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Huber, L C; Künzler, P; Boyce, S H; Michel, B A; Gay, R E; Ink, B S; Gay, S


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Abstract

OBJECTIVE: Biologicals have revolutionised the treatment of rheumatoid arthritis (RA). However, progressive joint destruction can still be observed in many patients and the search for novel molecular therapies targeting specific signalling pathways is ongoing. In the present study, we investigated the effects of GW282974, a novel compound directed against tyrosine kinase activity with respect to the potential suppression of inflammation and destruction. METHODS: Synovial tissue specimens were obtained from RA patients undergoing surgical joint replacement. Rheumatoid arthritis synovial fibroblasts (RASFs) were stimulated with cytokines and GW282974 was added in different concentrations. Gene expression was checked by TaqMan PCR, using 18S as housekeeping gene. Protein analysis was quantified by ELISA. Cell growth and proliferation was measured using the "ViaLight" proliferation assay. RESULTS: EGF had no effect on the gene expression profile of RASFs when used as single stimulatory agent. In combination with pro-inflammatory mediators however, EGF showed a synergistic effect. The expression of matrix metalloproteinases, inflammatory cytokines and cyclooxygenase-2 on mRNA levels was strongly increased, whereas the addition of GW282974 abrogated these effects in a dose-dependent manner. These data could be confirmed on protein/lipid levels analysing the supernatants of RASFs by ELISA. Similarly, cell growth and proliferation of RASFs were inhibited by GW282974 in a dose- and time-dependent manner. By contrast, no cytotoxic effects were seen within the concentrations used. DISCUSSION: GW282974 appears to interfere with the inflammatory and the destructive pathways in RASFs and might therefore be used as novel therapeutic strategy for the treatment of RA.
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Effects of a novel tyrosine kinase inhibitor in rheumatoid arthritis synovial fibroblasts

L C Huber,¹ P Küntzler,¹ S H Boyce,² B A Michel,¹ R E Gay,¹ B S Ink,² S Gay¹

ABSTRACT

Objective: Biologics have revolutionised the treatment of rheumatoid arthritis (RA). However, progressive joint destruction can still be observed in many patients and the search for novel molecular therapies targeting specific signalling pathways is ongoing. In the present study, we investigated the effects of GW282974, a novel compound directed against tyrosine kinase activity with respect to the potential suppression of inflammation and destruction.

Methods: Synovial tissue specimens were obtained from RA patients undergoing surgical joint replacement. Rheumatoid arthritis synovial fibroblasts (RASFs) were stimulated with cytokines and GW282974 was added in different concentrations. Gene expression was checked by TaqMan PCR, using 18S as housekeeping gene. Protein analysis was quantified by ELISA. Cell growth and proliferation was measured using the “ViaLight” proliferation assay.

Results: EGF had no effect on the gene expression profile of RASFs when used as single stimulatory agent. In combination with pro-inflammatory mediators however, EGF showed a synergistic effect. The expression of matrix metalloproteinases, inflammatory cytokines and cyclo-oxygenase-2 on mRNA levels was strongly increased, whereas the addition of GW282974 abrogated these effects in a dose-dependent manner. These data could be confirmed on protein/lipid levels analysing the supernatants of RASFs by ELISA. Similarly, cell growth and proliferation of RASFs were inhibited by GW282974 in a dose- and time-dependent manner. By contrast, no cytotoxic effects were seen within the concentrations used.

Discussion: GW282974 appears to interfere with the inflammatory and the destructive pathways in RASFs and might therefore be used as novel therapeutic strategy for the treatment of RA.

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that is characterised by synovial hyperplasia, altered immune responses and inflammation. The rheumatoid synovium is distinguished by the presence of a unique cell type, the RA synovial fibroblast (RASF), which shows morphological and molecular features of an activated phenotype.¹ ² These features include the expression of proto-oncogenes and alterations in the regulation of the cell cycle, leading to the release of pro-inflammatory mediators and matrix-degrading enzymes such as matrix metalloproteinases (MMPs) and cathepsins. These events ultimately result in the progressive destruction of tendons, articular cartilage, and bone. Anticytokine therapies and novel biologics have revolutionised the therapy of RA. In many patients however, progressive joint destruction can still be observed despite the combined use of novel biologics and immunosuppressive agents. While the development of pharmacologic inhibitors of MMPs has experienced a major setback due to the carcinogenicity of some compounds,³ intensive searching for novel substances that might target destruction and inflammation within the rheumatoid synovium is ongoing. We thus investigated the effects of GW282974, a quinazoline derivative directed against tyrosine kinase activity related to epidermal growth factor (EGF) receptor.⁴

