Exposure Biomarkers in Newborn Dried Blood Spots

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Abstract

Protein adducts in newborn dried blood spots (DBS) offer a means to assess prenatal exposure to chemical toxicants. Here we present a method for measuring 1,4-benzoquinone (1,4-BQ) adducts of hemoglobin (1,4-BQ-Hb) in newborn DBS. 1,4-BQ is a reactive electrophile, with both endogenous and exogenous origins. It is the metabolite of benzene that is thought to be responsible for leukemia in benzene-exposed persons.

In this study, 1,4-BQ-Hb levels were determined in globin from 50 adult DBS and 51 newborn DBS. The mean adduct level for newborns was 3.93 pmol/mg globin (SD=1.41), and the mean adduct level for adults was 1.84 pmol/mg globin (SD=0.63). The difference between the two groups was significant (*t-test, p<0.0001*). Reasons for the elevated adduct levels in newborns are being further explored.

Introduction

Robert Guthrie used newborn DBS in the 1960s for measuring phenylalanine as a biomarker for phenylketonuria [1]. This novel blood collection method fostered a national newborn screening program in the United States, where greater than 95% of newborns from all 50 states are tested for treatable metabolic disorders today [2]. Although analytical and immunochemical assays have been developed to measure proteins and peptides in DBS for a host of disorders [2], no one, to our knowledge , has reported an assay for measuring protein adducts in DBS as biomarkers of exposure to chemical toxicants.

Protein adducts in newborn DBS offer a means to measure prenatal exposures to chemical carcinogens integrated over the lifetime of the protein. Specifically, adducts of hemoglobin are of interest because hemoglobin is the most abundant protein in blood and has a residence time of about 63 days [3]. This allows for a substantial time window to evaluate exposures during a highly vulnerable stage of human development.

As a proof of concept , this study describes a method to measure 1,4-BQ-Hb in DBS from newborns and adults. These adducts were chosen because they are abundant in human blood, due to dietary and endogenous sources of 1,4-BQ [4], and because exposure to 1,4-BQ is a strongly suspected risk factor for leukemia [5].

Methods

DBS Samples

Sample Type	n	Description
Newborn DBS	51	Heel lancet DBS obtained from the North Carolina State Laboratory of Public Health
Adult Venous DBS (Adult V)	50	Frozen whole blood DBS from human volunteers spotted in 50 µl aliquots
Adult Finger Lancet DBS (Adult FL)	10	Freshly collected finger lancet DBS collected from adult volunteers
Sigma Globin Controls	20	Sigma-Aldrich human globin- not spotted onto specimen collection paper

Protein Isolation (Figure 1)

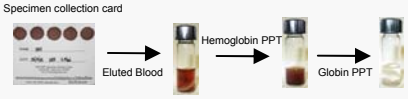


Figure 1 Isolation of Globin from DBS

- Entire DBSs were excised from the specimen collection card
- Proteins were eluted from the DBS with deionized water
- Hemoglobin was selectively precipitated using a mixture of ethanol and water
- Globin was isolated from hemoglobin using cold acidic acetone precipitation, with 96% purity (Figure 2)

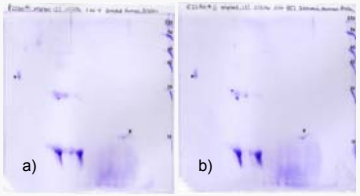


Figure 2 2D-gel comparison of a) conventionally isolated globin and b) DBS globin

Measurement of 1,4-BQ-Hb (Figure 3)

- 3 mg aliquots of Gb were spiked with 5 µg of isotopically labeled protein-bound internal standard [$^2H_3$]1,4-BQ-Hb
- Samples were reacted with methanesulfonic acid and trifluoroacetic anhydride
- Released trifluorothioacetate (HQ-S-TFA) was quantified using GC/MS in negative-ion-chemical-ionization mode [6]

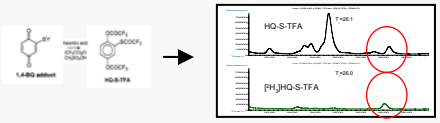
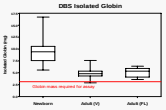


Figure 3 Measurement of 1,4-BQ adducts of hemoglobin

Results



Isolated Globin

Adults: 5.0 mg (SD=0.9)

Newborn: 9.4 mg (SD 2.4)

* Higher isolated globin masses in newborn DBS was attributed to both larger blood volumes in the newborn DBS and higher hemoglobin concentrations in newborns compared to adults

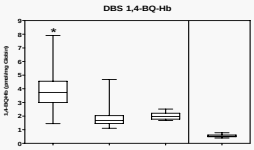
1,4-BQ-Hb

Newborn: 3.93 pmol/mg globin (SD=1.41)

Adults V: 1.84 pmol/mg globin (SD=0.63)

Adults FL: 2.01 pmol/mg globin (SD=0.26)

*Levels of 1,4-BQ-HB in adult V and adult FL DBS were not significantly different (t-test, p=0.41)



- Using our DBS protein isolation protocol, sufficient globin was isolated, with ~96% purity, to measure 1,4-BQ-Hb in all samples
- Levels 1,4-BQ-Hb in newborn DBS were significantly higher than adult DBS (multiple t-tests with Bonferroni correction)

Conclusions

Measurement of protein adducts in newborn DBS offers a non-invasive method to assess prenatal exposure to chemical toxicants during a highly susceptible stage of human development. The use of newborn DBS opens the possibility for retrospective molecular epidemiology, where archived DBS could be exploited for case control studies.

Levels of 1,4-BQ-Hb in newborn DBS were found to be significantly greater than adult DBS levels. Since newborn and adults have different variants of hemoglobin (HbF in newborns versus HbA in adults), higher adduct levels in newborns may be the result of the fetal hemoglobin variant being more reactive with 1,4-BQ. We are currently investigating this hypothesis.

References

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