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Effects of the Hypoxic Radiosensitizer Misonidazole on Normal and Irradiated Epidermis

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Misonidazole, a hypoxic cell radiation-sensitizer, has been used *in vivo* to analyze its effects on normal and x-irradiated epidermis. The drug action was evaluated 3 days after being implanted subcutaneously. The response of the epidermis and especially of the basal cells to the drug alone and in combination with x-radiation was studied. The ultramorphological analysis as well as quantitative data on epidermal thickness and keratinocyte volume showed that high doses of misonidazole impair the usual post-irradiation epidermal reaction, thus enhancing the involutional effects of radiation and inhibiting radiation-damage repair, even under conditions of normal oxygenation.

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Misonidazole (formerly Ro-07-0582), is an electron-affinic compound that has the property of increasing free radical production, thus enhancing the effects of ionizing radiation, and which can be effectively used in combination with conventional tumor radiotherapy. The potential value of this compound is that it can act as a selective radiosensitizer of tissues containing hypoxic tumor cells which are relatively resistant to the effects of ionizing radiation. (McNally et al. 1978, Hall & Biaglow 1977, Rauth et al. 1978). In addition to its radiosensitizing effect, misonidazole has also been shown to be toxic to cells (Moore et al. 1976, Brown 1977, Phillips 1977).

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Denekamp et al. (1974), using two different nitroimidazoles (metronidazole and misonidazole) shortly before irradiation, demonstrated that both drugs specifically sensitized epidermal cells made artificially hypoxic, but had no evident effect upon well oxygenated cells. Stone & Withers (1974), obtained an enhanced desquamation of normal skin after the administration of metronidazole immediately after irradiation.

The principal aim of this paper is to show the morphological alterations in the normal epidermis induced by misonidazole alone or in combination with radiation treatment under normal oxygenation conditions. Given the fact that during radiotherapeutic trials, misonidazole is administered during the course of a multifraction irradiation schedule, in which several hours or days elapse between fractions, the normal skin overlying the tumor will

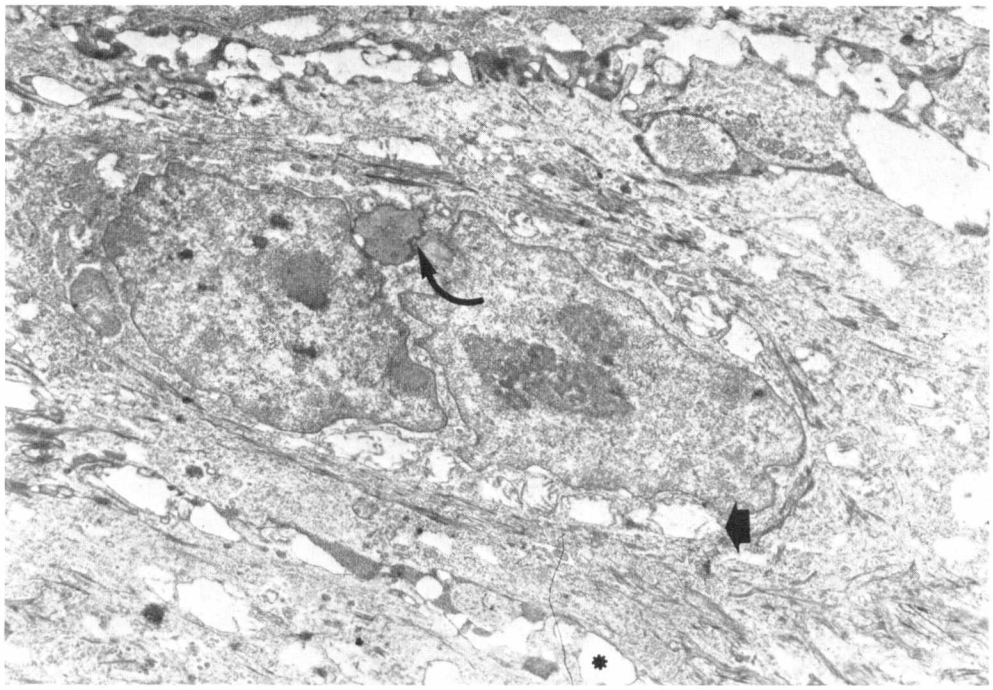

Fig. 1. Electron micrograph of basal cells from irradiated epidermis (I group in the text). Note features of radio-induced hyperactivity, i.e. cell and nuclear enlargement, fragmentation of nuclei, formation of lipid vacuoles (⌣), modification of mitochondrial structure (➨) and widened intercellular spaces (*). × 500.

be affected by radiation as well as by the prolonged action of the drug.

