ELSEVIER

European Journal of Pharmacology 314 (1996) 193–196

ejp

Action of anti-inflammatory drugs on interleukin-1β-mediated glucose uptake by synoviocytes

Alain Hernvann [a,b,*], Brigitte Bourely [a], Valérie Le Maire [a,b], Christian Aussel [a], Charles-Joël Menkes [c], Ohvanesse G. Ekindjian [a,b]

[a] *Laboratoire de Biochimie Cellulaire de l'Inflammation, Tour D4, 3ème Étage, Université Paris XI, rue J.-B. Clément, F-92296 Chatenay-Malabry Cédex, France*

[b] *Laboratoire de Biochimie A, 27 rue du Fbg Saint Jacques, 75014 Paris, France*

[c] *Rhumatologie A, Hopital Cochin, 27 rue du Fbg Saint Jacques, 75014 Paris, France*

Received 7 May 1996; revised 2 July 1996; accepted 5 July 1996

Abstract

Synovial cell cultures prepared from samples taken from osteoarthritic and rheumatoid patients were treated with different anti-inflammatory agents (cortisol, indomethacin, ibuprofen and piroxicam) to determine their 'anti-interleukin-1β' action, using inhibition of interleukin-1β-mediated glucose uptake stimulation as a biological test. Confluent cells were treated for 24 h with different concentrations of these drugs (10^{-5}, 10^{-6} and 10^{-7} mol/l) to study their effect on the inflammation process. 6 h before glucose uptake studies, interleukin-1β (1 ng/ml) was added. Whereas non-steroid anti-inflammatory agents were inefficient, cortisol inhibited the action of interleukin-1β on glucose uptake. In osteoarthritic cells, cortisol, 10^{-5} mol/l, reduced interleukin-1β-mediated glucose uptake by 27% after a 24-h incubation. In rheumatoid cells, stimulated 2-deoxy-D-glucose uptake was reduced by 40.6%. Results were similar when interleukin-1β and cortisol were added simultaneously, 6 h before glucose uptake was measured. This rapid effect of cortisol was protein synthesis-dependent (inhibited by cycloheximide). Cortisol decreased glucose uptake by synoviocytes by acting on basal and interleukin-1β-mediated glucose uptake. This effect was more pronounced in rheumatoid synovial cells. The inhibition of interleukin-1β-mediated glucose uptake could be proposed as a new model for studying the anti-interleukin-1β effects of anti-rheumatic drugs.

Keywords: Synovial cell; Human; Glucose uptake; Anti-inflammatory drug; Osteoarthritis; Rheumatoid arthritis

1. Introduction

Inflammation and joint destruction in rheumatic diseases, such as osteoarthritis and rheumatoid arthritis, are in part mediated by local cytokines. In this way, interleukin-1β was demonstrated to induce a wide range of effects in cultured synovial cells, including the production of collagenase, prostaglandin E_2 (Jorgensen et al., 1991), glycosaminoglycans (Yaron et al., 1987), cytokines (Bucala et al., 1991) and adhesion molecules (Lindsley et al., 1993) as well as DNA synthesis (Desmoulins et al., 1990). All of these effects require nutrient and energy supply. Taylor et al. (1988) demonstrated that cytokines were able to increase glycolysis and fructose 2,6-bisphosphate levels in rheumatoid synovial cells. More recently, we found that interleukin-1β strongly stimulates glucose uptake by osteoarthritic and rheumatoid human cultured synovial cells (Hernvann et al., 1992). This cytokine was a more potent regulator of glucose metabolism than insulin in these cells and acted by increasing the number of glucose transporter GLUT1. As cytokine receptor antagonists are being investigated for the treatment of rheumatic disease (Sipe et al., 1994), we investigated the possibility of using interleukin-1β-mediated glucose uptake by human cultured synovial cells to evaluate the anti-interleukin-1β activity of anti-rheumatic drugs. Three non-steroidal anti-inflammatory agents were used: ibuprofen, indomethacin and piroxicam, and one steroidal anti-inflammatory: cortisol.

2. Materials and methods

2.1. Sources

Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, trypsin and phosphate-buffered saline were from Eurobio (Les Ulis, France). Clostridia collagenase

* Corresponding author. Tel.: (33-1) 4683-5489; Fax: (33-1) 4234-1987.

