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Antioxidant N-acetyl cysteine reduces incidence and multiplicity of lymphoma in *Atm* deficient mice

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ABSTRACT

Hereditary human disorder ataxia telangiectasia (AT) is characterized by an extremely high incidence of lymphoid malignancies, neuromotor dysfunction, immunodeficiency and radiosensitivity. Cells from AT patients show genetic instability and a continuous state of oxidative stress. We examined the effect of long-term dietary supplementation with the thiol-containing antioxidant, N-acetyl-L-cysteine (NAC), on survival and cancer formation in *Atm* (AT-mutated) deficient mice, used as an animal model of AT. NAC was chosen because it is well-tolerated in animals and humans. It can be used by the oral route and for long-term at high concentrations. In addition, NAC suppresses carcinogenesis-associated biological markers in *Atm* deficient mice, such as DNA deletions and oxidative DNA damage (R. Reliene, E. Fischer, R.H. Schiestl, Effect of N-acetyl cysteine on oxidative DNA damage and the frequency of DNA deletions in atm-deficient mice, Cancer Res. 64 (2004) 5148–5153). In this study, NAC significantly increased the lifespan and reduced both the incidence and multiplicity of lymphoma in *Atm* deficient mice. The life span increased from 50 to 68 weeks and the incidence of lymphoma decreased by two-fold (76.5% versus 37.5%). Moreover, in mice with lymphoma, multiplicity of tumors decreased from 4.6 to 2.8 tumors per mouse. Thus, dietary supplementation with NAC may turn out to be protective against lymphomagenesis in AT patients.

1. Introduction

Ataxia telangiectasia (AT) is a hereditary human disorder characterized by a wide range of clinical manifestations, which include progressive neuromotor dysfunction, oculocutaneous telangiectasias, immunodeficiency, growth retardation, infertility, and very high incidence of cancer [1–3]. About 40% of AT patients develop cancer, mostly leukemia and lymphoma [4–6].

AT results from mutations in the *ATM* (AT-mutated) gene encoding a phosphatidylinositol 3-like kinase [7,8]. ATM is involved in multiple cellular processes including cell cycle checkpoint and repair responses to DNA damage caused by double-stranded breaks (DSBs) [9,10]. The lack of the functional ATM protein results in chromosomal instability (chromosome breaks, chromosome gaps, translocations, aneuploidy) [11–13] and hypersensitivity to DNA DSB-inducing agents, such as ionizing radiation [14,15] and radiomimetic chemicals [16–18]. In addition, ATM deficiency is associated with elevated oxidative stress [19]. Markers of oxidative stress and damage were found in AT patients [20,21], cell lines from these patients [22] and several tissues from *Atm* deficient mice [23–27]. These findings suggested that oxidative stress may be involved in the pathogenesis of AT and provided a basis for investigating the role of antioxidant therapy. In *Atm* deficient mice, NAC suppresses carcinogenesis-associated biological

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markers, such as DNA deletions and oxidative DNA damage [27]. EUK-189 improves performance on a rotarod and appears to retard ($p = 0.08$) development of thymic lymphomas [28]. Tempol when given from weaning but not from fertilization prolongs the latency of thymomas [29].

We conducted a 2-year study, where we examined the effect of chronic dietary supplementation with a well-established nontoxic antioxidant, NAC, on survival and cancer formation in *Atm* deficient mice. NAC, a low molecular weight thiol-containing molecule that is readily taken up by cells, detoxifies reactive electrophiles and reactive oxygen species (ROS), replenishes depleted glutathione (GSH) pools and has anti-carcinogenic properties [30]. NAC has wide applications in the clinical practice and is established as a safe drug even in long-term high dose applications [31–33]. NAC is used for the treatment of respiratory diseases as a mucolytic agent [34–36], for acetaminophen overdose, where it prevents GSH depletion in the liver [37], and is available as an over the counter dietary supplement. We found that NAC significantly increased the lifespan and decreased the incidence and multiplicity of lymphoma in *Atm* deficient mice.

