Ataxia-ocular motor apraxia syndrome: an investigation of cellular radiosensitivity of patients and their families

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Abstract

Although ataxia-ocular motor apraxia (AOA) has been described as a disease entity mimicking ataxia telangiectasia (AT), no radiobiological studies have been carried out on cells from patients with AOA to find their possible relationship to AT. In the present study, cultured fibroblasts from three patients with AOA and their asymptomatic relatives (parents and sibs) were, therefore, compared with those from a classical AT homozygote, an AT heterozygote, and four healthy subjects for cell survival after acute and chronic irradiation. While a moderately increased cellular sensitivity (compared to normal) was observed in two AOA patients and most of their relatives, the degree of their radiosensitivity was quite different from that of the AT homozygote after both acute and chronic irradiation. One AOA patient exhibited increased cellular sensitivity similar to that of a classical AT homozygote up to 4% survival level after chronic irradiation but not after acute irradiation. A comparison of peripheral blood lymphocytes from two AOA patients, an AT homozygote, and two normal controls for spontaneous and (acute) radiation induced chromosomal breaks also failed to show any similarity between AOA and AT. These data support the notion that AOA is different from classical AT, and may represent a distinct disease entity controlled by specific gene(s), or compound heterozygotes involving different AT genes promoting the manifestation of AOA characteristics.

Ataxia telangiectasia (AT) is an autosomal recessive "multisystem" disorder usually characterised by the occurrence of progressive cerebellar ataxia from early childhood, ocular-cutaneous telangiectasia, frequent sinopulmonary infection, and chromosomal instability.1-3 The most common laboratory findings in these syndromes include (1) decreased immunoglobulin synthesis, and reduced lymphocyte proliferation after mitogenic stimulation, (2) increased levels of serum α fetoprotein (AFP) and carcinoembryonic antigen (CEA), (3) cellular hypersensitivity to DNA damaging agents, particularly ionising radiation (acute and chronic), and (4) radiosensitive DNA synthesis.4-7 In addition, AT heterozygotes are highly susceptible to malignancies like lymphoma and leukaemia.8,9 AT heterozygotes are asymptomatic but carry an increased risk of developing various malignancies while their body cells often show a moderately enhanced sensitivity to radiation, particularly when given at a low dose rate.10,11 Several variant forms of AT showing differences with respect to the occurrence of ocular telangiectasia, various laboratory markers, and the degree of radiosensitivity have been described, suggesting a great deal of heterogeneity in the disease and making its classification somewhat difficult.12-15 Although some types of ataxia with ocular motor apraxia (AOA), as inherited disorders, were described earlier,16-17 Aicardi et al18 considered this syndrome as one mimicking AT but exhibiting certain properties indicating that it could be a unique disease entity. Aicardi et al18 emphasised that further studies were needed, particularly on radiosensitivity of these patients and their family members to establish or rule out a possible relationship of this syndrome with AT. Since the clinical description of the AOA syndrome, no radiobiological studies have been performed on cells from patients with this disease and their family members. Such studies would be useful in characterising the radiation sensitivity of the syndrome compared to classical AT heterozygotes and AT heterozygotes.

In the present study, we examined the sensitivity of cultured fibroblasts from two families each with probands showing AOA and their asymptomatic relatives, compared to that of a classical case of AT and its obligate heterozygote. The cellular response to irradiation of the AOA patients was clearly different from that in classical AT.

Materials and methods

Patients

Our three cases have been previously reported in abstract form.19 Briefly, all three patients, whose fibroblasts were analysed in this study, were between 10 and 15 years of age and had presented with gait ataxia and ocular motor apraxia in early childhood (before 2 years of age). None developed ocular telangiectasia and none of the patients had abnormally recurrent sinopulmonary infections. On examination, none was found to have pyramidal tract signs, peripheral neuropathy, sensory loss, pes cavus, scoliosis, EKG abnormalities, or extrapyramidal signs. All had normal serum AFP.
CEA, and immunoglobulins. In routine cyto-
genetic studies, none of these patients showed increased chromosomal breaks in blood lympho-
cytes. A slight decrease in mitogenic stim-
ulation of peripheral blood lymphocytes was
observed in one with phytohaemagglutinin
(PHA) and in two with pokeweed mitogen. In
general two had a static course of the disease
while one was still progressing at the latest
follow up.

The parents of one patient were first cousins
(family 2) and those of the two sibs with AOA
were second cousins (family 1). All parents
were asymptomatic and showed normal la-
boratory test results.

CELL CULTURES
Skin biopsies (after informed consent) were
obtained from three patients (two sibs from
family 1 and a girl from family 2) with AOA and
some of their family members (asymptomatic
parents and sibs as stated in the results). The
primary fibroblast cultures were developed by
growing the skin explants in Minimal Essential
Medium (MEM) supplemented with Earle's
salts, penicillin (100 U/ml), streptomycin
(100 μg/ml), glutamine (2 mmol/l), and 15% fetal
bovine serum in 25 cm² tissue culture flasks incubated at 37°C in a humidified (80%)
D, atmosphere with 5% CO₂, 95% air. Using the
same procedure, fibroblast cell strains were
developed earlier from a classical AT homo-
ygote, its obligate heterozygote, and four
healthy (Saudi) subjects.