PII S0014-2999(96)00541-9

(type ia), cycloheximide, cortisol, piroxicam, ibuprofen, indomethacin and 2-deoxy-D-glucose were from Sigma (St. Louis, MO, USA). Human recombinant interleukin-1β was from Genzyme (Boston, MA, USA). 2-Deoxy-D-[1-^{3}H] glucose (spec. act. 15–25 Ci/mmol) was from Amersham (Amersham, UK).

2.2. Synovial cell cultures

Human synovial cells were isolated from surgical samples of synovium obtained during joint surgery from patients with osteoarthritic joint disease or with rheumatoid arthritis. All operations were performed when the disease was active. The superficial layer of synovium was dissected, then enzymatically digested with collagenase and trypsin as previously described (Desmoulins et al., 1990). Cells were suspended in DMEM containing 15% fetal calf serum, penicillin, streptomycin and fungizone and were plated at 5×10^6 cells in 75-cm^2 culture flasks. Cells were cultured until confluence in DMEM with 10% fetal calf serum, at 37°C, in an atmosphere containing 5% CO_2. Medium was changed every 4 days. When confluence was attained, the cells were passaged, by using trypsin. 7×10^4 cells were suspended in 1 ml of DMEM with 10% fetal calf serum and placed in 2.5-cm^2 12-well plates at 37°C (5% CO_2). All experiments were performed on confluent cultures at first passage.

2.3. Pharmacological effect assessment

The growth medium of the cultures was replaced by fresh medium containing 2% fetal calf serum. Various concentrations (10^{-5}, 10^{-6} and 10^{-7} mol/l) of the anti-inflammatory drugs, piroxicam, ibuprofen, indomethacin and cortisol, were then added for 6 or 24 h. When used, interleukin-1β 1 ng/ml was then added, without changing the medium, for the last 6 h. At the end of the 6- or 24-h incubations, uptake assays were performed.

In some experiments, cells were incubated with 10^{-4} mol/l cycloheximide during the 6- or 24-h incubations.

2.4. 2-Deoxy-D-glucose uptake

2-Deoxy-D-glucose uptake assays were performed as previously described (Hernvann et al., 1992). Briefly, cultures were washed 3 times with 2 ml of PBS, pH 7.4, and incubated for 5 min with 1 ml phosphate-buffered saline (PBS) containing 0.5 mmol/l 2-deoxy-D-glucose (2 μCi/ml). Incubation medium was then removed and the dishes were rinsed 3 times with ice-cold PBS. The cell monolayers were dissolved in 1 mol/l NaOH and radioactivity was counted in a liquid scintillation counter. Total cell protein was assayed according to Lowry et al. (1951) with bovine serum albumin as standard. Results are expressed as 10^3 cpm/mg protein per 5 min.

2.5. Statistical analysis

Values are given as means ± S.E.M. of triplicate measurements. Comparisons were made using Mann-Whitney U-test. Differences with P values of less than 0.05 were considered significant.

3. Results

Interleukin-1β-mediated 2-deoxy-D-glucose uptake was more pronounced in osteoarthritic than in rheumatoid cells, +182 and +90%, respectively, after a 6-h incubation (Fig. 1). However, when absolute values were considered, the increase was similar.

Non-steroidal anti-inflammatory drugs, ibuprofen, indomethacin and piroxicam, whatever the concentration or incubation time with cells, 6 or 24 h, did not modify significantly interleukin-1β-mediated 2-deoxy-D-glucose uptake by osteoarthritic or rheumatoid cells (Table 1). In contrast, both in osteoarthritic and rheumatoid cells, a dose-effect was observed with cortisol. At 10^{-6} mol/l, and with a 24-h incubation, cortisol significantly reduced stimulated glucose uptake by 20.6 and 20.2% in osteoarthritic and rheumatoid cells, respectively. With 10^{-5}

Table 1
Effects of anti-inflammatory drugs on interleukin-1β-mediated glucose uptake by osteoarthritic (OA, $n = 3$) and rheumatoid (RA, $n = 3$) human cultured synovial cells

