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Effects of Mometasone Furoate on Human Keratinocytes and Fibroblasts in vitro

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Abstract

The long-term treatment of inflammatory skin diseases with topical glucocorticoids is limited by their side effects such as skin atrophy, delayed wound healing and striae distensae. Mometasone furoate (MF) is a newly synthesized glucocorticoid with the advantage of increasing efficacy and reducing the number of adverse effects. The aim of our study was to compare the effects of MF and conventional fluorinated corticosteroids on a human keratinocyte cell line (HaCat) and human skin fibroblasts in vitro. Monolayer cultures of these cell lines were exposed to different concentrations of the active compounds for 5 days to analyze the influence on morphology and proliferation. Chemotaxis of HaCat cells and fibroblasts was studied in blind-well Boyden chambers using collagen type I and fibroblast-conditioned medium as a chemoattractant. Additionally, fibroblasts were used to investigate the contraction of collagen gels since lattice contraction appears to model the contraction of skin wounds. All glucocorticoids tested influenced fibroblast and keratinocyte proliferation in a dose-dependent manner, yet the effect was clearly more marked with fluorinated corticosteroids than with MF. Similar effects were obtained using the chemotaxis assay. At low concentrations (10^{-9} *M*) MF exerted almost no influence, while the conventional fluorinated substances inhibited direct migration significantly. Contraction of collagen gels was inhibited completely by betamethasone valerate at high concentrations (10^{-5}–10^{-3} *M*), but only partially inhibited by MF at its highest concentration (10^{-3} *M*). Although MF reveals high anti-inflammatory activity similar to that known for conventional fluorinated derivatives of corticosteroids, the study shows that MF has less effect in the tested in vitro systems. Therefore, it remains to be seen whether these data might indicate the possibility of a dissociation between the inflammatory activity and the inhibition of the biosynthetic capacities of fibroblasts and keratinocytes by modification of the steroidal structure of corticosteroids.

Key Words

Glucocorticosteroids
Mometasone furoate
Keratinocytes
Fibroblasts

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Introduction

Topical corticosteroids are very potent drugs for the treatment of many acute and chronic inflammatory skin diseases. A variety of steroids have been developed for clinical use. Corticosteroids can be divided into different subgroups according to their potency, and there is a correlation between clinical efficacy and the risk of side effects. Therefore, long-term topical application of potent corticosteroids often leads to skin atrophy, delayed wound healing and striae distensae [1–3].

The basis of this phenomenon has been intensively investigated in recent years. Cutaneous atrophy following topical corticosteroid use appears to result from an inhibition of collagen and glycosaminoglycan synthesis [4, 5]. Additionally, it is a well-established fact that corticosteroids can inhibit cell proliferation and mitosis and have an inhibitory effect on general protein synthesis of keratinocytes and dermal fibroblasts [6]. Corticosteroids can also interfere indirectly with the biosynthetic capacities of these cells. This is due to the fact that corticosteroids affect the inflammatory system and inhibit the migration of inflammatory cells during inflammatory reactions [7].

Therefore, the ideal corticosteroid would be a potent agent with a low risk of adverse effects [8]. In recent years, a number of new corticosteroids have been developed. Mometasone furoate (MF) has been introduced as a very potent corticosteroid associated with a low risk of systemic and topical side effects [9, 10]. In the past, many clinical studies confirmed the efficacy in inflammatory skin diseases accompanied by low and rare adverse effects [11–16]. Until now, there have been only a few in vitro and in vivo investigations which may explain the effects of MF. Therefore, it was the aim of our study to use in vitro systems which allow the examination of the influence of various drugs on the biosynthetic capacities of keratinocytes and fibroblasts, and to obtain additional data concerning both newly developed corticosteroids and conventional fluorinated corticosteroids.

