Short report

Studies of cellular radiosensitivity in hereditary disorders of nervous system and muscle

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SUMMARY Skin fibroblasts from patients with familial dysautonomia, Duchenne muscular dystrophy and Charcot-Marie-Tooth disease show normal sensitivity to ionising radiation, as measured by post-irradiation clonal growth. Previous reports of cellular hypersensitivity to ionising radiation and other DNA-damaging agents in familial dysautonomia and Duchenne muscular dystrophy have not been confirmed.

Studies of cellular sensitivity to ultraviolet radiation in patients with xeroderma pigmentosum have shown a relationship between severity of ultraviolet effect and the occurrence of neurological manifestations, and have led to the suggestion that defective DNA repair may be involved in the pathogenesis of neurodegenerative disorders.¹ Tests of *in vitro* sensitivity to ultraviolet, ionising radiation or other DNA-damaging agents have shown abnormal responses, raising the possibility of impaired DNA reparative mechanisms, in ataxia telangiectasia,² Cockayne's syndrome,³ Huntington's chorea⁴ and Friedreich's ataxia.⁵ While the biological significance of some of these abnormal findings has yet to be demonstrated, the hypothesis that DNA repair defects in neurons might result in erroneous macromolecule synthesis, ultimately compromising function and viability, remains a plausible one.

Robbins and his colleagues have performed studies of cellular sensitivity to X-rays and the DNA alkylating agent N-methyl-N¹-nitro-N-nitrosoguanidine in a range of diseases of excitable tissue, both familial and sporadic.⁶ The inherited neuronal disorder familial dysautonomia⁷⁻⁸ and Duchenne muscular dystrophy⁹⁻¹¹ have been claimed to show an excessive response to these agents. In the present study fibroblasts from patients with these disorders and with Charcot-Marie-Tooth disease have been examined for evidence of increased sensitivity to ionising radiation.

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Methods

Patients

Forearm skin biopsies were obtained with informed consent from four patients with clinically typical Charcot-Marie-Tooth disease (all female, age range 15-58 years). Fibroblasts from four patients with familial dysautonomia (three males, one female, age 15-24 years) were obtained from the Institute for Medical Research, Camden, New Jersey, and fibroblasts from eight males with Duchenne muscular dystrophy (age 4-14 years) were supplied by Prof V Dubowitz, Department of Paediatrics. Six control fibroblast lines were obtained from healthy subjects (female, age 1-41 years) without evidence of any disease.

Cell culture

Cells were maintained in Eagle's medium supplemented by 10% newborn calf serum, penicillin (25 iu/ml), streptomycin (25 iu/ml) and glutamine (2mM). Cells were detached by means of 0.25% trypsin in Hank's basic salt solution (minus calcium and magnesium) and resuspended at appropriate density in Ham's F10 medium with 15% calf serum before X-irradiation in quadruplicate at doses of 137.5, 275, 412 and 550 rads, using a 7 meV linear accelerator. Clonal growth was then achieved in Ham's medium supplemented with 15% calf serum. Fibroblasts from patients with Charcot-Marie-Tooth disease, familial dysautonomia and Duchenne muscular dystrophy were irradiated and cloned in parallel with control cells. Cultures were sealed in polystyrene boxes, incubated for 14-20 days and stained with 0.4% Azur A. Colonies with more than 50 cells were counted as survivors; unirradiated cultures contained 10-40 such colonies. The fraction of cells surviving each dose of radiation was calculated taking into account the cloning efficiency of unirradiated cells, and for each cell line semilogarithmic survival curves were plotted. From these D₀ values (inverse of slope of linear

part of curve) were obtained. For each cell line between one and twelve replicate experiments were carried out.

Results

The Table summarises findings in 120 experiments in which the effects of X-irradiation on the clonal

growth of fibroblasts from patients with familial dysautonomia, Duchenne muscular dystrophy and Charcot-Marie-Tooth disease and from control subjects were measured. Overall mean D_0 values for the three test groups are similar and appear to show no difference from control values.