		6 h			24 h		
		10^{-7} mol/l	10^{-6} mol/l	10^{-5} mol/l	10^{-7} mol/l	10^{-6} mol/l	10^{-5} mol/l
OA cells	Indomethacin	+9.7 ± 2.8	+13.7 ± 3.5	+14.1 ± 1.9	+4.0 ± 2.1	+10.0 ± 1.2	+6.0 ± 1.5
	Piroxicam	−3.0 ± 0.6	−4.0 ± 6.1	−2.7 ± 7.9	+9.7 ± 6.4	−9.3 ± 2.2	−0.3 ± 6.3
	Ibuprofen	−7.0 ± 5.5	−11.3 ± 6.2	−7.3 ± 7.7	−7.3 ± 4.6	−5.0 ± 3.5	−6.7 ± 5.6
	Cortisol	−12.7 ± 3.9	−24.8 ± 4.1 [a]	−28.3 ± 5.1 [a]	−3.3 ± 1.4	−20.6 ± 4.3 [a]	−27.0 ± 4.1 [a]
RA cells	Indomethacin	−13.3 ± 11.7	+7.3 ± 5.8	+7.0 ± 6.5	+0.3 ± 1.7	+2.0 ± 3.6	+9.3 ± 0.7
	Piroxicam	−9.7 ± 1.2	+7.0 ± 1.7	−3.3 ± 3.2	−10.7 ± 0.9	+3.0 ± 3.8	+8.7 ± 2.3
	Ibuprofen	−4.3 ± 4.2	+3.3 ± 3.2	−6.3 ± 1.2	−2.0 ± 4.3	+8.3 ± 3.2	−9.3 ± 0.9
	Cortisol	−9.1 ± 1.8	−24.2 ± 3.5 [a]	−39.6 ± 7.3 [a]	−7.0 ± 2.4	−20.2 ± 4.8 [a]	−40.6 ± 5.7 [a]

Values represent the percentage of variation of interleukin-1β-mediated glucose uptake (mean ± S.E.M.). [a] $P < 0.05$ vs. untreated cells.

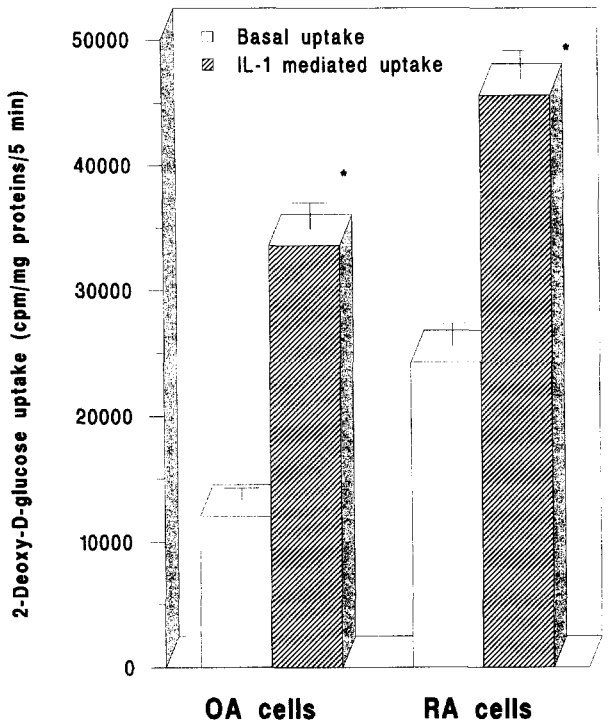

Fig. 1. Effect of interleukin-1β (1 ng/ml, 6 h) on 2-deoxy-D-glucose uptake by osteoarthritic (OA, $n = 3$) and rheumatoid (RA, $n = 3$) human cultured synovial cells (mean ± S.E.M.). * $P < 0.05$ vs. basal values.