Material and methods

Sixty-five-day-old Wistar rats were used. Thirty animals were submitted to the action of the drug misonidazole (1,2-(nitroimidazol-1-yl)3-methoxy-2-propanol), formerly Ro-07-0582 (kindly supplied by Roche Products Ltd., Welwyn Garden City, Hertdfordshire, England). The powder was directly implanted into the subcutaneous tissue (0.5 mg/g animal weight) through a small incision on the dorsal area. The same number of animals were subjected to a sham operation without the drug and used as controls.

Irradiation conditions

Seventy-two hours after the implantation of the drug, the left soles of all the animals were exposed to 8 Krad X-rays with a Philips radiotherapy machine operated at 250 Kv, 13 mA employing a 0.25 mm Cu and 1 mm Al filter (HVL 12.4 mm) at a dose rate of 500 rad/min. The rest of the animal's body remained shielded, and the right sole was used as non-irradiated control. All the animals were sacrificed 3 days after irradiation (6 days after the onset of the experiment), and the specimens were grouped as follows:

DI – Samples from animals treated with the drug and subsequent irradiation.

D – Samples from non-irradiated animals treated with the drug.

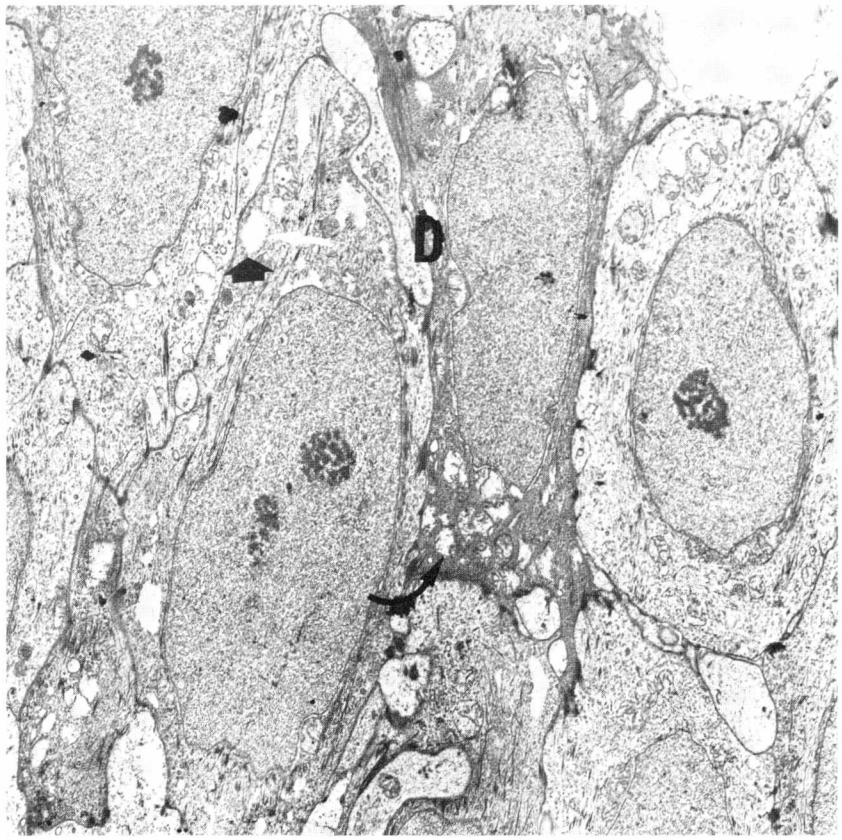

Fig. 2. Basal cells from non-irradiated epidermis, treated with misonidazole (D group in the text). Note empty areas in cytoplasm (➘) and swollen mitochondria (⌣). The outstanding feature is the formation of many electron dense cells (D) (dark cells) in the basal layer. × 4000.

I – Samples from irradiated animals without drug treatment.

C – Samples from non-irradiated animals receiving no drug treatment.

The skin of the soles of all groups was processed simultaneously for analysis by light and electron microscopy (Klein-Szanto et al. 1977). Ultra-thin sections were obtained from Epon blocks, contrasted with uranyl acetate and lead citrate, and photographed in a Philips 300 electron microscope. One or two-μm thin sections were also prepared and stained with PAS and Toluidine blue (Schroeder 1973). Three blocks were selected from each sample and, in total, 45 sections from each group were available for light microscopical quantitation. From each section, the average thickness of the epidermis was obtained by measuring it directly with the aid of a calibrated eyepiece.

In order to obtain the absolute volumes of basal cells, stereologic methods were applied. Determination of the numerical density (Nv) of cells was carried out following the procedure previously described (Klein-Szanto et al. 1977).

