Hydrolysis of Hydrocortisone 17-Butyrate 21-Propionate by Cultured Human Keratinocytes

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Hydrolysis of 17-butyrate 21-propionate (HBP) by human keratinocytes was studied using an in vitro system. In the culture of human keratinocytes 1.308 nmole/ml of hydrocortisone 17-butyrate (de-esterified HBP at the position of 21) was found at 1 h after the addition of 10 nmole/ml HBP. At 6 h most of HBP was de-esterified to become HB17 and a small amount of hydrocortisone 21-propionate (de-esterified HBP at the position of 17) was detected. Hydrocortisone (HC) was not detectable. These results indicate that when HBP is applied on the skin, it is hydrolyzed at the position of 21, and reaches the dermis where it finds the way to get into the systemic circulation. The intracellular concentration of HBP was estimated to be more than 5 times its extracellular concentration and its accumulation was higher than HC. These results were discussed in relation to the potentiation of local antiinflammatory action and the reduction of systemic effects of topical glucocorticoid.
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The topical corticosteroid therapy of skin aims at stronger antiinflammatory action on the skin and weaker systemic effects. It is known that esterification at 17- and 21-position of glucocorticoid enhances the local action by increasing the lipophilicity, and it is considered that the compound is converted to a physiological glucocorticoid by the metabolism in the skin.

Hydrocortisone 17-butyrate 21-propionate (HBP) is a synthetic glucocorticoid developed as a topical antiinflammatory drug (Fig. 1). The study of enzymatic hydrolysis of HBP using skin extract has shown that the acyl group of 21-position is primarily hydrolysed, that the substituent of 17-position is converted non-enzymatically to 21-position and that the compound is finally hydrolysed to hydrocortisone (Fig. 1) (1). After topical application the drug passes through the epidermis and reaches the dermis where it finds its way into the systemic circulation. It is necessary but not always easy to know how the drug is metabolized in the epidermis. The cultured keratinocytes can be used as a substitute of the epidermis in vivo (2). In this work, we studied the metabolism of glucocorticoid, especially the hydrolysis of the esters at 17- and 21-position by cultured human keratinocytes.

MATERIALS AND METHODS

Culture of human keratinocytes

Keratinocytes from adult human skin were isolated and cultured as described previously (3).

The human skin was treated with 0.25% trypsin (Difco) in Ca^{2+}- and Mg^{2+}-free phosphate buffered saline for 12 to 24 h in a refrigerator (4°C). An epidermal cell suspension was obtained by dissociating the epidermal sheet which had been peeled off the dermis with fine forceps. The culture was initiated by seeding 8×10^5 basal cells suspended in 4 ml of culture medium into a 60×15 mm plastic tissue culture dish. This was incubated in an atmosphere of 95% air and 5% CO_2 at 37°C. The

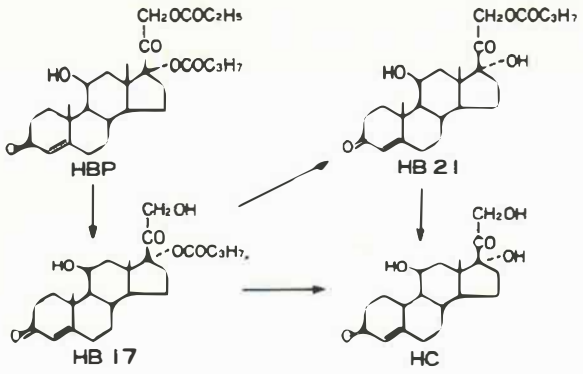

Fig. 1. Chemical structure and metabolic pathway of HBP and its metabolites.

medium consisted of Eagle's minimum essential medium (MEM) supplemented with 20% fetal bovine serum.

Treatment with steroids and HPLC analysis

HBP was a gift from Taisho Pharmaceutical Co. Hydrocortisone (HC) was purchased from Sigma Chemical Co. The stock solutions of steroids were prepared in absolute ethanol at 5×10^{-3} M, diluted with MEM to the final concentration of 10^{-5} or 10^{-6} M, and added to the 20 to 30 day-cultures which consisted of fully developed stratified sheets of keratinocytes showing various stages of differentiation (4). These cultures were washed four times with MEM and then MEM containing steroids was added. The MEM containing steroid was taken, the culture was washed with three changes of phosphate-buffered saline, and the cells were scraped off the dishes after a specified time of incubation.

