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# Epidermis is the Origin of High Creatine Kinase Levels in Skin Blister Fluid

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**Creatine kinase (CK) isoenzyme, CK-BB, known as the brain fraction, is not normally present in serum but predominates in several normal and malignant tissues and body fluids. We recently reported increased CK-BB levels in suction blister fluid. In the present study the cellular origin of the enzyme in skin was studied from homogenates of blister top epidermis and blister base dermis as well as from homogenates of split skin dermatome shavings and isolated keratinocytes. The CK-BB in human skin was derived almost exclusively from the epidermis. Enzyme determinations from various spontaneous bullae suggest that all types of skin blisters initially contain high CK-BB levels.** ***Key words: Suction blister; Keratinocyte; Enzyme.***

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Creatine kinase (CK; EC 2.7.3.2) plays a central role in the energy metabolism of skeletal muscle, cardiac muscle and brain tissue. CK is a dimeric molecule constituted of two subunits: M (muscle type) and B (brain type) subunits. The dimeric enzyme molecule can be arranged in three different cytoplasmic isoenzymes: CK-MM, CK-BB, and CK-MB (the myocardial type) isoenzyme, a hybrid dimer consisting of one M subunit plus one B subunit (1). The total CK activity is highest in the skeletal muscle. CK-MM is dominant in skeletal muscle, MB in cardiac muscle and BB isoenzyme in brain tissue. The total activity of CK in other tissues is much lower (2–5).

In addition to nervous tissue, CK-BB predominates in fetal tissues, uterus, prostata and gastrointestinal tract (1, 6, 7). Elevated concentrations of CK-BB have been found in sera from patients having common forms of cancer including adenocarcinomas of the prostate, breast, kidney, stomach and lung, especially small cell carcinoma (8–11).

We recently reported the presence of BB-isoenzyme as a major component of CK in skin blister fluid (12). In this study, results of further experiments characterising the distribution and origin of CK-BB in skin are reported.

## PATIENTS AND METHODS

Blisters (7 mm in diameter) were produced by a suction device within two hours, using suction pressure of 200 mmHg on healthy skin of male dermatological patients, aged 20 to 25 years (13). The blister roofs consisting of pure epidermis were cut off after blister aspiration. A dermal sample was taken by a 6 mm punch biopsy from unroofed blister bases. The wet-weighed tissue samples were homogenized in 2 ml cold PBS with Ultra Turrax Tissue Homogenizer for two minutes. After centrifugation (10 000 rpm; 20 min) the supernatants were collected for enzyme analysis. Results are expressed as U/g of wet weight tissue.

Two split skin slices were harvested using an electric dermatome from an intact donor site of a 60-year-old skin transplant patient. A superficial skin slice included the epidermis and part of dermal papillae and the deeper slice included dermis and dermal portions of epidermal appendages. The samples were processed as described above.

For the epidermal cell cultures the blister roofs were trypsinized in 0.02% EDTA solution for 45 min at 37°C. A sample of dispersed keratinocytes was counted and homogenized. The rest of keratinocytes were cultivated on collagen coated dishes in MCDB-151 medium supplemented with human AB serum, hydrocortisone and antibiotics (14). The culture media were changed every third day and collected. After three weeks the confluent keratinocyte cultures were collected by trypsinization, counted and homogenized.

Fluid samples were collected from bullae which had developed spontaneously or following thermal injury in ten patients. In three occasions blisters or larger bullae had developed around common or venereal warts after cryotherapy. For storage experiments small aliquots of fluid from newly induced suction blisters from five subjects were incubated at either 37°C or at room temperature (20°C), with no additives, for periods of 2 to 24 hours.

All collected fluid and tissue samples were frozen until

Table I. *CK activity and distribution of CK-isoenzymes in epidermal and dermal homogenates and in superficial and deeper skin slices*

| | Epidermis $n=5$ | Dermis $n=5$ | Superficial skin $n=1$ | Deeper skin $n=1$ |
|---|---|---|---|---|
| CK-total | $20.0 \pm 9.4^{ab}$ | $0.6 \pm 0.9$ | 19.6 | 7.4 |
| CK-BB | $10.7 \pm 4.0$ | $0.4 \pm 0.7$ | 16.2 | 2.6 |
| CK-MM | $9.2 \pm 6.3$ | $0.2 \pm 0.2$ | 3.3 | 4.7 |
| CK-MB | 0 | 0 | 0 | 0 |

[a] U/g.
[b] Mean ± SD.

analysed (within one week) for total CK and its isoenzymes. Total CK was determined according to Scandinavian recommendations (15) with automatic analyser (Boehringer Mannheim/Hitachi 705, FRG) using reagents from J. T. Baker, Holland. After electrophoretic separation of isoenzymes on cellulose acetate, the fluorescing bands were scanned according to the manufacturer's instructions (Helena Laboratories, USA).

