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NEAR-ULTRAVIOLET SENSITIVITY OF SKIN FIBROBLASTS OF PATIENTS WITH BLOOM'S SYNDROME

Irène Zbinden and Peter Cerutti

Department of Carcinogenesis
Swiss Institute for Experimental Cancer Research
CH-1066 Epalinges / Lausanne - Switzerland

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SUMMARY

Near-ultraviolet survival of colony forming ability was compared between fibroblast strains from normal individuals and seven strains from Bloom's Syndrome patients using monochromatic light at 313 nm. Six BS strains possessed abnormal survival properties. Two strains lacked the low dose shoulder, which is characteristic for normal fibroblasts, but their overall sensitivity was in the normal range. Four BS strains were hypersensitive and possessed biphasic survival curves with a sharp initial drop. They mimick in culture the characteristic solar sensitivity of BS patients.

INTRODUCTION

Bloom's Syndrome (BS) is an autosomal recessive disease with increased cancer incidence. Additional clinical symptoms are stunted growth, immunodeficiency and skin sensitivity to sun-light (1). In general, cultured BS fibroblasts possess normal sensitivity to far-ultraviolet (far-UV, wavelengths below 290 nm) at 254 nm (2,3). BS strain GM 1492 and two strains studied by Gianelli et al. (4) may represent exceptions. Near-ultraviolet (near-UV, wavelengths above 290 nm), in contrast to far-UV, induces DNA damage in part by indirect action. Indirect action is mediated by the formation of active oxygen species and induces a spectrum of lesions which is related to that of ionizing radiation (5-7). Since far-UV is removed from the solar spectrum which reaches the surface of the earth by the

NF, skin fibroblasts from normal donors ; BS, Bloom's Syndrome ; UV, ultraviolet light ; CE, cloning efficiency ; CFA, colony forming ability ; P, passage number.

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upper atmosphere it may be more revealing to use near-UV light in studies of the molecular basis of solar sensitivity. We report that six of seven BS strains tested possessed abnormal survival properties to near-UV at 313 nm. Four strains were hypersensitive and mimick in culture the solar sensitivity of BS patients, therefore.

MATERIALS AND METHODS

Cell Cultures

Skin fibroblasts from normal individuals, CRL 1121, CRL 1221, CRL 1222, were obtained from the American Type Culture Collection, Rockville, MD, USA. The skin fibroblast strains from patients with BS were received from the following sources : GM 1492, GM 1493, GM 2548, GM 2520 from the Human Genetic Mutant Cell Repository, Camden, N.J., USA ; H 46 from Dr. D. Bootsma, Rotterdam, Holland ; HG 916 and HG 369 from Dr. J. German, New York, USA. All cells were cultured in monolayers in Falcon plastic flasks or Petri dishes, in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 20% fetal calf serum. 100 I.U. ml^{-1} penicillin-G and 50 µg ml^{-1} streptomycin. The cloning efficiencies (CE) and passage numbers (P ; 1:2 split ratios) of the NF strains were : CRL 1121, CE 6.0-7.7%, P 18-24 ; CRL 1221, CE 16.6-19.2%, P 18-21 ; CRL 1222, CE 16.8-20.6%, P 17-18. For the BS strains they were : GM 2520, CE 17.3-21.6%, P 20-22 ; HG 369, CE 4.0-12.8%, P 20-21 ; GM 1492, CE 11.8-18.4%, P 21-22 ; GM 2548, CE 9.5-13.0%, P 16-19 ; GM 2548 $Cl_{30}J_3$, CE 11-13% ; H 46, CE 4.0-4.5%, P 13-14 ; GM 1493, CE 3.5-4.6%, P 13-14 ; GM 1493, CE 3.5-4.6%, P 13-14 ; HG 916, CE 3.1-6.8%, P 21-14.