Material and Methods

Corticosteroids

Following corticosteroids were used: MF (Essex, München, FRG), hydrocortisone (Sigma, FRG), clobetasol-17-propionate (Glaxo, Bad Oldesloe, FRG), and betamethasone-17-valerate (Essex, München, FRG). Corticosteroids were dissolved in 40% ethanol and then added to the cell medium. The maximum concentration of the solvent was 1%. Equivalent amounts of ethanol were used for controls.

Cell Culture

Human dermal fibroblasts were established from biopsies obtained from a normal adult donor and used in 4th to 10th passages. The long-lived human keratinocyte cell line HaCat was kindly provided by Prof. Fusenig, Heidelberg, Germany, who established and characterized this cell line [17]. The cells were kept in Dulbecco's Minimal Essential Medium (DMEM) supplemented with penicillin (400 U/ml), streptomycin (50 µg/ml), glutamine (300 µg/ml), and 10% fetal calf serum in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. To exclude the influence of intrinsic corticosteroid contained in fetal calf serum, we filtered the fetal calf serum with an activated charcoal filter. Cultures were examined by phase contrast microscopy.

Proliferation

Fibroblasts and keratinocytes were incubated with various corticosteroids and a portion was harvested by trypsinization. Cells were counted daily in a Coulter counter. Cell viability was examined using the trypan blue exclusion test [6].

Chemotactic Activity

The chemotactic response was studied in blind-well Boyden chambers with and without corticosteroids. Polycarbonate filters (13 mm in diameter, 8 µm pore size, Nuclepore Corp., Pleasonton, Calif., USA) were coated with gelatine (5 mg/l) as described by Postlethwaite et al. [18]. The lower compartment of the

chambers was filled with fibroblast-conditioned medium as a chemoattractant for fibroblasts or with collagen type I (100 ng/ml in DMEM) as a chemoattractant for HaCat cells, and the gelatine-coated filter was placed above. Fibroblast-conditioned medium was obtained as described previously [19] from confluent dermal fibroblasts which were incubated for 24 h and supplemented with serum free medium. Corticosteroid-treated cells were preincubated 4 h or 3 days with various corticosteroids dissolved in ethanol and then suspended in serum-free DMEM and added to the upper compartment of the chamber (4×10^5 cells/ml); controls were treated with equal concentrations of ethanol. After 4-hour incubation at 37°C, 95% air and 5% CO_2 filters were removed. Cells on the upper side of the filters were removed mechanically, and cells which had migrated to the lower side were fixed with methanol, stained with hematoxylin-eosin and counted in a 200-fold magnification field of a Leitz microscope. Each sample was assayed in triplicate, and cells were counted in five unit fields.

Contraction of Collagen Gels

Collagen gels were assayed according to Mauch et al. [20]. The lattices were prepared in bacteriological Petri dishes combining 1.6 ml of a ×1.5 concentrated medium containing fetal calf serum with 0.4 ml collagen solution (3 mg/ml) and 0.4 ml of cell suspension (1.5×10^5/ml). Different concentrations of glucocorticosteroids were added prior to the assay. The mixture polymerizes within 20 min at 37°C. The contraction of the collagen gel was analyzed by measuring the diameter of the collagen disk over time.

Results

Effects of MF and Other Glucocorticosteroids on Growth and Morphology of Human Dermal Fibroblasts and Keratinocytes

Glucocorticosteroids were added in various concentrations (10^{-5}, 10^{-7}, 10^{-9} *M*) to human fibroblasts and keratinocytes (HaCats). When cultures were examined by phase contrast microscopy, no alteration of cell morphology could be observed. Cell viability tested using the trypan blue test was not changed by corticosteroid treatment, even when used in high concentrations (data not shown).

With respect to cell proliferation corticosteroids reduce cell growth in a dose-dependent manner (fig. 1). High concentrations of corticosteroids (10^{-5} *M*) inhibit cell proliferation more than low concentrations (10^{-9} *M*, data not shown). However, this effect was clearly more marked with fluorinated glucocorticosteroids than with MF. While betamethasone showed a significant inhibitory effect at a low concentration (10^{-9} *M*) after 5 days, MF and hydrocortisone did not influence fibroblast or keratinocyte proliferation at the same concentration.

