DEFICIENT REPAIR OF ALKYLIATION DAMAGE OF DNA IN ALZHEIMER'S DISEASE AND AMYOTROPIC LATERAL SCLEROSIS CELLS

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Introduction

The etiology of amyotrophic lateral sclerosis (ALS) is still unknown. There are many pieces of evidence linking ALS, Alzheimer's disease and Parkinson's disease. These include the occasional clinical association of the conditions in the same patient and the same family, the endemic foci of all three diseases, and the presence of changes of one disease in the central nervous system of some patients dying of another of these diseases. There are several indications that alterations in genetic material may exist in these diseases, including decrease in nuclear and nucleolar size[1], alteration in chromatin of neurons[2] and decrease in transcriptionally active chromatin[3]. A possible DNA repair defect was implicated when it was observed that lymphocytes, lymphoblasts and skin fibroblasts from several different neurodegenerative disorders, including Alzheimer's disease and ALS were more sensitive to DNA alkylating agents[4,5]. It has also been reported that lymphocytes from Alzheimer's disease patients have increased sensitivity to bleomycin, 4-nitroquinoline-1-oxide and mitomycin C which produce various other forms of DNA damage[6]. We have further investigated the hypothesis that a DNA repair defect occurs in both of these diseases and offer direct evidence of a DNA repair defect in skin fibroblasts from ALS and Alzheimer's disease patients after exposure to an alkylating agent.

Materials and Methods

We have studied skin fibroblasts from 11 ALS patients, 9 Alzheimer's disease patients (3 of whom are pathologically proven), 13 normal controls, and 3 disease control patients. Two of the biopsy-proven Alzheimer's disease fibroblast lines were obtained from the Institute for Medical Research, Camden, New Jersey. The other autopsy-proven Alzheimer's disease cell line was obtained from a patient at the Medical Center Hospital of Vermont (MCHV) with a progressive dementia and typical brain pathology. The ALS biopsies were obtained at MCHV. The diagnosis of ALS was estab-

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lished on clinical, electrophysiological and histological criteria. The control skin fibroblasts were obtained from spouses and friends of similar age to the patients. The 3 disease controls had a non-Alzheimer's disease dementia, progressive external ophthalmoplegia and Friedreich's ataxia. Skin fibroblasts were maintained under routine tissue culture conditions, and used at approximately the same population doubling level (5-15), since various biochemical parameters, including DNA repair capacity, may change with increasing cell age[7].

A relatively crude estimate of fibroblast sensitivity to methyl methane sulfonate (MMS) was provided by studying cell survival 72 hours after exposure of the cultures to 800 μM MMS for one hour. Unscheduled DNA synthesis[8] and alkaline elution[9] were used to study DNA repair replication in skin fibroblasts following exposure to the alkylating agent, MMS. Assays of unscheduled DNA synthesis using confluent fibroblasts cultures permit the measurement of DNA replication not associated with DNA synthesis occurring before cell division, i.e., DNA repair synthesis. To accomplish this, confluent fibroblasts are treated for one hour with 100μM hydroxyurea followed by a four hour incubation with tritiated thymidine in the presence of 400 μM MMS, which causes single strand breaks and apurinic sites in DNA[10]. Incorporation of the tritium label into DNA was then determined by scintillation counting and the amount of DNA quantitated by measuring fluorescence after the binding of Hoechst dye #33258[11]. In the alkaline elution studies fibroblast cultures were exposed to 200 μM MMS for one hour, and allowed to attempt repair of alkylation damage for three hours before being studied.

The cell survival was expressed as a ratio of the number of cells present at 72 hours in MMS-exposed cultures compared to the number in control cultures. The unscheduled DNA synthesis response was expressed as the UDS repair index, calculated from the equation: uptake of 3H thymidine/μg DNA in MMS-exposed cultures divided by the same uptake in control (non-exposed) cultures. The alkaline elution results were expressed as strand break factor, given by the equation:

\[ SBF = \frac{\log_{10} \left( \frac{T}{U} \right)}{\log_{10} \left( \frac{T_x}{U} \right)} \]

where

\[ T = \%DNA retained on the filter of the treated sample after 10h. of elution; \]
\[ U = \%DNA retained on the filter of the untreated sample after 10h of elution; \]
\[ T_x = \%DNA retained on the filter of the sample treated with 400 rad x-ray after 10 h. of elution. \]

Results

The results of these studies are summarized in Table 1. The normal control and the disease control fibroblasts had similar responses to MMS. However, both the ALS and the Alzheimer's disease fibroblasts showed significantly reduced survival after exposure to 800 μM MMS. ALS and Alzheimer's disease fibroblasts had a reduced UDS repair index during exposure to 400 μM MMS, indicating a reduced amount of DNA repair. In the ALS and Alzheimer's disease fibroblasts, the Strand Break Factor was significantly increased three hours after exposure to 200 μM MMS, indicating a defect in the repair of strand breaks in these cells.