Near-Ultraviolet Survival Curves

NF or BS fibroblasts were usually plated at 400 and 800 cells per 6 cm Petri dish, respectively, and allowed to attach and grow for 13-16 h, before the media was replaced with 1 ml fresh DMEM (without serum). Highly radiation sensitive strains were plated at 600 and 1200 cells per dish at the low doses and 800 and 1600 cells per dish at the high doses. The covers of the dishes were replaced by a Kodacel plastic sheet which served as a stray light filter. The cultures were irradiated on a rotating platform with 313 nm light from a Schöffel GM 250 high intensity quarter meter grating monochromator (1180 grooves/mm). The entrance and exit slits were set at 3 mm resulting in a dose rate of 13-15 Jm^{-2} sec^{-1} depending on the lamp which was used. After irradiation the media were replaced with fresh DMEM containing 20% fetal calf serum. The media was changed every 5 days and after 15 to 20 days the cultures were fixed with methanol and stained with crystal violet and colonies containing more than 30 cells counted with the help of a dissecting microscope. Six replica plates were evaluated at each radiation dose. Colony forming ability (CFA) was calculated from the CE of the irradiated cells relative to sham-irradiated controls. Keeping the cultures in serum-free DMEM for 19 min., i.e. the maximal irradiation time, did not affect the CE of any of the NF and BS strains studied. Means of CFA of at least three independent experiments were plotted in the usual semilogarithmic fashion. Straight lines were fit by the method of least squares through all points along the exponential portions of the curves.

RESULTS

Near-UV survival of monolayer cultures of three NF and seven BS strains was investigated according to the following principal experimental design. Low numbers of cells were plated and after attachment irradiated with monochromatic light at 313 nm. The cultures were then allowed to grow in fresh complete media without replating for 10-14 days before the colonies were counted and the CFA calculated as a function of dose relative to sham-irradiated control cultures.

Figure 1 A contains the survival curves of three NF strains. They are characterized by a shoulder in the low dose region which is followed by an exponential portion. Substantial variation is observed in the slope of the exponential portions from -0.073 for CRL 1221 to -0.113 for CRL 1222. The survival curves for the three BS strains GM 2520, GM 1492 and HG 369 are given in Figure 1 B. The curve for GM 2520 is indistinguishable from those of NF, while the curves of GM 1492 and HG 369 lack the low dose shoulder. The curve of HG 369 contains an indication of an initial drop. The slopes of the exponential portions of the curves are in the normal range. Figure 1 C contains the curves of the BS strains HG 916, GM 1493, H 46 and Figure 1 D those of GM 2548 and a cloned substrain GM 2548 $Cl_{30}J_3$. The curves of these four BS strains are biphasic and contain two essentially exponential portions. The low dose portions possess steep slopes in the range of -0.197 to -0.243 while the slopes of the high dose portions are in the range of NF. As shown in Figure 1 D, no significant differences are observed between the curves of uncloned GM 2548 and its cloned derivative GM 2548 $Cl_{30}J_3$.

In Table 1 the following parameters of the survival curves are listed : D_{37} is the dose which is necessary to decrease the CFA to 37% relative to