Effects of MF and Other Glucocorticosteroids on Chemotaxis of Fibroblasts and Keratinocytes

To investigate whether corticosteroids were themselves chemoattractants for fibroblasts or keratinocytes, cells were tested with 10^{-5}, 10^{-7}, 10^{-9} *M* of the corticosteroids. None of the substances were able to induce chemotactic migration (data not shown).

Using the Boyden chamber assay with fibroblast-conditioned medium as a chemotactic stimulus, we determined the influence of corticosteroids on the chemotactic activity of dermal fibroblasts and human keratinocytes. At high concentration (10^{-5} *M*) all corticosteroids tested decreased chemotaxis of fibroblasts (fig. 2). Betamethasone and clobetasol showed a potent inhibitory effect of 50% chemotaxis reduction, while MF and hydrocortisone reduced migration in only 30% of the controls. At low concentration (10^{-9} *M*) migration of fibroblasts is significantly decreased by conventional fluorinated corticosteroids (betamethasone, clobetasol). In contrast, MF and hydrocortisone had no effect at low concentration (fig. 2).

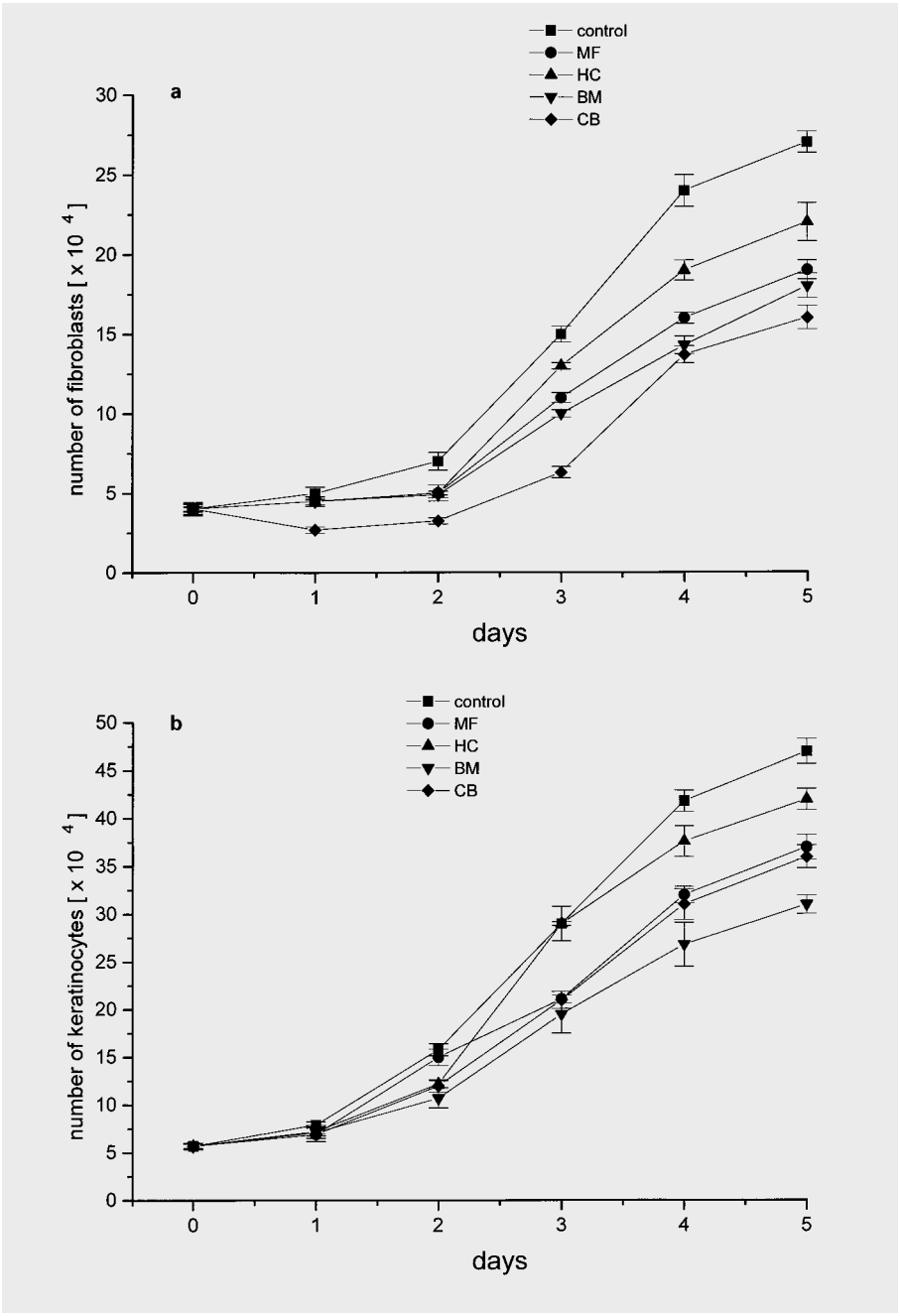
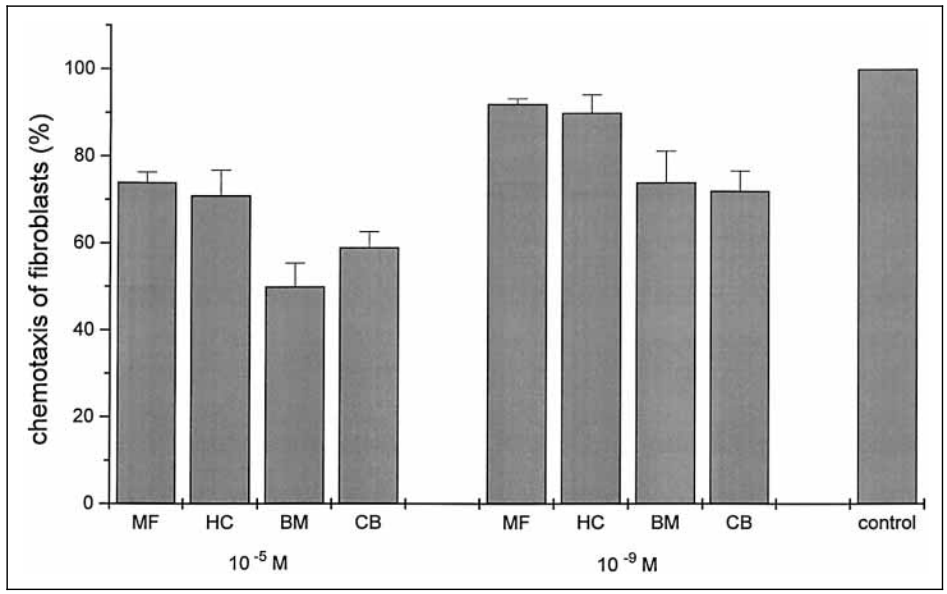


Fig. 1. Cell proliferation. High concentrations of glucocorticosteroids (10^{-5} *M*) inhibited cell proliferation of fibroblasts (**a**) and HaCat keratinocytes (**b**) more than low concentrations (10^{-9} *M*, data not shown) in a dose-dependent manner over a period of 5 days. HC = Hydrocortisone; BM = betamethasone; CB = clobetasol.