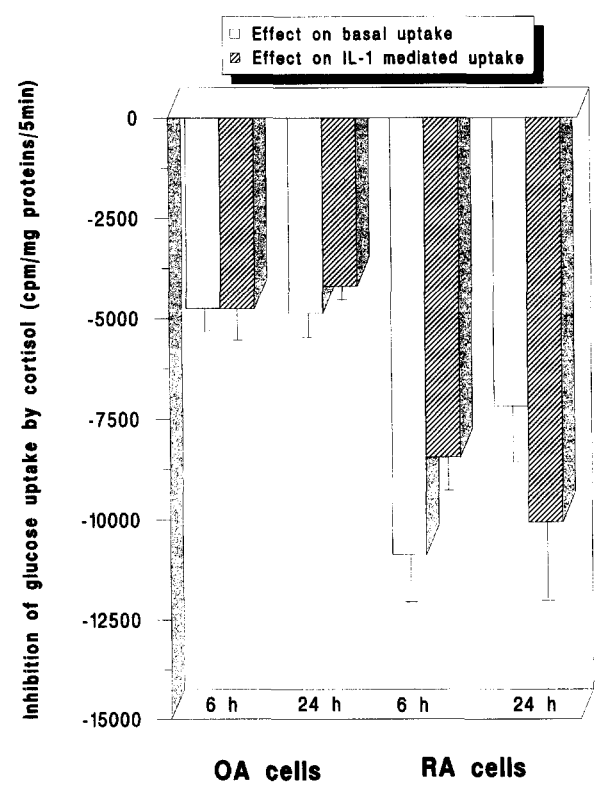

Fig. 2. Effect of cortisol (10^{-5} mol/l), after a 6- or 24-h incubation, on basal and interleukin-1β-mediated 2-deoxy-D-glucose uptake by osteoarthritic (OA, $n = 3$) and rheumatoid (RA, $n = 3$) human cultured synovial cells (mean ± S.E.M.).

mol/l cortisol, the effect was more marked, −27 and −40.6%, respectively. The anti-interleukin-1 effect appeared similar when cytokine and cortisol were added for a 6-h incubation. No effect was observed at a concentration 10^{-7} mol/l. The action of cortisol appeared to be protein synthesis dependent, since cycloheximide inhibited 92% (osteoarthritic cells) or 89% (rheumatoid cells) of the effect of this hormone.

The effect of cortisol at 10^{-5} mol/l was studied, to distinguish its effects on basal glucose uptake and specific interleukin-1β-mediated glucose uptake. Results in Fig. 2 show that cortisol similarly affected basal and interleukin-1β-mediated glucose uptake. Rheumatoid synovial cells appeared significantly more sensitive to cortisol than osteoarthritic cells.

4. Discussion

The destruction of articular joints in rheumatic diseases is related to an imbalance of cartilage breakdown and regeneration, due to a destructive action of cytokines via the production of degrading enzymes (Kirkham, 1991). Thus, the regulation of interleukin-1β remains a primary target for the treatment or rheumatic diseases (Sipe et al., 1994). In a previous study (Hernvann et al., 1992), we demonstrated that rheumatoid synovial cells, which produce a large quantity of interleukin-1β, were able to transport more glucose than osteoarthritic cells could. This could be related to a more intensive contact with interleukin-1β. Moreover, the cytokine appeared to stimulate glucose uptake by both cells: stimulation was higher in rheumatoid synoviocytes, but when absolute values (in cpm/mg protein per 5 min) were considered, the increase was similar to that in osteoarthritic cells. As interleukin-1β induces a wide range of effects that require nutrient and energy supply, actions on interleukin-1-mediated glucose uptake would be a reliable approach for the study of anti-rheumatic drugs.

Whereas non-steroid anti-inflammatory drugs possess a variable influence on some interleukin-1β-related effects, such as prostaglandin E_2 or collagenase synthesis (Arend and Dayer, 1990), they appeared to be inefficient on interleukin-1β-mediated glucose uptake in our model, although they decreased prostaglandin E_2 synthesis (to about 40%). The study of the action of non-steroid anti-inflammatory drugs on interleukin-1β-mediated 2-deoxy-D-glucose uptake is not appropriate for drugs which act predominantly on cyclooxygenase pathways. Pelletier et al. (1993) have shown that non-steroid anti-inflammatory drugs are able to reduce the interleukin-1 receptor level in articular chondrocytes. A similar action on synoviocytes would be insufficient to inhibit stimulated glucose uptake, since a number of interleukin-1β receptors would remain to have a stimulating effect.

In contrast, cortisol decreased glucose uptake by syn-

oviocytes by acting on basal and interleukin-1β-mediated glucose uptake. This effect was more pronounced in rheumatoid synovial cells. There are different possible mechanisms of action of this hormone. As for the non-steroid anti-inflammatory drugs, the hypothesis of an inhibition of prostaglandin production (Bailey, 1991) should be rejected since, under our experimental conditions, cortisol reduced slightly the production of prostaglandin E_2 (data not shown). Moreover, in a previous work, we demonstrated that exogenous prostaglandin E_2 was ineffective on 2-deoxy-D-glucose uptake (Hernvann et al., 1992). A 6-h incubation with cortisol appeared sufficient to inhibit the effect of interleukin-1β on 2-deoxy-D-glucose uptake. Cortisol could act by modifying the intracellular movement of the glucose carrier, decreasing the number of carriers in plasma membranes and increasing the number in the intracellular pool, as shown in a near cellular model (Horner et al., 1987).