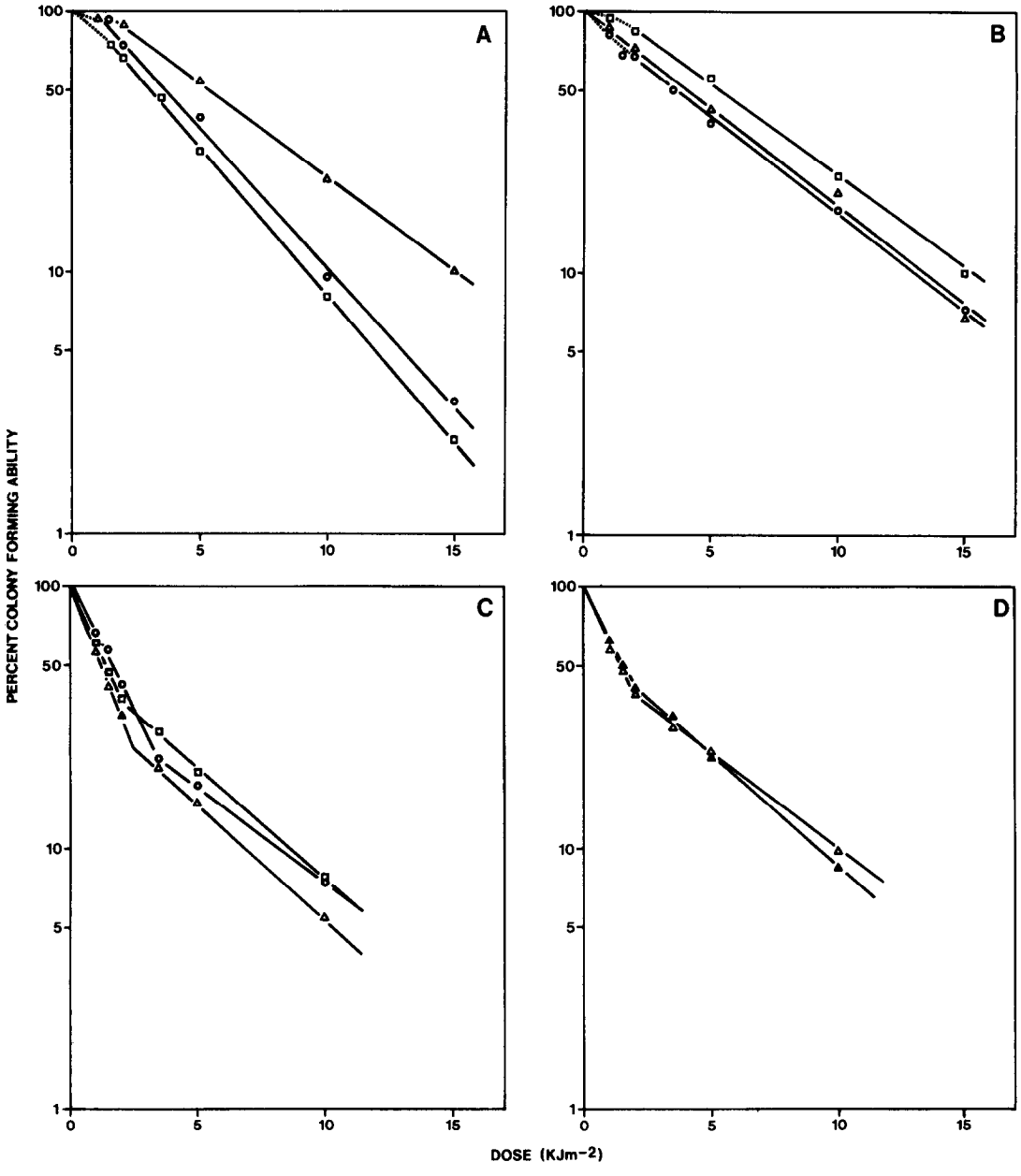

FIGURE 1 Survival of colony forming ability of monolayer cultures of normal and Bloom's Syndrome fibroblasts irradiated with monochromatic light at 313 nm (for experimental conditions see under "Materials and Methods"). A. NF strains : Δ, CRL 1221 ; O, CRL 1121 ; ▭ , CRL 1222. B. BS strains : Δ, GM 1492 ; O, HG 369 ; ▭ , GM 2520. C. BS strains : Δ, H 46 ; O, GM 1493 ; ▭ , HG 916. D. BS strains : Δ, GM 2548 ; ▲, subclone GM 2548 $Cl_{30}J_3$.

sham-irradiated controls and allows a comparison of the overall radiosensitivity of the different cell strains. D_0 is the dose required to

TABLE 1.

PARAMETERS OF NEAR-ULTRAVIOLET SURVIVAL CURVES OF NORMAL AND BLOOM'S SYNDROME SKIN FIBROBLASTS*

		D_{37}†	D_0†	n
NF	CRL 1221	7.3 ± 0.2	5.8 ± 1.1	1.36 ± 0.25
	CRL 1121	4.9 ± 0.6	4.0 ± 0.3	1.25 ± 0.11
	CRL 1222	4.3 ± 0.6	3.8 ± 0.4	1.15 ± 0.07
BS	GM 2520	7.3 ± 0.7	6.1 ± 0.7	1.22 ± 0.02
	GM 1492	5.8 ± 1.0	5.6 ± 0.9	
	HG 369	5.5 ± 1.0	5.8 ± 0.4	
	HG 916	2.0 ± 0.3	a) 2.1 ± 0.3	
			b) 5.1 ± 0.4	
	H 46	1.7 ± 0.3	a) 1.8 ± 0.2	
			b) 4.8 ± 0.6	
	GM 1493	2.3 ± 0.2	a) 2.2 ± 0.3	
			b) 5.9 ± 0.7	
	GM 2548	2.2 ± 0.3	a) 2.4 ± 0.4	
			b) 5.9 ± 0.7	
	GM 2548 $Cl_{30}J_3$	2.4 ± 0.3	a) 2.2 ± 0.4	
			b) 5.0 ± 0.6	

* Irradiation of monolayer cultures with monochromatic light at 313 nm (plus Kodacel) in DMEM (without serum)

† In KJm^{-2}; for biphasic survival curves a) refers to the D_0 value for the low dose branch and b) to the D_0 value for the high dose branch