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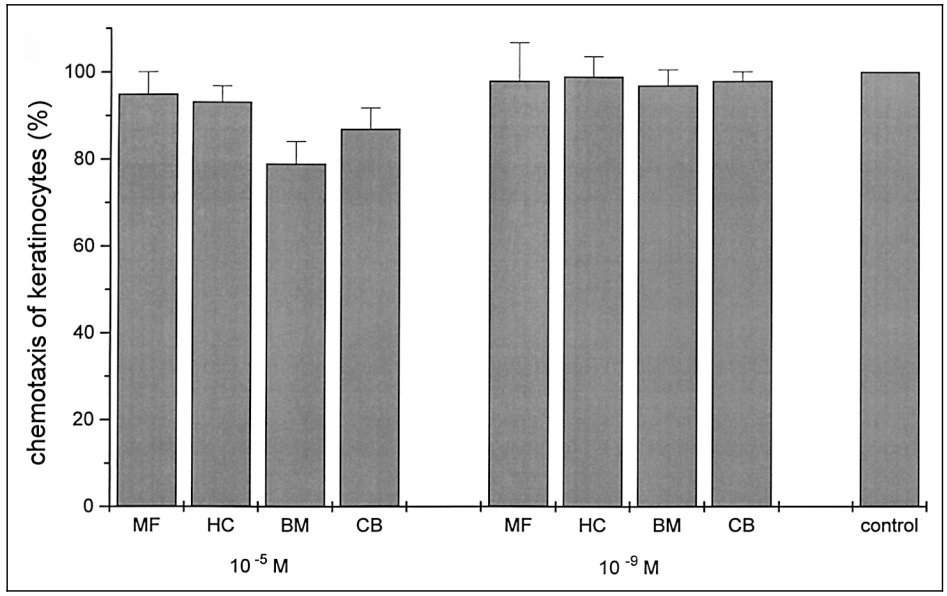

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Fig. 2. Chemotaxis of human fibroblasts. Chemotaxis to fibroblast-conditioned medium was inhibited by glucocorticosteroids in a dose-dependent manner. Cells were exposed for 3 days or 4 h (data not shown) to various compounds at concentrations of 10^{-5} and 10^{-9} *M*. The chemotactic activity is expressed as the percentage of response of untreated controls (100%) For abbreviations see figure 1.

Fig. 3. Chemotaxis of HaCat cells. Using high concentration (10^{-5} *M*) of glucocorticoids, only betamethasone and clobetasol decreased chemotactic response of HaCat cells to collagen type I as a chemoattractant. Lower concentrations (10^{-9} *M*) did not influence the chemotaxis of keratinocytes significantly. For abbreviations see figure 1.

The chemotactic migration of keratinocytes was not influenced by any of the corticosteroids at low concentration (10^{-9} *M*, fig. 3). Using 10^{-5} *M*, only betamethasone inhibited keratinocyte migration, while MF and hydrocortisone were not active (fig. 3).

