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CYTOPLASMIC AND MITOCHONDRIAL ISOENZYMES OF MALATEDEHYDROGENASE IN PSORIATIC EPIDERMIS

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Abstract. In order to study mitochondrial activity in psoriatic and uninvolved epidermis, and the effect of dithranol on mitochondria in psoriatic epidermis, analyses of malatedehydrogenase (MDH) isoenzymes in epidermal homogenates were made. MDH has both mitochondrial (M-MDH) and cytoplasmic (C-MDH) isoenzymes which can be separated electrophoretically. It was found that mitochondrial MDH could readily be shown in the supernatant of the homogenate of psoriatic epidermis while ultrasonic treatment of the homogenate of normal epidermis was necessary to release M-MDH from the mitochondria. Two bands of M-MDH were found in psoriatic epidermis but only one band in normal epidermis. In 2 psoriatic patients out of 14, a weak, more anodal, fraction of C-MDH was found. After 2 weeks of dithranol and steroid treatment, no effect on the M-MDH was found in the psoriatic epidermis.

Psoriasis is often regarded as a metabolic disease the exact nature of which is unknown. In spite of several extensive studies of different kinds, nothing specific about psoriasis has been shown conclusively (1). Psoriasis is probably an inherited disease, most likely dominant with incomplete penetrance of the gene. No genetic marker has been found, however, by which we can trace asymptomatic carriers of the "psoriatic gene". Several means, partially successful, are available for the treatment of psoriasis, though the mechanism of their action is not fully understood.

An inherited metabolic disease is generally due to the absence of an enzyme, which results in the accumulation or deficiency of a certain metabolite. An enzyme may, however, occur in different molecular forms, so-called isoenzymes, which catalyze the same chemical reaction but may be separated, for instance by electrophoresis. Diseases may be caused by a lack of one such isoenzyme (13). Isoenzymes are genetically determined and a characterization of isoenzymes may be valuable in genetic studies of patients with inherited diseases.

The intracellular localization or origin of different isoenzymes may vary. Malatedehydrogenase (MDH), isocitric acid dehydrogenase (ICDH) and glutamate-oxaloacetate-transaminase (GOT) have one or several isoenzymes that are mitochondrial and one or several cytoplasmic, non-mitochondrial, isoenzymes. To study the relative activities of mitochondrial and cytoplasmic isoenzymes thus may give some information about mitochondrial activity. Red blood cells have naturally no mitochondrial isoenzymes.

One of our most effective agents against psoriasis, dithranol, has been shown to form molecular complexes with deoxyribonucleic acid (DNA) (14) and to induce respiration-deficient mutants in yeast (7). There is no data to indicate that these mutations are in the mitochondrial DNA and not in the chromosomal DNA (16). It is therefore of interest to study the relative activities of mitochondrial and cytoplasmic isoenzymes with the assumption that the mitochondrial isoenzyme is coded by the mitochondrial DNA and the cytoplasmic isoenzyme by the chromosomal DNA, and that dithranol has a similar effect on epidermal cells as it has on yeast.

In the present study small pieces of isolated epidermis have been used. Initially it was planned to study the isoenzymes of MDH, ICDH and GOT. However, the activity of ICDH was too low for isoenzyme studies of such small samples. The activity of GOT is also somewhat low for such studies with the technique we used, but two fractions can sometimes be obtained.

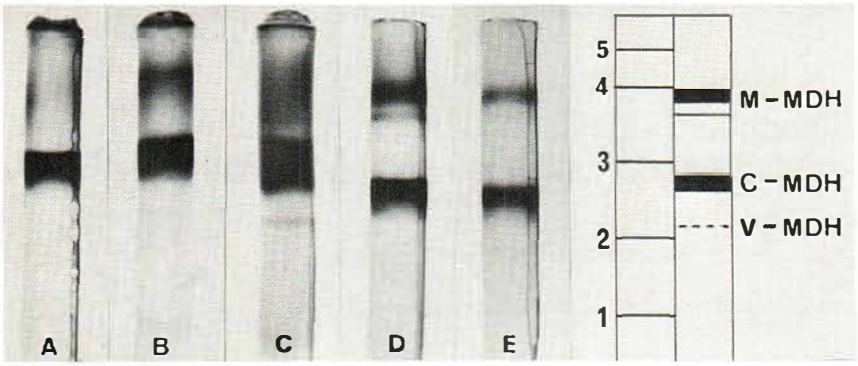

Fig. 1. Disc-electrophoretic separation of MDH. (*A–C*) belong to the first and (*D*) and (*E*) to the second series of experiments (see text). (*A*) Uninvolved epidermis from a psoriatic patient. Only the cytoplasmic MDH-fraction is seen. (*B*) Psoriatic epidermis with C-MDH and a diffuse M-MDH band. (*C*) Psoriatic epidermis with a variant band (V-MDH) underneath the C-MDH (*D*) Psoriatic epidermis. One major and one minor M-MDH fraction in addition to the C-MDH (*E*) Uninvolved epidermis from a psoriatic patient. C-MDH and only one M-MDH fraction are seen. The schematic drawing shows in the right column the different MDH-fractions in relation to the position of the LDH-fractions indicated in the left column.

MATERIAL AND METHODS

In all, 14 patients with psoriasis vulgaris were used in the present investigation. Epidermal specimens were taken from uninvolved skin and from psoriatic lesions before the treatment started. One side of the patient was treated with a dithranol paste (conc. 0.05–0.25 %) and the other half with a steroid ointment (Celestona val.). No UV-treatment was given during this period. Epidermal specimens were then taken from psoriatic lesions on both the dithranol- and the steroid-treated side.