For HPLC analysis 2.0 ml of MEM containing steroid was mixed with 10 ml of ethyl acetate, and was shaken vigorously for 10 min. The cells were homogenized in 2.0 ml of MEM and were mixed with 10 ml of ethyl acetate. 5.0 ml of the organic phase was taken, and dried under a nitrogen stream.

The dried samples were dissolved in 0.1 ml methanol with 10^{-5} M diphenyl as an internal standard, and 0.02 ml of them were injected into a Hitachi 4×15 mm column packed with Nucleosil 5 C18, and eluted with methanol/water/acetate (54.4/45/0.5 by volume) at a flow rate of 0.5 ml/min at room temperature. The HPLC system consisted of a Hitachi model 655 liquid chromatograph with variable wavelength UV monitor. The identification of the peak was established by absorbance at 254 nm of the chromatographed standard compounds. The integrated peak areas were determined by chromatopac C-R1A (Shimadzu). Known amounts of each compound were dissolved in methanol, and subjected to HPLC analysis. Calibration curve (area versus concentration) was made and the amounts of HBP, hydrocortisone 17-butyrate (HB17), hydrocortisone 21-butyrate (HB21) and HC were calculated by comparing the area value of HPLC profiles.

Determination of cell volume

The intracellular water volume was estimated by measuring the equilibrium uptake of ^{3}H-3-O-methyl-D-glucose (90 Ci/mM, New England Nuclear). The culture of keatinocytes was incubated with 1 μCi/ml of ^{3}H-3-0-Methyl-D-glucose in medium for 20 min, removed from the medium, and rapidly washed three times with ice-cold phosphate buffered saline. The cells were then digested at 37°C with 0.25 ml 1 N NaOH for 1 h. 0.1 ml aliquot of the medium and the solubilized cells were mixed with a scintillation cocktail (toluene containing 0.6% PPO and 0.025% POPOP) with the aid of NCS tissue solubilizer, and was counted in an Isocap liquid scintillation spectrometer.

RESULTS

Effect of serum

HBP can be hydrolyzed by serum. Therefore fetal bovine serum in the culture medium was evaluated for its esterase activity to hydrolyse HBP (Table I). At 1 h after the addition of 1 nmole/ml HBP, 0.116 nmole/ml of HB17 was formed in MEM supplemented with 20% fetal bovine serum. This was about 40% of HB17 in MEM found in the culture of human

Table I. *Metabolites of HBP (1 nmole/ml) in the culture medium (nmol/ml)*

HK = human keratinocyte, MEM = Eagle's MEM only, MEM/20 = Eagle's MEM c 20% fetal bovine serum, nd = not detected

		HBP	HB21	HB17	HC
HK	1 h	0.295	nd	0.278	nd
MEM	1 h	0.501	nd	nd	nd
MEM/20	1 h	0.496	nd	0.116	nd
HK	6 h	nd	nd	0.470	nd
MEM	6 h	0.625	nd	nd	nd
MEM/20	6 h	0.189	nd	0.387	nd

Table II. *Metabolites of HBP (10 nmole/ml) in the culture medium of human keratinocytes (nmol/ml)*

HK = human keratinocyte, MEM = Eagle's MEM only, nd = not detected

		HBP	HB21	HB17	HC
HK	1 h	4.257	nd	1.308	nd
MEM	1 h	5.174	nd	nd	nd
HK	6 h	1.521	0.127	3.512	nd
MEM	6 h	4.498	nd	0.393	nd

keratinocytes. Therefore four washes of the culture with MEM were done strictly before the addition of HBP to the culture, whereas no HB17 was detected in MEM.

Metabolism of HBP and HC by keratinocyte

Table II shows the amounts of HBP and its metabolites in the medium after the addition of 10 nmole/ml HBP. At 1 h 1.308 nmole/ml of HB17 was found in the culture of human keratinocytes. At 6 h most of HBP was deesterified at the position of 21 to become HB17, and a small amount of HB21 was also detected in the culture. HC was not detectable. When HBP was incubated in MEM, no metabolite of HBP was found at 1 h and 0.393 nmole/ml of HB17 was found at 6 h. Decomposition of HC in the culture of keratinocytes, which was examined by taking samples at 24 and 48 h after the addition of 10 nmole/ml HC, was very slight until 48 h. The concentration of HC was slightly less in the culture than in MEM porobably due to the uptake by the cultured keratinocytes which was not included in the regular measurement of the HBP metabolites (Table III).