Effects of MF and Other Glucocorticosteroids on Contraction of Collagen Gels

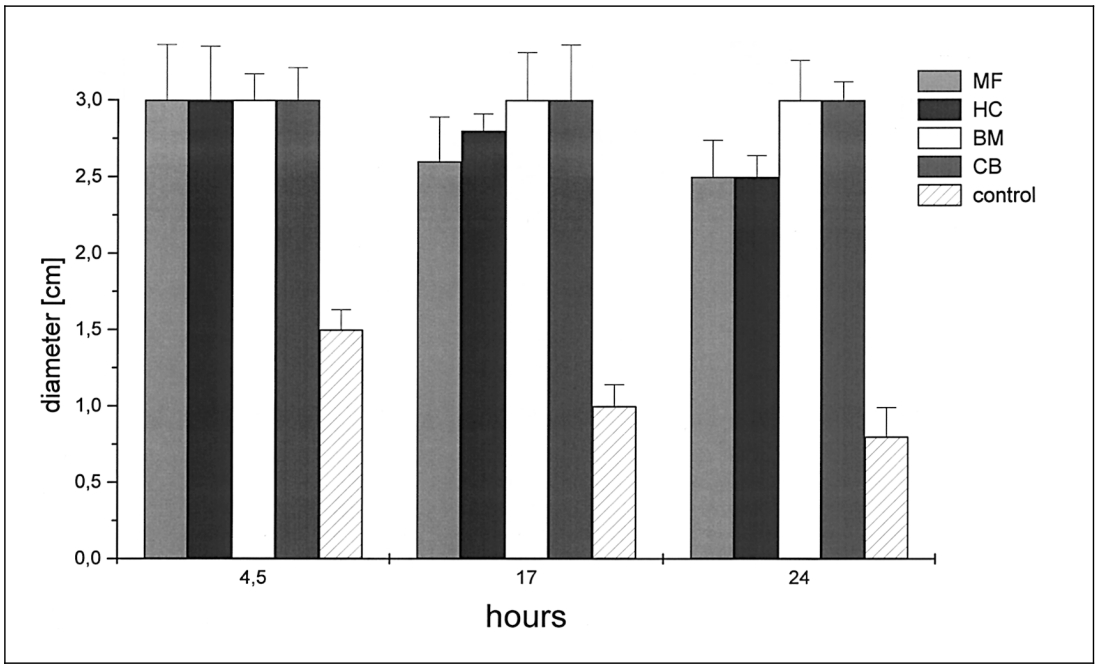
When human fibroblasts are seeded in Petri dishes containing a collagen solution, fibroblasts contract the collagen gel over time. At 10^{-3} *M* collagen gel contraction was completely inhibited by betamethasone, but only partially by MF or hydrocortisone (fig. 4a). All glucocorticosteroids reduced contraction of collagen gels after 4.5 h at a concentration of 10^{-5} *M* (fig. 4b). After 24 h ethanol-treated fibroblasts decreased the diameter of collagen gels to 80% contraction, while corticosteroid-treated fibroblasts decreased collagen gel contraction to only 50% contraction. No significant difference between the tested corticosteroids could be observed.

Discussion

Conventional fluorinated glucocorticosteroids are known to induce strong anti-inflammatory activity, but this effect is accompanied by the risk of side effects [1–3]. In the past, clinical trials have shown that the anti-inflammatory potential of MF is similar to triamcinolone [13, 14] and stronger than the efficacy of betamethasone [9, 10]. Therefore, MF was introduced as a member of the subgroup of glucocorticosteroids with high clinical efficacy. In contrast to conventional potent glucocorticosteroids, MF is thought to be a glucocorticosteroid with low risk of skin atrophy, delayed wound healing and other complications [11–16] comparable to hydrocortisone. Belsito et al. [21] reported a relative lack of systemic effects of MF on Langerhans cells in mice after topical application as compared to other glucocorticoids.

Skin atrophy and delayed wound healing are results of disturbed cell function and metabolism of fibroblasts and keratinocytes. Conventional corticosteroids are well-known inhibitors of fibroblasts and keratinocyte proliferation and mitosis [6]. As shown in prior experiments all glucocorticosteroids in our study inhibited fibroblast and keratinocyte proliferation in a dose-dependent manner. However, at low concentrations hydrocortisone and MF had no influence on cell proliferation, while betamethasone and clobetasol showed a significant inhibitory effect. The inhibition of cell proliferation is not caused by toxicity of the corticosteroids since cell morphology and cell viability are not influenced.

The chemotactic response of fibroblasts and keratinocytes to various chemoattractants is thought to control the tissue repair process during wound healing, and is probably also important for the continuous remodelling of connective tissue. In our experiments corticosteroids were shown to reduce chemotaxis of keratinocytes and fibroblasts without influencing random migration. These results are in agreement with other reports [7]. Chemotaxis of fibroblasts was inhibited more than migration of keratinocytes even at low concentrations of potent corticosteroids and might, therefore, play an important role in explaining delayed wound healing which is often observed during systemic or local treatment with corticosteroids. At high concentrations betamethasone and clobetasol showed a stronger inhibitory effect than hydrocortisone and MF. Additionally, betamethasone and clobetasol inhibited cell migration of fibroblasts and keratinocytes significantly even at low concentrations, while MF and hydrocortisone had no effect at the same concentration.