## RESULTS

### *CK and CK-isoenzymes in epidermis, dermis and skin slices*

The total CK activity in epidermal blister roof homogenates was $20.0 \pm 9.4$ U/g (mean ± SD) compared to $0.6 \pm 0.9$ U/g in dermal homogenates. The corresponding CK-BB activities were $10.7 \pm 4.0$ U/g and $0.4 \pm 0.7$ U/g, and CK-MM values $9.2 \pm 6.3$ and $0.2 \pm 0.2$ U/g, respectively (Table I). The total activity of CK was 19.6 U/g in surface skin layer homogenate compared to 7.4 U/g in deeper layer. CK-BB activity was 16.2 U/g in the superficial and 2.6 U/g in the deeper skin layer (Table I). Thus the proportion of CK-BB in the surface sample was 82.6%, and 35.1% in the deeper sample.

### *CK and CK-isoenzymes in keratinocyte cultures*

Neither keratinocyte culture media (day 3–27) nor homogenates of cultured keratinocytes (day 21) contained appreciable amounts of CK, although two trypsin-treated homogenates from freshly isolated keratinocytes did contain measurable amounts of CK activity. The CK-BB proportions in these keratinocyte homogenates were 41% and 67%, respectively (Table II). Culture medium contained no CK.

### *CK and CK-BB in spontaneous bullae*

In 11 spontaneous bullae the median proportion of CK-BB was 17%. When the data were arranged into two groups according to age of bullae, the median CK-BB was 53% in 2–24 hour bullae, and 2% in older bullae, which had been aspirated on days 2 or 4 after their formation. On two occasions no traces of CK-BB were detectable. On the other hand, the total CK level (median 166 U/l and 155 U/l) was approximately the same in both groups (Table III).

### *Effect of storage on CK and CK-BB activities*

When blister fluid samples were stored at 37°C for 6 hours the total CK activity was lowered from the 0-hour level to 9% and the CK-BB activity disappeared almost completely. Storage at room temperature caused a slower decrease and by 24 hours the mean corresponding residual CK and CK-BB activities were 21% and 13%, respectively (Table IV).

## DISCUSSION

Our recent study (12) appears to be the first report of the existence of CK-BB in human skin blister fluid, but it was not yet clear whether this isoenzyme was derived from the epidermis, dermis or both. The present observations strongly suggest that CK-BB is of epidermal origin and is released into dermal fluid from the epidermal keratinocytes.

Total CK and CK-BB concentrations in epidermal blister roof homogenates were clearly higher (about 30 times) than in dermal blister base extracts (20.0 and 10.7 vs 0.6 and 0.4 U/g, respectively) (Table I). Low dermal activities were probably derived from the dermal portions of epidermal appendages. This result is further substantiated by the difference of the total CK and CK-BB levels in the superficial and deep skin slices (Table I). These results obtained using two unrelated methods for skin splitting indicate that the origin of high CK and CK-BB levels in skin blister fluid is the epidermis. Our results do not exclude the

Table II. *Total CK activity and distribution of CK-isoenzymes in homogenates from isolated keratinocytes*

| | | CK (U/$10^6$ cells) | MM (%) | MB (%) | BB (%) |
|---|---|---|---|---|---|
| Before culture | Exp. 1 | 79 | 33 | 0 | 67 |
| | Exp. 2 | 59 | 59 | 0 | 41 |
| Culture day 21 | Exp. 1 | <1 | – | – | – |
| | Exp. 2 | 0 | – | – | – |