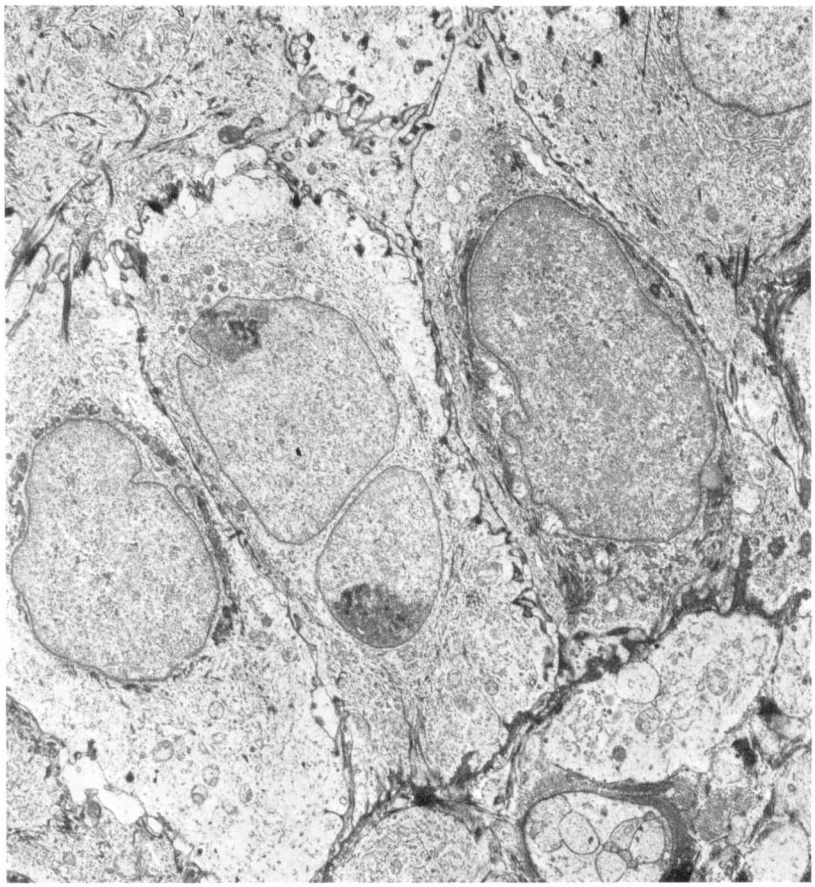

Fig. 3. Electron micrograph of basal cells from irradiated and misonidazole-treated epidermis (DI in the text). Except for a few lobulated nuclei, no remarkable evidence of radio-induced alterations can be noted.

Results

Morphologic observations

Direct observation of the experimental animals treated with misonidazole clearly showed a smaller body size as well as a remarkable alteration in the hair growth pattern. The ultrastructural analysis of the control epidermis (group C), showed the characteristic features of normal sole epidermis. Tissues from group I (irradiated animals without drug treatment) showed alterations in the cell membrane system. The most remarkable feature was the formation of large basal cells with lobulated or fragmented nuclei and lipid vacuoles near infoldings of the nuclear membrane (Fig. 1).

Non-irradiated, drug-treated animals (group D) exhibited basal keratinocytes with swollen mitochondria. Some dark cells exhibiting swollen membraneous organelles and an electron dense cytoplasm with a compact arrangement of ribosomes and fibrils could be seen.

Samples from group DI, pretreated with misonidazole and irradiated 3 days later, showed few radio-induced alterations. Only some cells with lobulated nuclei were observed. In a few cells it was possible to observe nuclear membrane disintegration and cytoplasmic disorganization (Fig. 3). Some dark epithelial cells were observed, but the electron density of these cells was somewhat less marked than in the cells of the previous group.

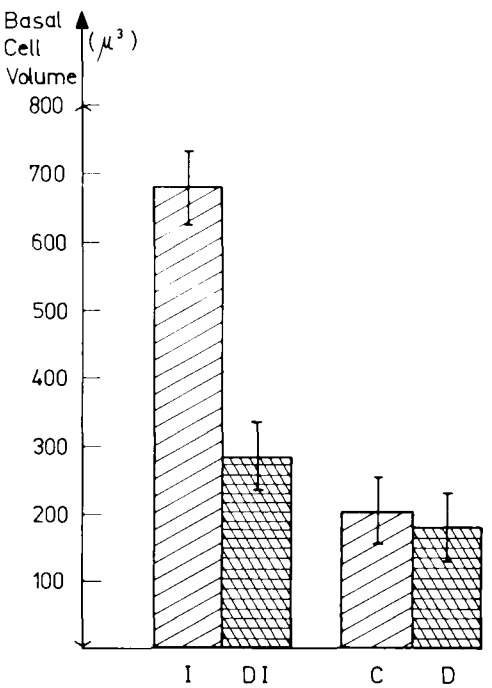

Fig. 4. Absolute volume of basal cells from:
I – irradiated cells 3 days after irradiation with 8 Krad, without misonidazole.
DI – irradiated cells 3 days after 8 Krad and 6 days after a treatment with misonidazole.
C – non-irradiated cells, not pretreated with misonidazole.
D – non-irradiated cells, 6 days after treatment with misonidazole.
Vertical bars represent standard errors.