EGF is a protein growth factor that has been detected within the synovial fluid of RA patients in high concentrations.⁵ Moreover, EGF was identified as important factor that stimulates the release of inflammatory mediators and induces the growth of synovial cells,⁶ ⁷ probably by acting in a synergistic manner with the key pathogenetic mediators of RA.⁸–¹⁰ EGF-receptor (EGFR, or HER), however, forms a family of surface receptors with tyrosine kinase activity including ErbB1 and ErbB2.¹¹ Activation of EGFR has been linked to various cellular processes including proliferation, differentiation, and angiogenesis.¹² In addition, EGFR appears to induce the expression of cyclooxygenase (COX)-2 in different tissues, thus leading to the production of bioactive lipids and potent inflammatory mediators, for example prostaglandin (PG)E₂.¹³ In the context of RA, it was also shown that ErbB2 is highly expressed on the cell surface of synovial cells making it a potential target for future therapies.

In recent years, specific therapies against the EGFR family have emerged. Numerous studies performed in vivo and in vitro have demonstrated that the interruption of signalling by EGFR inhibitors directed against the extracellular (monoclonal antibodies) or intracellular domain of the receptor (tyrosine kinase inhibitors) results in inhibition of cell growth and viability.¹⁴ ¹⁵ Furthermore, a growing body of evidence suggests the presence of a functional crosstalk between EGFR and COX-2 pathways (reviewed in Mann et al and Dannenberg et al)¹⁶ ¹⁷ which would additionally increase the impact of specific therapeutic interference in the setting of rheumatoid arthritis.

In the present study, we addressed this issue by analysing the expression pattern of several MMPs, IL-6, cathepsin K and COX-2 in RASFs upon incubation with growth factors and the small molecule inhibitor GW282974.

MATERIALS AND METHODS

Reagents

GW282974 was provided from GlaxoSmithKline (Stevenage, UK). Epidermal growth factor (EGF),
tumour necrosis factor α (TNFα) and interleukin (IL)-1α were all purchased from R&D Systems (Abingdon, Oxfordshire, UK).

**Cells/cell culture**

Synovial tissue specimens were obtained from RA patients (n = 5, Clinic of Orthopedic Surgery, Schulthess Hospital Zurich, Zurich, Switzerland) undergoing surgical joint replacement. All RA patients fulfilled the American College of Rheumatology (ACR) criteria for RA. All tissue analyses were performed according to the regulations by the ethical committee Zurich. RASFs were isolated from synovial tissues, digested by collagenase, and used from passages 4–8 as described previously. RASFs were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco). DMEM was supplemented with 10% heat-inactivated foetal calf serum (FCS), 15 mmol/litre HEPES, 2 mmol/litre l-glutamine, 50 µg/ml streptomycin, 100 U of penicillin, and 2 µl/ml fungizone.

For stimulation, 0.5 × 10⁵ RASFs were stimulated with IL-1β (1 ng/ml), TNFα (10 ng/ml) and/or EGF (1 ng/ml) for 48 h. Afterwards, the supernatants were collected and RNA was isolated. The number of cells was determined by the CASY-1 cell counter (Schärfe System, Reutlingen, Germany). GW282974 was added 1 h before incubation with cytokines.

**TaqMan reverse transcriptase (RT)-PCR**

Total RNA was isolated using an RNEasy kit (Qiagen, Basel, Switzerland), converted into cDNA, and gene expression was quantified by RT-PCR as described previously. Gene transcript levels of MMP-1, -3, -13, -14, IL-6, cathepsin K and COX-2, as well as 18S rRNA were quantified by gene assays (Applied Biosystems, Foster City, California, USA) using RT-PCR.

**Fluorescence activated cell sorting (FACS)**

Flow cytometry was performed using a FACScan flow cytometer (Becton Dickinson, Basel, Switzerland). A minimum of 1 × 10⁴ cells per sample were acquired in list mode and analysed using Cell quest software (Becton Dickinson Immunocytometry System, San Jose, California, USA).

