Research article

Deletion of GCN2 affects whole body and tissue response to asparaginase

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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
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 corn cob 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).
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
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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