Table III. *Total CK and CK-BB activities in early bullae (patients 1–5) and old bullae (patients 6–10)*

| Patient no. | Condition (site) | Blister age | CK (U/l) | CK-BB (%) |
|---|---|---|---|---|
| 1 | Frictional[a] (foot) | 2 h | 2 675 | 75 |
| 2 | Dyshidrotic (hand) | <1 day | 166 | 53 |
| 3 | Bullous impetigo (wrist) | 1 day | 277 | 79 |
| 4 | Burn blister (thigh) | 1 day | 66 | 22 |
| 5 | Cryoblister (hand) | 1 day | 163 | 20 |
| Median | | | 166 | 53 |
| 6 | Frictional[a] (foot) | 2 days | 140 | 17 |
| 7 | Cold blister (ear lobe) | 2 days | 174 | 2 |
| 8 | Cryoblister (hand) | 2 days | 96 | 0 |
| 9 | Cryoblister (penis) | 2 days | 195 | 0 |
| 10 | Burn blister (hand) | 2 days | 113 | 6 |
| 10 | Burn blister (hand) | 4 days | 170 | 2 |
| Median | | | 155 | 2 |

[a] Epidermolysis bullosa simplex; blister provoked by 1 h march.

Table IV. *Effect of sample storage on total CK and CK-BB activities at 37°C and at room temperature*

ND = not determined

| | Exp. | | Storage time 0 h (U/l) | 2 h | 6 h | 12 h | 24 h |
|---|---|---|---|---|---|---|---|
| | | | | (percent of 0-hour activity) | | | |
| Total CK | 1 | 37°C | 221 | ND | 8 | 5 | 1 |
| | 2 | 37°C | 286 | ND | 6 | 5 | 0 |
| | 3 | 37°C | 75 | 33 | 13 | ND | ND |
| | 4 | 20°C | 170 | ND | 82 | 76 | 18 |
| | 5 | 20°C | 280 | ND | 89 | 43 | 25 |
| CK-BB | 1 | 37°C | 134 | ND | 0 | 0 | ND |
| | 2 | 37°C | 209 | ND | 0 | 0 | ND |
| | 3 | 37°C | 31 | 16 | 2 | ND | ND |
| | 4 | 20°C | 96 | ND | 75 | 77 | 14 |
| | 5 | 20°C | 239 | ND | 80 | 28 | 12 |

potential presence of minor amounts of CK-MM in the epidermis.

Fresh homogenates of trypsinized blister roof keratinocytes contained CK and its major fraction was CK-BB. Whether the minor CK-MM fraction in keratinocyte extracts was a contamination from dermal fluid, could not be established. On the other hand, cultured keratinocytes and the culture media were not found to contain appreciable amounts of CK (Table II). Keratinocyte cultures initiated from suction blister roof epidermis are composed only of epidermal cells lacking any dermal cellular components. These preliminary culture experiments suggest that keratinocytes in vitro, devoid of dermal influence and under the employed conditions may not be able to synthetize detectable amounts of CK or its isoenzymes. An alternative explanation is that because of rapid inactivation of extracellular CK, freshly synthetized CK-BB becomes immediately destroyed in culture fluid.

Storage of blister samples at 37°C caused rapid activity decrease of total CK and particularly of CK-BB, but slower declines at room temperature (Table III). These results on extravascular skin fluid are in agreement with reports indicating that CK-BB is thermolabile and loses activity rapidly at 37°C (16). Also, CK-BB is cleared rapidly from blood, having a biological half-life of only about 3 h (1).

While clearly detectable in 2–24 h old bullae, CK-BB was practically lacking in older spontaneous bullae. The highest CK level and a high proportion of CK-BB was found in an unbroken shallow 3 cm wide friction blister. This bulla, aspirated soon after its formation, was deliberately provoked by a one-hour march in a young conscript whose suspected clinical diagnosis of epidermolysis bullosa simplex was confirmed by the natural friction test (Table III). In fluid from old spontaneous bullae CK-MM was consistently the predominant component, and in cases showing no CK-BB, CK-MM comprised almost 100% of total CK activity as in serum. Robson & Heggers (17), who also studied CK isoenzyme distribution by electrophoresis in frostbite blister fluid in 10 patients, found consistently only the CK-MM component. However, in view of our present results, all types of skin blisters are likely to contain CK-BB provided the fluid is taken soon enough after blister formation.

In about half of the spontaneous bullae and also in suction blisters there was a minor extra band, migrating cathodal to the CK-MM band. This band may represent a mitochondrial CK, which is an atypical band migrating at this region and is occasionally seen

in serum samples (18). Adenylate kinase, which could be an alternative explanation for this band, was tested in three samples containing this cathodic band but was not demonstrable (15, 19).

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