in energy processing. In general, CR induces a shift from carbohydrate to lipid metabolism (Jensen et al., 1987). In particular, CR reduces circulating blood glucose and insulin while upregulating pathways associated with glucose synthesis (e.g., gluconeogenesis and lipid and amino acid catabolism) (Hagopian et al., 2003) and downregulating pathways associated with glucose catabolism (e.g., glycolysis and the Krebs cycle) (Feuers et al., 1989). Given these observations, it is reasonable to hypothesize that treatments reducing glycolytic activity independent of reduced caloric intake may trigger CR-like responses and qualify as potential CRMs (Hipkiss, 2007).

The first proposed CRM was 2-deoxy-D-glucose (2DG) (Lane et al., 1998). 2DG is an analog of glucose with a hydrogen atom instead of a hydroxyl group at its C2 position, and it can be absorbed by the intestines, circulated, and taken up by cells via glucose transport mechanisms. Within cells 2DG is converted to 2-deoxyglucose 6-phosphate by hexokinase in the first step of glycolysis. This is as far as glycolysis with 2DG can proceed, as 2-deoxyglucose 6-phosphate inhibits phosphoglucose isomerase at the second step of glycolysis and impedes processing of glucose to fructose 6-phosphate. Because of this inhibition, 2DG has been used for over 50 years as an experimental tool to evaluate glycolysis in organisms from bacteria to humans (Wick et al., 1955; Brown, 1962). Additionally, fluorodeoxyglucose (FDG), where a hydrogen atom on 2DG is replaced with the positron-emitting isotope fluorine-18, is routinely used in medical imaging (PET scanning) in living systems (Basu and Alavi, 2008).

2DG is a potential CRM because its effects on metabolism may upregulate stress responses that protect against many aging processes to enhance longevity without a reduction in food intake. Supporting data for this hypothesis include studies where 2DG has been shown to recapitulate key hallmarks of CR such as reduced body temperature (Ingram et al., 2006b), increased torpor (Dark et al., 1994), reduced heart rate (Wan et al., 2003), and reduced circulating levels of glucose and insulin (Lane et al., 1998; Wan et al., 2003; Wan et al., 2004). 2DG has also been shown to confer functional benefits that parallel those seen with CR including inhibition of tumor growth (Gridley et al., 1985; Zhu et al., 2005) and increased stress resistance to neurotoxins and cold shock (Duan and Mattson, 1999; Yu and Mattson, 1999; Guo and Mattson 2000; Wan et al., 2004). 2DG has also shown potential benefits in the treatment of seizures in rodents (Stafstrom et al., 2009). While the downstream mechanisms elicited by 2DG-induced glycolytic inhibition have not been fully established, animals treated with 2DG have been observed to show increased levels of glucocorticoids (Weidenfeld et al., 1994; Wan et al., 2004) and heat shock proteins (Wan et al., 2003)—again paralleling effects of CR (Sabatino et al., 1991; Selsby et al., 2005).

Ultimately the validation of a potential CRM depends on its ability to extend lifespan. To this end, a recent study in nematodes (*Caenorhabditis elegans*) demonstrated that 2DG could extend both mean and maximum lifespan (Schulz et al., 2007). While longevity studies in lower organisms are commonly conducted as a screen prior to testing in mammals, it is important not to extrapolate such findings to higher organisms without further investigation. Indeed, an earlier pilot evaluation of 2DG as a CRM found that when young male Fischer-344 (F344) rats were fed diets supplemented with 2DG for 12 weeks, some doses resulted in deaths with evidence of cardiac pathology (Lane et al., 1998). Given that other studies have reported favorable preliminary toxicity results with 2DG (Stafstrom et al., 2008), and the promising findings regarding the potential efficacy of 2DG as a CRM, the current studies were conducted to further evaluate chronic consumption of 2DG in rats. First, we conducted a survival study in male F344 rats and found evidence of cardiotoxicity at two doses and increased mortality at a dose of 0.2 g/kg (0.4% by weight in the diet). To rule out source-specific and strain-specific effects, a second study evaluated 2DG from two different sources in both Brown Norway (BN) and F344 rats. This study further confirmed our initial results with additional evidence of cardiac toxicity occurring in 2DG-treated rats of both strains and from both sources.

Materials and methods

Materials

Animals. In the lifespan study male F344 rats were obtained from Charles River Laboratories (Raleigh, NC). Male F344 and BN rats were obtained for the second and third study from Harlan Sprague–Dawley (Indianapolis, IN).

2DG. For the first study, 2DG was obtained from Sigma/Aldrich (St. Louis, MO; 2DG-S). For the second study, 2DG was obtained again from Sigma/Aldrich and also from a second source, Regent Chemical Products (Quebec, Canada; 2DG-R). The two sources differ in their production methods; 2DG-S is synthetically derived while 2DG-R is naturally derived from the exoskeletons of crayfish. For the third study, 2DG was obtained from Sigma/Aldrich.

Methods

Animals. For the survival study rats were individually housed in stainless steel cages with *ad libitum* access to a standard chow diet (Teklad NTP-2000, Harlan Teklad, Madison, WI) and tap water until 6 months of age. This study was conducted under contract at TherImmune Research Corporation, Gaithersburg, MD. The room was maintained at 65–75 °F with 30–70% relative humidity and a 12:12 h light:dark cycle. At 6 months of age, the animals were randomized into three diet groups: (1) Control ($n=45$); (2) 0.25% 2DG ($n=45$); and (3) 0.4% 2DG ($n=45$). All groups were granted *ad libitum* access to their diets. 2DG (Grade II, CAS #154-17-6; ≥98% pure by gas chromatography) was mixed with the diet at the indicated dry weight percentages (0.25% or 0.40%). Body weight and food intake were measured weekly during weeks 1–14 and every 4 weeks thereafter. Core body temperature (CBT) was measured by thermistor probe or subcutaneous implant (BioMedic Data System, Inc. Monitoring System) when body weights were collected. Blood glucose and insulin levels (collected by orbital sinus puncture under CO_2/O_2 anesthesia) were acquired during weeks 1, 13, and 39 at 06:00 h (±1:00 h).

In the second study 3-month old male F344 rats ($n=44$, initial weight range = 242–304 g) and BN ($n=44$, initial weight range = 201–285 g) rats were housed individually in clear plastic cages on a 12:12 h light:dark schedule at the Holabird NIA/NIH research facility in Baltimore, MD, with food and water available *ad libitum*. After a 6-day habituation period, the rats were weighed and randomly assigned to dietary treatment groups whereby 6 each of the F344 and BN rats were designated to receive NIH-31 standard rodent chow (Harlan Teklad, Indianapolis, IN, USA) supplemented by dry weight with 0.6%, 0.2% or 0.04% Sigma 2DG. Six more animals from each strain were assigned to equivalent dose levels of the diet supplemented with the Regent 2DG, and eight animals of each type were allocated as control animals to receive unsupplemented NIH-31 chow. Throughout the duration of the study, all rats had *ad libitum* access to weighed amounts of their prescribed diet and water. At the