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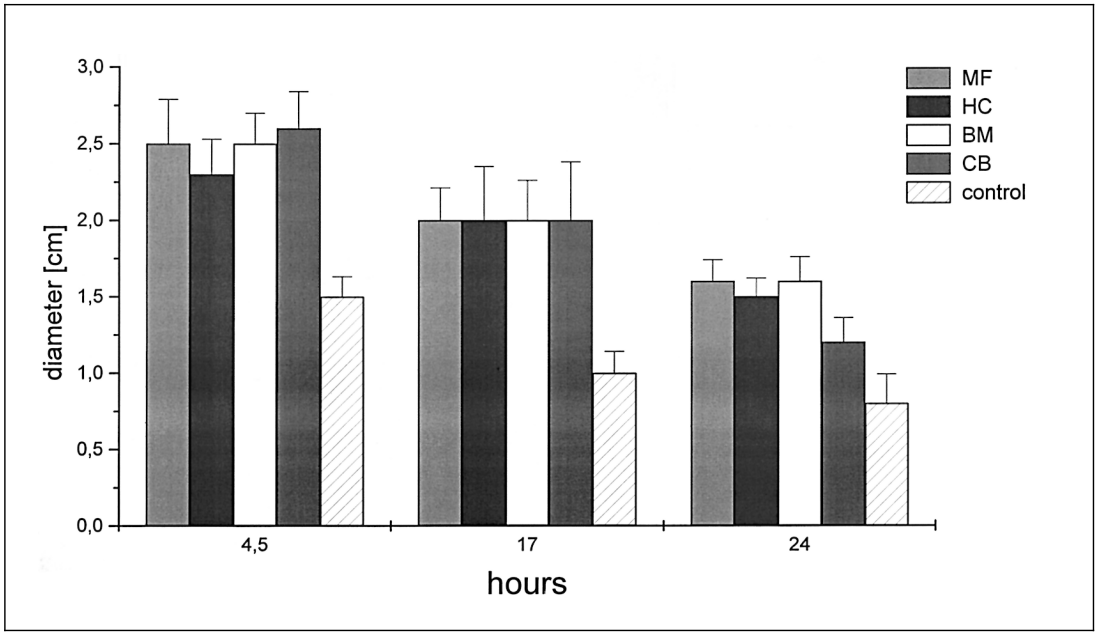

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Fig. 4. Contractions of collagen gels by human fibroblasts. Human fibroblasts were seeded in Petri dishes (diameter 3 cm) containing a collagen solution. Glucocorticosteroids inhibited contraction of collagen gels: at 10^{-3} *M* betamethasone and clobetasol inhibited collagen gel contraction completely, while MF and hydrocortisone showed only a partial inhibition (**a**). No significant difference between the tested corticosteroids could be observed at concentrations lower than 10^{-5} *M* (**b**). For abbreviations see figure 1.

These results might explain the observation of minor side effects induced by MF and hydrocortisone.

Contraction of collagen gels by fibroblasts represents another function of fibroblasts which is an important part of wound contraction in wound healing [20]. Contraction of collagen gels was inhibited completely by high concentrations of conventional fluorinated corticosteroids, but only partially by hydrocortisone or MF.

Most of the effects of MF on the metabolism of fibroblasts and keratinocytes are similar to those of hydrocortisone. In contrast, conventional fluorinated corticosteroids were found to be most active in affecting fibroblast and keratinocyte metabolism. It turned out that MF has less effect in the systems tested, although it reveals a high anti-inflammatory activity similar to that known for conventional fluorinated derivates of corticosteroids. It, therefore, remains to be seen whether this data might indicate the possibility of a dissociation between the anti-inflammatory activity and the inhibition of the biosynthetic capacities of fibroblasts and keratinocytes by modification of the steroidal structure of corticosteroids.

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