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Research article

Deletion of GCN2 affects whole body and tissue response to asparaginase

Rana Jaber Tarish Al-Baghdadi[1] Tracy G. Anthony[2]

[1-]*Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.*
[2-]*Department of Nutritional Sciences, New Jersey, Institute for Food, Nutrition and Health, Rutgers Cancer Institute of New Jersey, Rutgers, The State University of New Jersey, New Jersey; USA.*
Corresponding Author Email: Rana.tarish@qu.edu.iq

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Abstract

Asparaginase (ASNase) treatment results in the synthesis of some factors such as activating-transcription-factor-4 (ATF4). The eIF2-ATF4 pathway is essential for cell survival during amino acid starvation conditions. This requires the eukaryotic-initiation-factor-2 (eIF2) kinase called general control nonderepressible 2 kinase (GCN2). Our objective and hypothesis are addressed in our aim to describe the liver response to ASNase in mice deleted for Gcn2 and Atf4 and either treated with phosphate buffered saline (PBS) or asparaginase (ASNase) for 8 days. We found that deletion of Gcn2 and/ or Atf4 affect body weight and fat and lean content. The results showed that $Atf4^{-/-}$ mice had significantly less fat mass than WT and $Gcn2^{-/-}$ mice even before the startup of the study. Also, WT mice experienced minimal change in body weight and body composition, but $Atf4^{-/-}$ and $Gcn2^{-/-}$ mice both lost substantial amounts of body weight and body fat without altering lean mass. Moreover, $Gcn2^{-/-}$ mice showed high significant increment in liver and pancreas weight when treated with ASNase compared to the other groups. Lastly, spleen weight was significantly lower in all treated groups, except Atf4 null mice, compared to their own control groups. In conclusion, this research provides insight into the importance of the genetic background of patients in choosing ASNase as a treatment.

Keywords: Asparaginase, ATF4, GCN2, eIF2, AAR

Introduction

One of the frequent causes of death in those under age 20 is acute lymphoblastic leukemia (ALL). Asparaginase (ASNase) is a drug that is used to treat ALL (1), and it is widely recommended as it improves remission induction rate (2). Nevertheless, ASNase has many deleterious side effects such as liver failure (3). ASNase works to decrease some amino acids causing amino acid deprivation (4). It was found that ASNase reduces liver protein synthesis by increasing phosphorylation of eukaryotic initiation translation factor 2 (eIF2) (5) via the general control nonderepressible 2 kinase (GCN2) (6). Phosphorylation of eIF2 by GCN2 dampens global protein synthesis rates while simultaneously promoting gene-specific translation of protein factors. This GCN2-eIF2-ATF4-driven adaptive mechanism is described previously (7). ATF4 is described in many research articles as a master regulator of metabolism in response to many cellular stressors (8). It was found to play critical role in amino acid deficiency as a member of the GCN2-eIF2-ATF4 AAR pathway (9). The goal of this project is to determine whether the role

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of GCN2 is fully mediated by ATF4 or shared with other factors. This will help to better identify the causes of drug toxicity and perhaps reveal new treatment and toxicity prevention approaches.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 225

Animals

Mice from Jackson Laboratories, Bar Harbor, ME were used in these experiments. All mice were individually housed in clear plastic cages with corncob bedding and freely provided commercial diet. Protocols of animal use were according to Rutgers (IACUC). Animals were bred and maintained at the Rutgers Bartlett animal care facility.

Design

Mice were administered once-daily intraperitoneally with 3 IU/g BW of ASNase (Deerfield, Illinois) after the start of the light cycle as previously detailed (10). The doses are based on our previous work as described in (5) and enzyme activity was determined prior to injection by the Nesslerization technique by detecting the level of ammonia as was described (4, 9).

Sample Collection.

Body weight was recorded daily and at the point of euthanasia. Mice from all treatment groups were euthanized by decapitation ~8 h after the eighth daily injection. Tissues including liver, pancreas, and spleen were rapidly dissected and rinsed in ice-cold PBS, blotted and weighed. The ethics protocols regarding animal care and use of Rutgers University/ NJ/ USA (IACUC) was followed during this study.

Body composition

Body composition of live mice prior first injection and before euthanasia was determined by magnetic resonance using an EchoMRI instrument (Echo Medical Systems, Houston, TX, USA).

Statistics

Two-way ANOVA was employed to do statistics for this study with $P<0.05$. This was also followed by Tukey's post-hoc test.

Results

The results showed that Atf4$^{-/-}$ mice had significantly less fat mass than wild type (WT) and *Gcn2*$^{-/-}$ mice, that is consistent with its reported lean phenotype (Fig. 1A). The study also showed that following the drug treatment, WT mice experienced minimal change in body weight (Fig. 1B) and body composition (Fig. 1C-D), especially at the fat and lean mass levels, but *Atf4*$^{-/-}$ and *Gcn2*$^{-/-}$ mice both lost substantial amounts of body weight and body fat without altering lean mass. Moreover, *Gcn2*$^{-/-}$ mice showed high significant increment in liver weight when treated with ASNase compared to the other groups (Fig. 1E). Similar results were shown when pancreas weight was checked under the treatment (Fig. 1E). Lastly, spleen weight was significantly lower in all treated groups, except *Atf4* null mice, compared to their own control groups as shown in the same figure listed above (Fig. 1E).

