

Effect of Grenz Rays on Langerhans' Cells in Human Epidermis

BERNT LINDELÖF, STURE LIDÉN and ANNE-MARIE ROS

Department of Dermatology, Karolinska sjukhuset, Stockholm, Sweden

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In order to investigate the effect of grenz rays on Langerhans' cells in human epidermis, five healthy volunteers were treated with a single dose of 4 gray of grenz rays on a limited area of buttock skin. Biopsies were obtained before irradiation and from the irradiated site 30 min, 6 hours, 24 hours, 1 week and 3 weeks after X-ray therapy. There was a slight reduction after 30 min and a very pronounced reduction of OKT-6 positive cells regarded as Langerhans' cells, 1 and 3 weeks after irradiation. *Key words: Bucky rays; Langerhans' cell; Monoclonal antibody.* (Received March 26, 1984.)

B. Lindelöf, Department of Dermatology, Karolinska sjukhuset, S-104 01 Stockholm, Sweden.

It is only within the past 5 years that the important role of Langerhans' cells in the immune system of epidermis has been recognized, though these cells have been known for a long time. For summary see (1).

A large amount of work has been performed concerning the effects of ultraviolet light on Langerhans' cells. As regards X-rays on the other hand, little is known (2-5). In mice soft X-rays reduce Langerhans' cells in a dose-dependent manner (4, 5).

Long wave X-rays for the treatment of cutaneous diseases have now been used for more than 60 years after Bucky's introduction of grenz rays. The X-rays of long wavelength used in dermatology are the following: Soft X-rays (average wavelength 0.015 nm), superficial X-rays (average wavelength 0.05 nm) and grenz rays (average wavelength 0.2 nm). Synonyms for grenz rays are Bucky rays and ultrasoft rays and those are the waves closest to the ultraviolet light in the spectrum of electromagnetic radiation.

The aim of the present study was to investigate the effect of a single dose of grenz rays on the number of OKT-6 positive cells in normal human skin. The dose of grenz rays selected was 4 gray which is a dose commonly used in the treatment of benign skin disorders.

MATERIALS AND METHODS

Subjects

Five healthy caucasian volunteers, 1 male and 4 females, 36 to 69 years old participated in the study.

Grenz rays

The grenz rays machine factors were: 10 kV, 10 mA, half-value layer 0.03 mm Al. Focus-skin distance 10 cm. Beryllium window.

Experimental procedure

Each subject was exposed to grenz rays in one single dose of 4 gray in a circular area, 5 cm in diameter, of buttock skin (untanned). Biopsies were obtained from the buttock before irradiation and from the irradiated site, 30 min, 6 hours, 24 hours, 1 week and 3 weeks after the X-ray therapy.

Histology techniques

Punch biopsies (3 mm) were taken under local anesthesia with lidocain without epinephrine. Each specimen was immediately frozen on solid carbondioxide and stored at -80°C until use. The biopsies were cryostat sectioned at 6–8 μm and every 5th section was mounted on cooled glass slides.

OKT-6 labelling

The slides were incubated in OKT-6, a monoclonal antibody (Ortho Pharmaceutical Corp., Raritan, New Jersey). OKT-6 reacts with the majority of thymocytes and with Langerhans' cells (6).

Subsequently, a highly sensitive immunoperoxidase technique was employed (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame). Briefly, tissue sections were layered with a biotin-labelled secondary antibody and this introduced biotinyl residues into the section at the location of the primary antibody. An avidin: biotinylated horseradish peroxidase complex was then added and the tissue antigen was localized by incubation in a peroxidase substrate (amino-aethyl carbazol). As a control the whole staining procedure was performed but with omission of the primary antibody.

Cell quantification

The slides were examined at a magnification of 500 \times using an ocular square grid. From each biopsy a total of 40 grid fields were examined. This represented 10 mm of skin surface length. Only dendritic cells with visible nucleus were counted as positives, isolated dendrites were not scored. One investigator (B. L.) examined all samples which were counted blind.

RESULTS

The results are summarized in Table I. In the majority of the counted sections the distribution of OKT-6 positive cells was regular and the cells were easily identified. After

Table I. Number of OKT-6 positive cells/10 mm of skin surface length in sections of human epidermis after a single dose of 4 gray of grenz rays

Subj.	Age/Sex	Time interval between exposure and biopsy					
		Before	30 min	6 h	24 h	1 w	3 w
1	38/M	154	147	159	157	84	62
2	36/F	188	145	127	149	67	43
3	64/F	168	118	137	145	52	48
4	69/F	146	112	97	116	52	45
5	63/F	134	107	76	53	22	34
	Mean	158	125.8	119.2	124	55.4	46.4
	\pm SD	20.8	18.3	32.9	42.6	22.9	10.2
	$p <$		0.05	0.05	NS	0.001	0.001

30 min we found a slight reduction ($p < 0.05$) of OKT-6 positive cells and after 1 and 3 weeks the reduction was highly statistically significant ($p < 0.001$ paired *t*-test).

The skin exposed to grenz rays did not show any erythematous reaction or other signs of inflammation.

DISCUSSION

Our study shows that grenz rays reduce the number of OKT-6 positive cells in human epidermis. The OKT-6 antibody is both a specific and sensitive probe for Langerhans' cells and indeterminate cells in the skin. The topic has been reviewed by Murphy 1982 (6). This reduction of the Langerhans' cell population as revealed by the OKT-6 technique is not necessarily due to a physical absence of Langerhans' cells but could also be due to a loss or modulation of surface markers. Probably the reduction is due to both a physical absence and a depletion of surface markers. The time interval between exposure and biopsy could be important in such a way that a slight reduction of Langerhans' cells due to loss of surface markers may be seen a short time after irradiation, whereas the reduction may be due to physical absence later on. Such a loss of surface markers could explain the slight reduction of Langerhans' cells that we observed as early as 30 min after grenz ray therapy.

Grenz ray therapy has been a useful therapeutical modality in dermatology for many decades. The grenz rays (ultrasoft X-rays, Bucky rays) normally used in dermatology have photon energies between conventional X-rays and ultraviolet light. The wavelengths are 7.5–40 nm.

Early investigators found that the number of Langerhans' cells decreased under conditions which stimulated melanocytes, i.e. ultraviolet light and X-rays (2, 3). In a recent study (5) the influence of soft X-rays (80, 1 200, 1 600 and 3 200 R; 30 kV; 1 gray \cong 100 R) was studied in the guinea pig and mouse system. In the mouse system, 1 week after exposure to 800 and 1 200 R, ATPase and Ia-positive cells, in a more or less parallel manner, were reduced to 74% and 70%, respectively, and 4 weeks after exposure to 49% and 43%, respectively. In the guinea pig system, similar results were obtained. 8 weeks after exposure to 1 200 R, pre-treatment values were reached again.

Our observation, that grenz rays reduce the number of epidermal Langerhans' cells, indicates that alteration of the immunologic properties of epidermis may contribute to the good effect noted in various inflammatory skin disorders. Further work is now in progress to collect more information about the effects of grenz rays on various immunological parameters of the skin.

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