Ionizing Radiation and Lucanthone Enhance the IgG Content of Burkitt’s Lymphoma Cells

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Abstract

Ionizing radiation or lucanthone treatment of CRL-1647, Burkitt’s lymphoma cells, increased their content of IgG three fold. Radiation induced increases in IgG cell content relative to IgM persisted for several cell generations. IgM remained the predominant immunoglobulin after either treatment. Elevated AID, activation induced cytidine deaminase, was not found after 5 Gy.

However, 8 µM lucanthone for 48 h increased cellular AID cell content three fold and, as previous results showed, progeny of lucanthone survivors exhibited increased levels of AID many generations later.

Keywords: Lucanthone; Ionizing radiation; Class switch recombination (CSR); Activation induced deoxycytidine deaminase (AID)

Introduction

Lucanthone, a thiaxanthenone, once widely used to treat schistosomiasis, induced 3.6 fold increases in immune globulin G (IgG) relative to immune globulin M (IgM) in CRL-1647 Burkitt’s lymphoma cells [1]. This increase persisted for many generations and was accompanied by 7 fold increases in cellular activation induced cytidine deaminase (AID) [1]. AID is a key factor in Ig class switching from IgM to IgG.

Here we examined the effects of ionizing radiation on the ratio of IgG/(IgG+IgM) (Figure 1) and the content of AID in CRL-1647 cells. Lucanthone increased both by 48 h (Figures 1 and 2).

The novel results with ionizing radiation described later raise general questions about the mechanisms responsible for changes in IgG/(IgG+IgM). AID seems not alone in altering it. While class switch recombination by AID is being very actively pursued, other mechanisms, induced by clinically available tools, deserve attention.

Materials and Methods

Cells

CRL-1647 Burkitt human lymphoma cells were purchased from American Type Culture Collection (ATCC), Manassas, VA 20108. They were grown in suspension at 37ºC in Roswell Park Memorial Institute 1640 medium with 10% fetal bovine serum in 8% CO₂ in a humidified atmosphere. The cell culture doubling time was 24 hours. Media and sera were from ATCC.

Cell lysates

Cells were sedimented from phosphate buffered saline without Ca⁺⁺ or Mg⁺⁺, resuspended in lysis buffer with 10 µM Aprotinin and sonicated with 20 one-second strokes, leaving 1%-2% unbroken cells. Lysates of 10⁵ to 10⁶ cells that were clarified by centrifugation at 15,500 g for 12 min contained approximately 1 µg/µl of protein.

Western blots

For most experiments, 7 cm minigels, purchased from BioRad Laboratories, Los Angeles, CA were used. Buffer without SDS or methanol, containing 25 mM Tris, pH 8.3 and 192 mM glycine were used for gel electrophoresis and Western blot transfer.

Detection of IgM and IgG in cell lysates was made in Western blots.

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Received August 31, 2018; Accepted September 14, 2018; Published September 19, 2018

Citation: Bases R, Lekhraj R (2018) Ionizing Radiation and Lucanthone Enhance the IgG Content of Burkitt’s Lymphoma Cells. J Bioanal Biomed 10:105-107. doi:10.4172/1948-593X.1000216

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using goat anti human IgG precoupled to horseradish peroxidase (SC 2453) (Santa Cruz Biotechnology, Inc, Santa Cruz California, 95060).

This antibody reacts with IgM and more strongly with IgG. An advantage is that both IgG and IgM can be determined in the same cell lysate aliquot in the same gel lane. Quantitation is made by reference to immunodensities of reference standards in the gel. To characterize the relative abundance of IgG and IgM in an aliquot, relative immunodensities were determined: IgG/(IgM+IgG). Lucanthone increased cellular AID content as well. Ionizing radiation induced the IgG increase with little or no participation by AID, suggesting a role for other enzyme activities. AID due to lucanthone may be supplementary to the radiation induced activity. Radiation might be inducing a more primitive APOBEC cytidine deaminase [2].

Lucanthone's action in inducing abasic cites in cell DNA [3] and DNA strand breaks [4] likely caused a different spectrum of strand break species than encountered after radiation. DNA nicks separated by 250 nucleotides on opposite strands can strongly mediate class switch recombination by AID [5]. Double strand break response factors influence end joining features of IgH class switch [6].

DNA strand species determinants of the AID response by lucanthone and APOBEC cytidine deaminases after radiation are as yet unknown.

Lucanthone was formerly used to safely treat schistosomiasis in hundreds of thousands of patients [7]. More recently, we found that lucanthone was a clinically useful adjuvant to radiation therapy in the treatment of brain metastases [8]. Patient serum levels of 8 µM, the concentration used in the present study, were maintained for several weeks without incident, when care was taken to avoid interference from other medications.

Roles for lucanthone and radiation in clinical macroglobulinemias are worth considering. Such treatment might restore enhanced levels of IgG, consistent with in vitro results described here.

**Author Contributions**

- Conception and design: Bases R.
- **AID, IgG and IgM assays:** Bases R and Lekhraj R.
Grant Support

This work was supported by the Rome Sisters Foundation for Cancer Research, by grants to R. Bases.

References

1. Bases R, Lekhraj R (2017) Enhanced content of IgG in Burkitt’s lymphoma cells after treatment with the topoisomerase II inhibitor, lucanthone. J Bioanal Biomed 9: 186-193.
2. Liu MC, Liao WY, Buckley KM, Yang SY, Rast JP, et al. (2018) AID/APOBEC-like cytidine deaminases are ancient innate immune mediators in invertebrates. Nat Commun 9: 1948-1959.
3. Mendez F, Goldman JD, Bases RE (2002) Abasic sites in DNA of He La cells induced by lucanthone. Cancer Investigation 20: 983-991.
4. Bases R, Leifer A, Rozycki H, Blake C, Neubort S (1977) Effects of lucanthone on the sedimentation properties of DNA from He La Cells. Cancer Res 37: 2177-2181.
5. Ling AK, Socc Le MX, Chen AY, Hung L, Martin A (2018) Double stranded DNA break polarity skews repair pathway choice during intrachromosomal and interchromosomal recombination. Proc Natl Acad Sci USA 115: 2800-2805.
6. Panchakshari RA, Zhang X, Kumar V, Du Z, Wu P-C, et al. (2018) DNA double strand break response factors influence end-joining features of IgH class switch and general translocation junctions. Proc Natl Acad Sci USA 115: 762-767.
7. Blair DM (1958) Lucanthone Hydrochloride: A Review. Bull World Health Organ 18: 989-1010.
8. Del Rowe JD, Bello J, Milnick R, Sood B, Fillippi C, et al. (1999) Accelerated regression of brain metastases in patients receiving whole brain radiation and the topoisomerase II inhibitor lucanthone. Int J Radiation Oncol Biol Phys 43: 89-93.