Epidermis was separated in vivo by the suction blister method according to Kiistala & Mustakallio (10), removed and immediately frozen. As a rule the pressure within the suction cup of the Dermovac® was held at 200 mmHg for 1 to 1.5 hour before a blister was formed. The total wet weight of the epidermal specimens obtained was between 1 and 10 mg. The specimens were homogenized in a ground glass homogenizer with a small amount of phosphate buffer added. In the second series of experiments the samples were further treated in an ultrasonic disintegrator (MSE 100 W). Both types of homogenate were centrifuged 3 000 r.p.m. for 5 min and about 0.040 ml of the supernatant was subjected to disc electrophoresis.

In the first series of experiments the ultrasonic disintegrator was not used and separation was achieved by the usual disc-electrophoretic method of Davies (6). In the second series of experiments samples that were ultrasonically disintegrated were used and separation was made with a modified disc-electrophoretic method according to Clarke (3). With this method no large-pore gel was used. Instead, the sample was mixed with a sucrose solution and added to the electrophoresis tubes and subsequently covered with a small amount of small-pore gel. In both series the gel had a pH of 9,5. The visualization of the MDH fractions was achieved by the same method as that used by Goldberg (8).

RESULTS

In the first series of experiments with 10 patients before and after treatment for 2 weeks the specimens were homogenized only with a ground glass homogenizer and the usual disc-electrophoretic method was used. The samples obtained from the psoriatic epidermis showed, by this method, consistently 2 MDH fractions before treatment but also after treatment. The treatment had not given total healing although different degrees of improvement were evident. Neither the dithranol nor the flubenisolon-17a-valerate (Celestona val.) had affected the isoenzyme pattern. In the sample from the uninvolved epidermis only one MDH fraction was found in 8 patients but in 2 patients a very weak second fraction was also observed.

The consistently found MDH fraction occurred at the same position as lactate-dehydrogenase 3 (LDH 3) or slightly on the LDH 2 side of this fraction. This MDH fraction is generally regarded as the cytoplasmic or soluble isoenzyme of MDH and is called C-MDH. The second fraction found mainly in the psoriatic lesions occurs in the vicinity of LDH-4 but somewhat blurred. This isoenzyme is regarded as of mitochondrial origin and is called M-MDH.

In 2 of the 10 patients a third MDH fraction was observed in the samples from the psoriatic lesions. This fraction occurred close to the position of LDH-2.

In the second series of experiments with 4 psoriatic patients the epidermal specimens were further homogenized by ultrasonics and the modified disc-electrophoretic method according to Clarke (3) was used. This method gave a better separation both of LDH- and MDH-fractions. C-MDH occurred at a position between LDH-2 and LDH-3. The mitochondrial fraction of the sample from the psoriatic lesion consisted of one major band and one minor band on the anodal side of the major band. These M-MDH bands occurred in the vicinity of the LDH-4 fraction. In the sample from the uninvolved skin the major M-MDH band had nearly the same intensity as in the sample from the psoriatic epidermis but only in one patient was a minor M-MDH band observed. The ratio of the activity of the C-MDH and M-MDH in uninvolved and psoriatic epidermis seemed not to be significantly different. Normal epidermis from 4 non-psoriatic subjects showed a pattern similar to that of uninvolved epidermis of the psoriatic patients.

DISCUSSION

In the present study pure epidermal preparations obtained from suction blisters were used. Suction blistering occurs at the dermo-epidermal junction without profound alterations in the microscopic structure (10) or vitality (9) of the epidermis. The amount of tissue in each preparation was a few milligrams. With regard to the identity of the two major MDH bands I refer to earlier papers by other authors (4, 12).

From the results of the first series of experiments without ultrasonic disintegration of the specimens one was inclined to believe that there was practically no mitochondrial MDH in the normal or uninvolved epidermis, while the M-MDH was readily demonstrable in the preparations from the psoriatic lesions. When ultrasonic disintegration was used, about the same ratio of mitochondrial to cytoplasmic MDH-activity was obtained for normal and psoriatic epidermis. A very rough estimate of the activities indicates that about one-third of the total MDH-activity lies in the mitochondrial fraction. From these findings the following conclusion may be drawn: Either the "psoriatic" mitochondria are damaged and their mitochondrial enzymes have leaked out, or the larger amount of fibrillar material in the normal epidermal cells compared with the psoriatic cells protects the mitochondria during the homogenization in a ground glass homogenizer. Electron microscopic findings (2, 11) seem to support the latter explanation although a combination of the two mentioned factors cannot be ruled out.

Multiple forms of M-MDH have earlier been described in different animal species (15) and as a genetic variant in man (4). In the psoriatic epidermis at least two M-MDH fractions are generally found while usually only one M-MDH form is found in the normal or uninvolved epidermis. At present it is difficult to explain this difference between psoriatic and normal epidermis but it may be possible that a strong induction of, or requirement for, MDH may be necessary to achieve synthesis of the second form of M-MDH. There may be a similar explanation for the extra MDH fraction on the anodal side of the C-MDH observed in the psoriatic epidermis of 2 patients, although a genetic variant cannot be excluded. A rare genetic variant of C-MDH has earlier been described in this region of the isoenzyme pattern (5). The minor M-MDH band and the V-MDH fraction may of course also be due to the activity of proteolytic enzymes in the preparation or other factors that may be caused by the preparation procedure.

No effect of dithranol or steroids on the mitochondrial MDH was noticeable. Had an interaction of dithranol with DNA, giving a decrease in mitochondrial enzymatic activity, been of importance for the healing effect of dithranol, one would expect to see a decrease in M-MDH after only a week or two. It has, however, been suggested on a genetic basis (4) that the M-MDH is not coded by the mitochondrial DNA. Thus the findings of the present investigation do not rule out a direct effect of dithranol on mitochondrial DNA as the primary event in the healing of dithranol-treated psoriatic lesions.

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