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, oculocutaneous telangiectasia, frequent sino-pulmonary 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) radioresistant DNA synthesis.4-7 In addition, AT homozygotes 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 homozygotes 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 cytogenetic studies, none of these patients showed increased chromosomal breaks in blood lymphocytes. A slight decrease in mitogenic stimulation 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 laboratory 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%) atmosphere with 5% CO₂, 95% air. Using the same procedure, fibroblast cell strains were developed earlier from a classical AT homozygote, its obligate heterozygote, and four healthy (Saudi) subjects.

IRRADIATION AND CELL SURVIVAL ASSAY

Early passage (4–5) fibroblast cells from different persons were grown in Ham’s F12 containing 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 × 10⁵ cells/ml 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. Following 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) already 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/colony 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 source (International Neutronics, 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 radiation dose point and percent survivors determined by a comparison with the respective unirradiated controls. Both the crude survival curves and D₂₀ 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 presented in fig 1. The D₂₀ values (radiation doses resulting in 10% survival) as estimated by eye from these curves were 4·0–5·2 Gy for the controls, 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 membrane.
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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 D10 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.20

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 cytogenetic 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

Figure 2  Survival curves of fibroblasts from the four healthy subjects, a classical AT homozygote, an AT heterozygote, the AOA patients, and their asymptomatic family members (family 1) after chronic irradiation.

Normal range
Classical AT
Obligate AT heterozygote
Proband 1
Proband 2
Mother of probands 1 and 2
Father of probands 1 and 2

Chronic gamma radiation dose in Gy

Percent survival

0.1

0

10

1

Figure 3  Survival curves of fibroblasts from the four healthy subjects, the classical AT homozygote, an AT heterozygote, an AOA patient (proband 3), and his asymptomatic parents after chronic irradiation.

Normal range
Mother of proband 3
Father of proband 3
Classical AT
Obligate AT heterozygote
Proband 3

Chronic gamma radiation dose in Gy

Percent survival

0.1

0

10

1

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 cytogenetic 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).
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 a 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-ocular motor apraxia who lacked telangiectasia even after the age of 10 years. Some AT variants were characterised 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 showed 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 $D_{22}$ value similar to their mothers. In the case of family 2, only the mother showed an AT heterozygote-like radiosensitivity 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 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 the idea of AOA being 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 their 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.