Mean values with standard deviations are listed

decrease the CFA from a given point on a linear portion of the survival curve to 37% of that point; two values are given for the BS strains with biphasic curves. The extrapolation number n corresponds to the y-intercept obtained by back extrapolation of the linear portions of survival curves with a low dose shoulder. From the D_{37} doses it is evident that four of the seven BS strains tested are two to three times more sensitive to 313 nm radiation than NF. The hypersensitivity of the four BS strains lies in the low dose region as is documented by the $D_{0,a}$ values for these portions

of the survival curves. In contrast, the $D_{0,b}$ values for the high dose branches of the curves were all in the normal range. While the D_{37} and D_0 values were normal for BS strains GM 1492 and HG 369 the curves reproducibly lacked the low dose shoulders which are characteristic for the NF strains.

DISCUSSION

Our results indicate that six of the seven BS strains tested possessed abnormal near-UV survival curves. Four BS strains (HG 916, H 46, GM 1493, GM 2548 and its subclone GM 2548 $Cl_{30}J_3$) were hypersensitive as reflected in their D_{37} values and mimick in culture the increased skin sensitivity of BS patients to solar radiation. The hypersensitivity of these four strains is due to a steep initial drop in their survival curves. No relationship is discernible between the CE of the individual fibroblast strains and their near-UV sensitivity. It should be noted that the CE's of some of the BS-strains with clearly abnormal survival properties are quite comparable to NF (e.g. GM 2548, CE 9.5-13% ; HG 369, CE 4.0-12.8% ; GM 1492, CE 11.8-18.4% ; see "Materials and Methods"). The observed differences in the near-UV survival properties of individual BS strains may reflect genetic heterogeneity. A BS strain which was hypersensitive both to irradiation at 254 nm and 313 nm was recently investigated by P. Smith and M. Paterson (personal communication).

The biphasic survival curves observed for four BS strains are reminiscent of Ataxia telangiectasia fibroblasts exposed to bleomycin (8,9), skin fibroblast strain 11961 obtained from a sun-sensitive patient (10), but also of chinese hamster cells irradiated with X-rays under conditions which gradually changed from aerobic to hypoxic as a function of radiation dose (11). Several alternatives have to be considered for the interpretation of the biphasic survival curves : (1) The cultures contain highly sensitive

and more resistant genetic subpopulations. This hypothesis was experimentally tested for strain GM 2548. The fact that the survival curve of the cloned substrain GM 2548 $Cl_{30}J_3$ retained the biphasic character and was almost identical to the curve of the parent strain argues against this hypothesis (see Figure 1 D). (2) An abnormality in the cell cycle parameters of BS fibroblasts relative to NF could be responsible for the observed results. Some BS cultures may contain increased portions of non-cycling cells (12) which may be more sensitive to near-UV killing than rapidly dividing cells. (3) A phototoxic factor is produced more efficiently in BS than in NF cultures. The formation by near-UV of toxic oxidation products of tryptophan, tyrosine and riboflavin in culture media and of endogenous flavins is known (13,14). These products are formed via the intermediacy of active oxygen species. A deficiency in their detoxification in BS would result in increased concentrations of such cytotoxic components. Recent cytogenetic data from our laboratory support the notion that active oxygen species may play a role in the pathology of BS. A clastogenic factor was identified in the media of BS skin fibroblast cultures whose activity could be reduced substantially by the addition of bovine superoxide dismutase (15). Studies of the formation of DNA single strand breaks in BS fibroblasts relative to NF by monochromatic light at 313 nm revealed that breaks were induced more efficiently in seven of eight BS strains (16). BS strain GM 2520 possessed normal survival, normal near-UV induced strand breakage and only very low clastogenic activity. These observations point towards a common etiology for the observed abnormalities in BS fibroblasts and support the hypothesis that a defect in oxygen metabolism may be responsible for the formation of elevated concentrations of DNA lesions in BS fibroblasts. (4) The possibility cannot be excluded that a deficiency in the repair of near-UV induced DNA lesions could be responsible for the ab-

normal survival properties of a majority of BS strains. While the low dose shoulders in the survival curves of the NF strains may reflect repair processes the biphasic curves of the BS strains are difficult to explain on the basis of a repair deficiency, however. It should be noted that no evidence for a repair deficiency has been discovered in BS despite considerable efforts by several laboratories (3, 17-22).

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