IRRADIATION AND CELL SURVIVAL ASSAY
Early passage (4–5) fibroblast cells from differ-
ent persons were grown in Ham's F12 con-
taining the same supplements as in MEM.
For acute irradiation, late log phase cells were
harvested after trypsinisation and 2 ml aliquots
of the cell suspension containing 2 × 10⁶ cells/mi in 15 ml tubes were exposed to γ rays from
a Cs¹³⁷ source (Gamma Cell 1000, Atomic
Energy of Canada Ltd) at a dose rate of 8 Gy/
minute. The tubes with cells were kept on ice
for five minutes before irradiation and during
the entire period of handling until plated. Fol-
lowing irradiation, cells were diluted and
seeded at appropriate densities (200 to 20 000/
plate depending upon plating efficiency and
radiation doses) together with a feeder layer of
normal human fibroblasts (60 000/plate) al-
ready inactivated by 50 Gy γ irradiation. The
cells were then incubated for a period of three
weeks, with a weekly change of growth medium,
to obtain macroscopic colonies (>50 cells/col-
ony counted as a survivor). Phosphate buffered
saline was used to wash the colonies which
were then stained with crystal violet and scored
as survivors. For chronic irradiation, confluent
cell cultures in 100 mm dishes were irradiated,
inside a CO₂ incubator, using a medical γ sour-
cise (International Neutrons, California,
USA) at a dose rate of 0.008 Gy/minute over
a period of 2.5 to 30 hours. After irradiation
the growth medium was renewed and the cells
were left in the CO₂ incubator overnight. Cells
treated with different doses of radiation were

then trypsinised, harvested, diluted, and seeded
at appropriate densities (200 to 20 000/plate)
together with a feeder layer as in the case of
acute irradiation. All subsequent procedures
involved in cell plating and counting surviving
colonies were the same as in acute irradiation.

Survival curves were drawn based on colony
counts from at least four dishes for each ra-
diation dose point and percent survivors de-
termined by a comparison with the respective
unirradiated controls. Both the crude survival
d curves and D₅₀ values (estimated radiation dose
resulting in 10% survival) were compared to
ascertain the relative radiosensitivity of the cell
strains.

Results
Survival curves obtained after acute irradiation
of fibroblasts from two (brother and sister)
AOA patients, their asymptomatic parents, and
a sister (family 1), a classical AT homozygote,
and four healthy subjects (controls) are pre-
sented in fig 1. The D₅₀ values (radiation doses
resulting in 10% survival) as estimated by eye
from these curves were 4–0–5.2 Gy for the con-
trols, 2–4–3.3 Gy for the two probands and their
mother, while only 1.06 Gy for the classical AT
homozygote. The radiation response of the
father and the sister of the probands overlapped
with the control range. These results clearly
distinguished the AOA patients showing only a
moderately increased radiosensitivity from the
classical AT homozygote exhibiting a greatly
enhanced sensitivity after acute irradiation. Fig
2 illustrates the survival curves obtained after
chronic irradiation of fibroblasts from the mem-

![Figure 1](http://jmg.bmj.com/) Survival curves obtained after acute gamma
irradiation of fibroblast cells from four healthy subjects, one
classical AT homozygote, two patients (probands 1 and 2)
with AOA, and asymptomatic parents and a sister of the
patients (family 1).
bers of family 1 (proband, parents, and a sister), the classical AT homozygote, and an obligate AT heterozygote as well as the four control subjects. The estimated $D_{10}$ values in Gy were 1·3 for the AT homozygote and proband 1 (sister), 2·8 for proband 2 (brother) and the mother, 3·3 for the sister, 4·0 for the father, 4·5 for the AT heterozygote, and 7·0–9·4 for the controls. The results of chronic irradiation showed a similar radiosensitivity of the classical AT homozygote and proband 1 at least up to 4% survival level, while the asymptomatic parents and the sister clearly exhibited an intermediate radiosensitivity compared to the classical AT homozygote and the controls.

Fibroblasts from another AOA patient, his asymptomatic parents (family 2), a classical AT homozygote, an AT heterozygote, and the four healthy subjects were also compared for survival after chronic irradiation. The survival curves presented in fig 3 showed an AT heterozygote-like intermediate radiosensitivity of the proband and his mother, while the radiation response of the father was indistinguishable from that of the controls. Acute irradiation (data not shown) showed only a moderately increased radiosensitivity of the proband in this family.