2. Materials and methods

2.1. Mice and NAC treatment

C57BL/6Jp^{un}/p^{un} mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). *Atm* deficient mice were created previously [38] and crossed into a mixed C57BL/6J–129/Sv background. The *ATM* mutation was crossed into the C57BL/6Jp^{un}/p^{un} genetic background by six backcrosses. The resulting p^{un}/p^{un}, $Atm^{+/-}$ mice had a genetic background containing 99.992% of C57BL/6J and were morphologically similar to the parental C57BL/6Jp^{un}/p^{un} strain. Genotyping for *ATM* was carried out by PCR, as described by Liao et al. [39]. Mice were bred in the institutional specific pathogen free animal facility under standard conditions with a 12 h light/dark cycle, and were given a standard diet (Harlan Teklad No 8656) and water ad libitum. Pregnancy was timed by checking for vaginal plugs, with noon of the day of discovery counted as 0.5 days post coitum (dpc). Similarly, the time of birth of a litter was timed with the noon of discovery counted as 0.5 days post partum (dpp).

A treatment group ($Atm^{-/-}$ $n = 22$, $Atm^{+/+}$ $n = 18$) was chronically exposed to NAC (Sigma, St. Louis, Mo, USA) via drinking water from fertilization throughout life, while control groups ($Atm^{-/-}$ $n = 34$, $Atm^{+/+}$ $n = 32$) received regular water. $Atm^{+/-}$ female mice were mated with $Atm^{+/-}$ males and a group of dams were given free access to drinking water supplemented with 40 mM NAC starting at 0.5 dpc then constantly during pregnancy and lactation. NAC supplemented water or regular water was changed once a week. After weaning the treatment group received the same dose of NAC in the drinking water. The average consumption of NAC supplemented water was 4 ml per mouse per day, which yields a dose of 1 g NAC/kg body weight/day, not accounting for spill. The same NAC dose previously given to pregnant mice inhibited formation of oxidative DNA lesions and bulky DNA adducts in wild-type mouse fetuses exposed to cigarette smoke [40] and suppressed DNA deletions and oxidative DNA damage in $Atm^{-/-}$ offspring [27]. The study was terminated after 2 years of observation. Euthanized mice that showed obvious tumors or sickness and dead mice were preserved in 10% buffered formalin (Sigma).

2.2. Histopathological analysis

Formalin preserved animals were presented to the UCLA Division of Laboratory Animal Medicine Diagnostic Service Laboratory for gross and histologic examination. A key to the animal identification and treatment was not provided. A gross examination was performed on all mice used in this study. With the exception of the brain, sections of all thoracic and abdominal organs were examined histologically. The selected tissues were sliced, placed in tissue cassettes and submitted to Pathology Inc. (Torrance, CA, USA) for paraffin embedding and sectioning. Each paraffin block was sectioned to 4 μm and stained with hematoxylin and eosin (H&E). Each tissue was examined by light microscopy and all pathology was recorded. In the cases of lymphoma, if the thymus was the primary tissue involved, the diagnosis of thymic lymphoma was made. Additionally, a diagnosis of leukemia was included when large numbers of neoplastic cells were present within the vascular circulation.

2.3. Statistical analysis

Kaplan–Meier analysis and the log-rank test were performed for survival analysis. χ^2 analysis and ANOVA followed by the post hoc Fisher's LSD test (also called least significant difference method) were performed to compare differences between tumor incidence and tumor multiplicity, respectively. $p < 0.05$ was considered significant.

3. Results

3.1. NAC increased the lifespan of $Atm^{-/-}$ mice

NAC was administered via drinking water from fertilization throughout life. We began the treatment from fertilization because NAC given during embryonic development suppresses DNA deletions and oxidative DNA damage [27] that may contribute to carcinogenesis later in life. Drinking water containing 40 mM NAC was given to $Atm^{+/-}$ female mice after mating with $Atm^{+/-}$ males starting at 0.5 dpc then during pregnancy and lactation. We obtained 13% $Atm^{-/-}$ offspring (55/423) and an average of 5.0 pups per litter from untreated dams, compared to 12.4% $Atm^{-/-}$ offspring (45/364) and 4.6 pups per litter from NAC treated mice. There was no significant reduction in the frequency of $Atm^{-/-}$ offspring or in the number of pups per litter suggesting that NAC was not toxic during embryo and early postnatal development. After weaning, $Atm^{-/-}$ and $Atm^{+/+}$ littermates received NAC supplemented or regular water. Most untreated as well as NAC treated wild-type mice survived until termination of the 2-year study (Fig. 1). The mean survival of untreated $Atm^{-/-}$ mice was 50 weeks and NAC treated $Atm^{-/-}$ mice 68 weeks ($p = 0.03$). Thus, NAC dietary supplementation increased the life span of $Atm^{-/-}$ mice by about 18 weeks (4.5 months).