Table III. *Amount of HC (10 nmole/ml) in the culture medium of human keratinocytes (nmole/ml)*

HK = human keratinocyte, MEM = Eagle's MEM only

		HC
HK	24 h	7.184
MEM	24 h	7.943
HK	48 h	6.974
MEM	48 h	8.031

Table IV. *Intracellular and extracellular distribution of HBP (HB17) and HC in the culture of human keratinocytes at 10 min of incubation at 0°C after the addition of 1 nmole/ml HBP or HC*

Cell: nmole/dish, MEM: nmole/ml, nd = not detected

	HBP	HB21	HB17	HC
HBP, 1 nmole/ml				
Cell	0.084	nd	0.096	nd
MEM	0.919	nd	nd	nd
HC, 1 nmole/ml				
Cell	nd	nd	nd	0.023
MEM	nd	nd	nd	0.494

Uptake and intracellular concentration

The uptake of HBP and HC in keratinocytes was estimated by the determination of the intracellular HBP and HC. Table IV shows the distribution of steroids in the cells and MEM after treatment for 10 min at 0°C. The estimated intracellular water volume by the uptake of ^{3}H-3-0-methyl-D-glucose was 0.0348 cm^3/dish. When 1 nmole/ml HBP was added to the culture, 0.181 nmole of HBP was taken up by the cells (sum of HBP and HB17). The intracellular concentration was estimated to be 5.19 nmole/cm^3. When 1 nmole HC was added to the culture, 0.023 nmole HC was taken up by the cells, and the intracelular concentration was estimated to be 0.671 nmole/cm^3. The ratio of intracellular-/extracellular concentration was 5.64 in HBP and 1.36 in HC. The intracellular concentration of HBP was 7.7 times higher than that of HC.

DISCUSSION

The process of enzymatic hydrolysis of hydrocortisone 17, 21-diesters is as follows (1): the acyl group of 21-position is primarily hydrolyzed, thereafter the substituent of 17-position is translocated nonenzymatically to 21-position and then the compound is finally hydrolyzed to hydrocortisone (Fig. 1). The hydrolysis of HBP by cultured keratinocytes progressed rapidly to formation of HB17. A small amount of HB21 was detected after prolonged incubation but HC was not detectable. To exclude the possibility that HC may be destroyed immediately after its formation, 10 nmole/ml HC was incubated with the keratinocytes. A slight decrease of the concentration of HC in MEM with keratinocyte was observed between 24 and 48 h. It is conceivable that HBP applied to the skin is absorbed and converted to HB17 in the epidermis, which reaches the dermis.

It is generally assumed that steroids are transported across the cell membrane by simple diffusion (5). The lipophilicity of the steroids accounts for the ratio of intracellular and extracellular concentration (6). The study of the intracellular concentration of the steroids showed the accumulation of HBP and HC in the keratinocytes. The intracellular concentration of HBP was more than five times of the extracellular concentration, and was higher than that of HC.

The potency of hydrocortisone molecule can be increased by some modifications. Esterification of glucocorticoid at the position of 17 and 21 enhances the local antiinflammatory action by increasing the lipophilicity. It is desirable that the esterified portions are removed before HBP gets into systemic circulation. This study showed that epidermal keratinocytes de-esterify HBP at the position of 21 but not at the position of 17. All these

results further support feasible characteristics of HBP as a glucocorticoid for topical cutaneous use.

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REFERENCES

1. Misaki T, Yamada M, Hirai R, Komori M, Washitake M, Ozawa Y, Koyama I, Yoshizawa H. Enzymatic hydrolysis of hydrocortisone diesters in skin. Yakuzaigaku 1982; 42: 92 (in Japanese).
2. Kitano Y, Okada N, Nagase N. Metabolism of an aromatic retinoid Ro 10-9359 by cultured human epidermal keratinocytes. Arch Dermatol Res 1982; 273: 327.
3. Kitano Y, Okada N. The course of culture of human keratinocytes: Increase of heat-labile fraction of glucose-6-phosphate dehydrogenase. Acta Derm Venereol (Stockh) 1981; 61: 385.
4. Kitano Y, Endo H. Differentiation of human keratinocytes in cell culture. In: Seiji M, Bernstein IA, eds. Biochemistry of cutaneous epidermal differentiation. Tokyo: University of Tokyo Press. 1977; 319.
5. Giorgi EP, Stein WD. The transport of steroids into animal cells in culture. Endocrinology 1981; 108: 688.
6. Ponec M, Kempenaar JA. Biphasic entry of glucocorticoid into cultured human skin keratinocytes and fibroblasts. Arch Dermatol Res 1983; 275: 334.