In conclusion, cortisol, at pharmacological concentrations, has an inhibitory effect on basal and interleukin-1β-mediated 2-deoxy-D-glucose uptake by human cultured synoviocytes, whereas non-steroid anti-inflammatory drugs were without any effect. This model is useful to study the action of drugs, which could be used in the treatment of rheumatic diseases since, during inflammation, synoviocytes present an active metabolism. The study of glucose uptake, in vitro, by human synovial cells from osteoarthrosic or rheumatoid arthritic patients could be proposed as a new and complementary approach to study the anti-interleukin-1 activity of drugs usable in the treatment of osteoarthritis or rheumatoid arthritis.

Acknowledgements

This work was supported by grants from the 'Caisse Régionale d'Assurance Maladie (CRAM)'. We are grateful to the personnel of the Department of Orthopedic Surgery of the Cochin Hospital for providing tissue specimens.

References

Arend, W.P. and J.M. Dayer, 1990, Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis, Arthritis Rheum. 33, 305.

Bailey, J.M., 1991, New mechanisms for effects of anti-inflammatory glucocorticoids, BioFactors 3, 97.

Bucala, R., C. Ritchlin, R. Winchester and A. Cerami, 1991, Constitutive production of inflammatory and mitogenic cytokines by rheumatoid synovial fibroblasts, J. Exp. Med. 173, 569.

Desmoulins, D., C. Pontezière, J. Agneray, O.G. Ekindjian and M.J. Cals, 1990, Effects of human recombinant interleukin-1 beta on rheumatoid and non rheumatoid human synovial cell growth, Cell. Mol. Biol. 36, 309.

Hernvann, A.H., C. Aussel, L. Cynober, N. Moatti and O.G. Ekindjian, 1992, Interleukin-1β, a strong mediator for glucose uptake by rheumatoid and non-rheumatoid cultured human synoviocytes, FEBS Lett. 303, 77.

Horner, H.C., A. Munck and G.E. Lienhard, 1987, Dexamethasone causes translocation of glucose transporters from the plasma membrane to an intracellular site in human fibroblasts, J. Biol. Chem. 262, 1769.

Jorgensen, C., J. Angel and C. Fournier, 1991, Regulation of synovial cell proliferation and prostaglandin E_2 production by combined action of cytokines, Eur. Cytokine Netw. 2, 207.

Kirkham, B., 1991, Interleukin-1, immune activation pathways, and different mechanisms in osteoarthritis and rheumatoid arthritis, Ann. Rheum. Dis. 50, 395.

Lindsley, H.B., D.D. Smith, C.B. Cohick, A.E. Koch and L.S. Davis, 1993, Proinflammatory cytokines enhance human synoviocyte expression of functional intercellular adhesion molecule-1 (ICAM-1), Clin. Immunol. Immunopathol. 68, 311.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with Folin phenol reagent, J. Biol. Chem 193, 265.

Pelletier, J.P., R. McCollum, J. Di Battista, L.D. Loose, J. Cloutier and J. Martel-Pelletier, 1993, Regulation of human normal and osteoarthritic chondrocyte interleukin-1 receptor by antirheumatic drugs, Arthritis Rheum. 36, 1517.

Sipe, J.D., J. Martel-Pelletier, I.G. Otterness and J.P. Pelletier, 1994, Cytokine reduction in the treatment of joint conditions, Mediat. Inflamm. 3, 243.

Taylor, D.J., R.J. Whitehead, J.M. Evanson, D. Westmacott, M. Feldmann, H. Bertfield, M.A. Morris and D.E. Wolley, 1988, Effect of recombinant cytokines on glycolysis and fructose 2,6 bisphosphate in rheumatoid synovial cells in vitro, Biochem. J. 250, 111.

Yaron, I., F.A. Meyer, J.M. Dayer and M. Yaron, 1987, Human recombinant interleukin-1β stimulates glycosaminoglycan production in human synovial fibroblast cultures, Artrhitis Rheum. 30, 424.