Discussion

The present findings indicate that cells from patients with familial dysautonomia, Duchenne muscular dystrophy and Charcot-Marie-Tooth disease are no more sensitive than control fibroblasts to the sublethal effects of ionising radiation. The range of D_0 values found in all groups was similar to that reported as being normal in previous studies^{5 12 13} and no single cell strain examined here appeared to be abnormally sensitive.

The pathogenesis of familial dysautonomia is unknown, and the suggestion that defective DNA repair might be involved is an attractive one. Neuropathological studies show premature loss of neurons in autonomic ganglia and spinal sensory ganglia, with secondary changes in ascending spinal tracts and peripheral nerves.¹⁴⁻¹⁸ Spinal ganglion cell loss is a striking feature in the known or presumed DNA repair deficient disorders xeroderma pigmentosum and ataxia telangiectasia, as well as in Friedreich's ataxia, where increased cellular X-ray sensitivity has been shown.⁵ The earlier study of familial dysautonomia cells by Robbins and his colleagues⁷ used virus-transformed lymphocyte cell lines, the post-irradiation viability of which was determined by trypan blue dye-exclusion, while the occurrence in familial dysautonomia of hypersensitivity to a chemical mutagen was indicated by later findings⁸ using fibroblasts. The present study does not support the supposition that defective DNA repair might occur in familial dysautonomia. Similarly the previous reports from Robbins and his colleagues suggesting the occurrence of cellular hypersensitivity to DNA-damaging agents in Duchenne muscular dystrophy⁹⁻¹¹ have not been confirmed. Such discrepancies emphasise the need both for caution in interpreting experimental results suggesting abnormal cellular responses to DNA-damaging agents, and for multiple parallel experiments of the type devised by Teo and colleagues.¹⁹

Cells from Charcot-Marie-Tooth disease were examined because of the supposed clinical interface of this disorder, through cases of Roussy-Levy type, with Friedreich's ataxia (cf for example ref. 20), which has previously been shown to exhibit mild cellular hypersensitivity to ionising radiation.⁵ No comparable abnormality was detected.

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Table Radiosensitivity of test and control fibroblasts

Familial dysautonomia		
Case	Age	D_0 (rads)
GM0732	15 years	148 ± 17 (6)
GM2341	17 years	171 ± 0.3 (2)
GM2342	19 years	141 ± 3.5 (3)
GM2343	24 years	146 ± 10 (8)
Overall mean		151 ± 6.7 (19)
Charcot-Marie-Tooth Disease		
Case	Age	D_0 (rads)
Ho	58	138 ± 6.0 (13)
Hu	35	144 ± 12 (4)
Wal	15	139 ± 15 (3)
War	57	148 ± 10 (8)
Overall mean		144 ± 4.0 (28)
Duchenne muscular dystrophy		
Case	Age	D_0 (rads)
A	9	123 ± 2.9 (7)
SW	4	147 ± 1.4 (3)
M	?	124 ± 6.0 (3)
SM	5	135 ± 7.4 (4)
W	7	154 ± 4.0 (3)
R	14	137 ± 8.6 (5)
J	6	162 ± 1.4 (3)
K	5	140 ± 3.2 (4)
Overall mean		141 ± 4.0 (32)
Controls		
Case	Age	D_0 (rads)
H	1	124 ± 4.0 (3)
D	11	109 ± 1.5 (4)
R	15	192 ± 4.2 (5)
B	30	159 ± 9.0 (12)
G	39	121 ± 2.1 (12)
P	41	160 ± 4.3 (5)
Overall mean		144 ± 12 (41)

D_0 values are doses in rads which reduce survival of fibroblast clones from any point on the exponential part of the survival curve to 37% of the value at that point, and are given with standard errors and (in parentheses) number of experiments. For experimental details see methods.

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