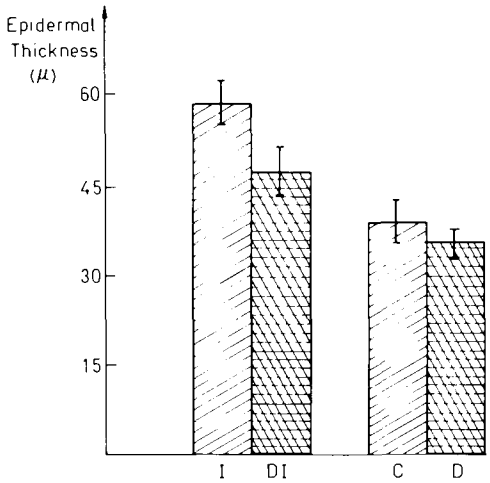

Fig. 5. Absolute epidermal thickness. (I, DI, C, D as in Fig. 4.).

Quantitative results

The mean cell volume in group C was 204 μm^3. Cells from group I showed a mean cell volume of 680 μm^3. Cells from group D exhibited a mean cell volume of 188 μm^3, which is even lower than seen in the control group (the difference is not statistically significant). Finally, cells from group DI, from tissues exposed to drug action and irradiation, had a mean cell volume of 280 μm^3 (Fig. 4). This implies a decrease of 109% (statistically significant) in the mean cell volume between groups I and DI and a decrease of 8% between C and D. A one-way analysis of variance, in which the four groups were contrasted, demonstrated that the cell volume was significantly modified by treatment. Comparison of the epidermal thicknesses of the non-irradiated group indicated an 8.8% decrease produced by misonidazole. When comparing the same parameter in the irradiated group, a 19% statistically significant decrease could be observed in the drug-treated animals (Fig. 5).

Discussion

Experimental work on hypoxic skin has demonstrated that substantial sensitization can be achieved *in vivo* with misonidazole (Denekamp et al. 1974).

The present results suggest that normal epidermis was affected by protracted misonidazole action. The ultrastructural analysis showed that the irradiated and drug-treated epidermis (DI) reacted differently from the epidermis exposed to radiation only. Some of the cellular alterations that have been already described as post-irradiation effects were seen in this group (de Rey & Cabrini 1973). On the other hand, some characteristic images attributed to cell reactions induced by ionizing radiation, such as nuclear fragmentation, cell enlargement and abundance of tonofilaments seen in group I were absent in group DI.

In animals treated with misonidazole (no irradiation), the epidermis exhibited some organelle alterations and the formation of a large number of electron dense cells. Although hyperactive electron dense epithelial cells have been described in several hyperplastic stratified squamous epithelia (Klein-Szanto & Nettesheim 1980, Klein-Szanto et al. 1980), other types of dark electron dense cells have been seen in irradiated oral epithelium and interpreted as degenerative cells (Liu et al. 1975). The dark cells seen after misonidazole treatment showed numerous signs of involution and were very similar to those described by Liu et al. (1975).

The morphologic evidence of the drug action coincides with the quantitative findings. The epidermal cells were larger and the epidermis was thicker when the animals were exposed to radiation alone. The drug also affected the non-irradiated epidermis, i.e., the keratinocytes' volume, as well as the thickness of the epidermis with misonidazole, were lower than the control values.

As previously reported (de Rey et al. 1979), irradiated keratinocytes show early changes in some morphometric indicators of cellular hyperactivity, i.e., increased basal cell volume and epidermal thickness that are quite evident after 72 h with 8 Krad. The cell hyperactivity has been considered as an attempt to bring about cellular recovery. After this acute phase, the cells either reverted to the initial normal condition or progressed showing changes in other morphometric indicators of cell degeneration or cell death. The present data indicate that the protracted action of misonidazole decreases the cell's capacity to react and possibly repair radiation-induced damage. The sensitization of the epidermis by this hypoxic sensitizer is probably due to the partially hypoxic condition of this tissue under normal circumstances (Brown 1975). The drug interferes with the usual cellular reparative reaction, triggered by ionizing radiation, and manifests itself at the morphological level by a lack of epidermal hypertrophy and hyperplasia. This effect is probably due to an uncoupling of oxidative phosphorilation which alters the generation of ATP, thus affecting the cell's repair capacity. The high doses employed, and the unconventional administration route of misonidazole, make it difficult to extrapolate some of these data to human medicine. The present data and especially the observations of a lack of reactive capacity of the drug-treated epidermis would indicate a toxic action of high doses of misonidazole on epidermal cells, even under conditions of normal oxygenation.

Acknowledgments

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