A limited cyogenetic analysis performed on PHA stimulated peripheral blood lymphocytes from two probands (family 1), a classical AT homozygote, and two controls (whose survival curves have been shown in fig 2) failed to show an AT-like increase in the frequency of spontaneous chromosomal breaks or a translocation involving chromosomes 7 and 14 (found in 5% of AT cells) in the two probands (data not shown).

Discussion
The data obtained in the present study indicated a moderately increased radiosensitivity of fibroblasts from the two AOA patients (family 1) after acute irradiation. However, chronic irradiation appeared to be more useful in clearly distinguishing their enhanced sensitivity compared to the control subjects and also in showing an intermediate radiosensitivity (between classical AT and controls) of their family members (father, mother, and sister in family 1 and mother in family 2). It was interesting to note that fibroblasts from one of the two sibs (the sister in fig 2) with AOA exhibited a biphasic survival curve which was identical to the survival curve of AT up to 4% survival level after chronic irradiation. This is the patient who also showed a progressive course of the disease. The lowest $D_{10}$ values (2·4 and 1·3 Gy respectively) shown by the cells from this patient after both acute and chronic irradiation indicated that she was the most radiosensitive of all. Thus, her greatly increased radiosensitivity could be a factor in disease progression. A possible correlation of the degree of radiosensitivity and the state of neurodegenerative process has been reported in ultraviolet radiation sensitive xeroderma pigmentosum (XP) patients in which the most UV sensitive cases were the ones showing early onset of neurological disease.

The cell survival data obtained with both acute and chronic irradiation of the three patients with AOA did not suggest that any one of them represented classical AT. This was also evident from a cyogenetic analysis of family 1 with the two patients (sibs) showing neither an AT-like increase in spontaneous chromosome breakage in PHA stimulated peripheral blood lymphocytes nor a translocation involving chromosomes 7 and 14.

Aicardi et al proposed that AOA which differed in several clinical aspects from classical AT could be a distinct disease entity. However, in the absence of radiobiological data for their
patients a possible relationship of the disease with AT could not be ruled out. Indeed, there are suggested AT variants which reflect a great deal of genetic heterogeneity with considerable variation in clinical features and laboratory findings as well as in the degree of cellular radiosensitivity. In particular, some of these variants have been reported to exhibit an intermediate radiosensitivity. However, such variants show both ataxia and telangiectasia unlike patients with ataxia-oculomotor apraxia who lacked telangiectasia even after the age of 10 years. Some AT variants were characterized by highly enhanced radiosensitivity like classical AT but without telangiectasia. In these cases the role of genes other than AT needs to be ruled out. Our data, particularly on one family with two AOA cases showing increased cellular sensitivity to chronic irradiation in both probands and family members, need careful interpretation. If the parents, both showing an intermediate radiosensitivity after chronic irradiation, were AT heterozygotes (for the gene of a specific complementation group), the probands showing an AT homozygote-like response should have been found in the family, after both acute and chronic irradiation, but none was. One possibility is that the parents were AT heterozygotes for different AT genes and the probands were double heterozygotes, thus showing different levels of radiosensitivity; that is, they were more sensitive than asymptomatic parents. While this may be true for proband 1 of family 1, it does not seem to be the case for proband 2 of this family and the proband of family 2 who showed a D$_{20}$ value similar to their mothers. In the case of family 2, only the mother showed an AT heterozygote-like radiosensitivity only after chronic irradiation while the father did not. It is possible that these patients were heterozygous for one AT gene inherited from their mothers resulting in a similar response to chronic irradiation. However, these data do not answer the question as to why the offspring showed AOA while their radiosensitive parents did not. The second possibility is that the parents carried different AT genes in heterozygous states and the AOA patients were compound heterozygotes with a varying degree of radiosensitivity but promoting the occurrence of AOA. This appears unlikely in view of the results obtained with two AOA patients exhibiting radiosensitivity only like their mothers. The third possibility is that genes for radiosensitivity (AT or not) in combination with some other gene (in the homozygous state) result in the manifestation of AOA. This would mean that parents were heterozygous for that (hypothetical) gene and, hence, remained asymptomatic even though they were radiosensitive. None of these possibilities can be confirmed or ruled out without molecular probing for AT genes when they become available and a rigorous pedigree analysis enabling the identification of the possible additional genes. Meanwhile, both the data showing a variable radiosensitivity of AOA patients after acute and chronic irradiation and a preliminary cytogenetic analysis showing no AT-like chromosomal abnormality may support AOA as a disease entity distinct from classical AT.

Patients with AOA have been described in Japanese publications as well as in the West. Whether or not the AOA syndrome represents a variant form of AT, compound AT heterozygosity, or the carrier state of other genes in combination with AT genes, the frequency and distribution of this disease in different populations should be carefully studied as cellular and chromosomal radiosensitivity may increase its susceptibility to malignancies. In Saudi Arabia, the frequency of this syndrome among patients with degenerative ataxia appears to be relatively high and these cases may serve as unique material for further genetic and epidemiological studies.

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