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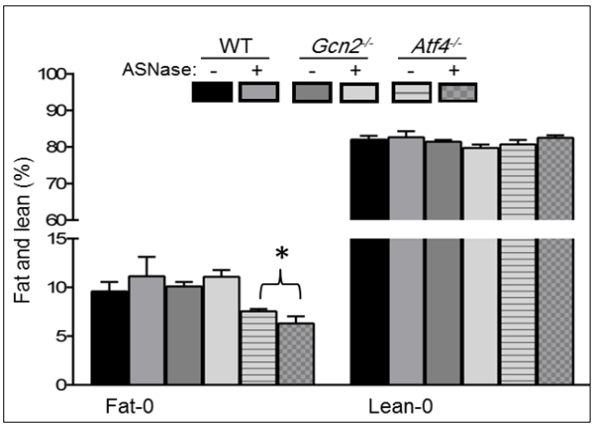

Figure 1-A: Fat and lean percentage of body composition in wild type mice, *Gcn2*$^{-/-}$ mice, and *Atf4*$^{-/-}$mice at day 0 (before the beginning of the experiment). The asterisk refer to presence of statistical differences at P<0.05.

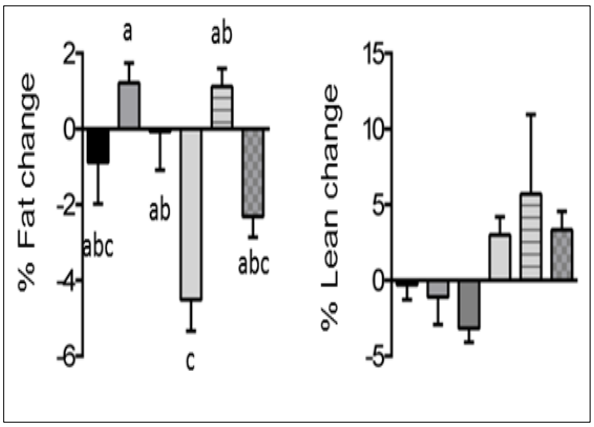

Figure 1-B and C: Fat and lean percentage of body composition in wild type mice, *Gcn2*$^{-/-}$ mice, and *Atf4*$^{-/-}$mice at day 8 of the experiment before the euthanization (last day of the experiment). The different letters refer to the presence of statistical differences at P<0.05

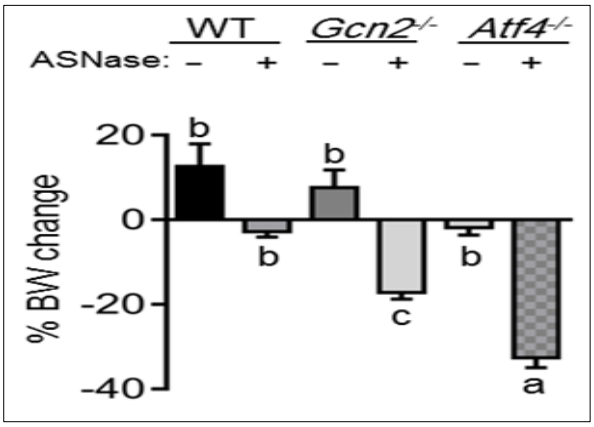

Figure 1-D: Percentage of body weight change in wild type mice, *Gcn2*$^{-/-}$ mice, and *Atf4*$^{-/-}$ The different letters refer to the presence of statistical differences at P<0.05

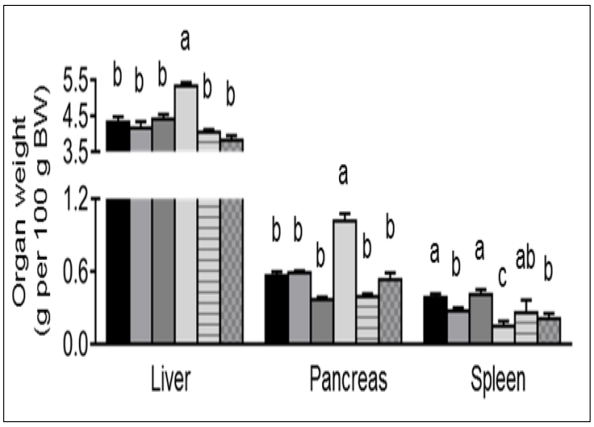

Figure 1-E: Percentage of organs (liver, pancreas, and spleen) weight to body weight in wild type mice, *Gcn2*$^{-/-}$ mice, and *Atf4*$^{-/-}$ .The different letters refer to the presence of statistical differences at P<0.05

Discussion

ATF4 is a master regulator of metabolism and thermogenesis in ways that are for the most part mysterious. This study shows that Atf4$^{-/-}$ and Gcn2$^{-/-}$ mice share somatic but not tissue-specific responses to ASNase; which means they respond almost similarly at the body weight levels but not at the tissue level. We sought to understand the role of ATF4 relative to GCN2 in liver adaptation to ASNase treatment. To accomplish this, we injected ASNase at 3 international units per gram body weight (3 IU/g BW) into WT, Gcn2$^{-/-}$, and Atf4$^{-/-}$ mice once daily for 8 days using phosphate buffered saline (PBS) as a control. Before treatment commenced, *Atf4*$^{-/-}$ mice had significantly less fat mass than WT and *Gcn2*$^{-/-}$ mice, that is consistent with its reported lean phenotype (11). This might be attributed to the global deletion of *Atf4* that increases energy expenditure, resulting in a lean phenotype, and

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compromises bone development (10,11,12,13). On the other hand, liver and pancreas weight show significant increase in *Gcn2* null mice that might be attributed to the ASNase treatment that causes hepatitis as well as pancreatitis as discussed previously (4, 5). While ASNase effect on the spleen was clear in WT and Gcn2 deleted mice, which might be related to the inhibiting effect of ASNase on the immune system (4). In conclusion, for the first time effect of ASNase on *Atf4*$^{-/-}$ animals was reported in the current study and this may mean that those animals behave similar to the *Gcn2*$^{-/-}$ mice under ASNase. Further studies are required to understand asparaginase whole effect on Gcn2 null and Atf4 null mice especially at the level of toxicity.

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