**Detection of apoptosis**

A total of 0.5 × 10⁵ cells were examined for apoptosis using Annexin V-PE according to the manufacturer’s protocol. Briefly, adherent cells were trypsinised, centrifuged at 500 g for 10 min, and resuspended in 100 µl of FACS binding buffer (BD Pharmingen, San Diego, California, USA). Annexin V-PE was added directly to the cells. After incubation for 15 min at room temperature, the cells were analysed by FACS (FACScalibur, Becton Dickinson Mansfield, Massachusetts, USA). Treatment with FasL (100 ng/ml) was used as positive control.

**“ViaLight” proliferation assay**

Cell viability was assessed with the ViaLight HS Kit (Cambrex, Verviers, Belgium), according to the manufacturer’s protocol. This assay is based upon the bioluminescent measurement of ATP levels. Briefly, unstimulated cells were cultivated in 12-well plates at a density of 4000 cells/well, lysed at defined timepoints, and ATP was extracted. After addition of the substrate, the generated luminescence signal was measured in a SIRIUS Luminometer (Berthold Detection Systems, Pforzheim, Germany).

**Immunooassay**

Levels of MMP-1 and prostaglandin E₂ (PGE₂) protein were determined by quantitative colourimetric sandwich ELISA (MMP-1: Amersham Biosciences, GE Healthcare UK Limited; PGE₂: R&D Systems) according to the manufacturer’s instructions. Concentrations were calculated using a standard curve generated with specific standards provided by the manufacturer. IL-6 was measured using a Luminex assay developed “in house” at GlaxoSmithKline. IL-6 standard was from R&D Systems.

**Statistical analysis**

All data are expressed as mean (SD). Statistical analysis was performed using the GraphPad Prism Software, 4.08 (Graph Pad Software Inc., San Diego, California, USA). For analysis between different groups the Mann–Whitney U test was used, and a value of p < 0.05 was considered statistically significant.

**RESULTS**

**Stimulation of RASFs**

To determine the ability of RASFs to respond upon stimulation with epidermal growth factor (EGF) and pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and interleukin (IL)-1β, we first examined the expression levels of matrix metalloproteinases (MMP)-1, -3, -13, -14, cathepsin K, IL-6, -8 and COX-2. As shown in fig 1, the combination of EGF (1 ng/ml), IL-1β (1 ng/ml) and TNFα (10 ng/ml) reached the highest expression levels of MMP-1 (fig 1A), MMP-3 (fig 1B), MMP-13 (fig 1C), IL-6 (fig 1D) and COX-2 (fig 1E) when checked by RT-PCR 24 h after stimulation. Addition of EGF as a single stimulatory agent revealed only marginal effects at the dosages used. By contrast, no effect was observed for MMP-14 and cathepsin K (data not shown). Interestingly, EGF alone showed virtually no stimulation of RASFs in all experimental settings (data not shown). When used in addition to TNFα and IL-1β, however, synergistic effects could be seen. In particular, the mRNA expression levels for MMP-1 were increased by 25 (16)–fold (p = 0.03) when RASFs were stimulated with IL-1β and TNFα, whereas combined stimulation of EGF with IL-1β and TNFα increased the mRNA levels of MMP-1 by 42 (4)-fold. Similarly, mRNA for MMP-3 was elevated by 976 (952) and by 1884 (692)-fold, respectively. MMP-13 was significantly up-regulated by 39 (17) (p = 0.01) and by 53 (4)-fold under combined stimulation. IL-6 was increased by 199 (94) (p = 0.01) and by 247 (125)-fold, respectively. The expression levels of COX-2 mRNA were increased by 728 (158) (p<0.001) and by 1070 (35)-fold under additional stimulation by EGF. The COX-2 gene expression under combined stimulation reached significance (p = 0.04) when compared to the stimulation with proinflammatory mediators alone.

These data could be confirmed on protein levels for MMP-1 (fig 2), showing an increase from 2.9 (2.1) to 20 (14) ng/ml upon combined stimulation.

**Inhibitory effects of GW282974**

Several studies have demonstrated that interfering with the EGF-mediated signalling might inhibit the invasiveness of cancer cells, probably by interfering with EGFR autotransphosphorylation and subsequent PI3K/AKT activation. Since PI3K are also involved in the activation of MMPs, we next analysed the effects of the newly developed compound GW282974 on the expression levels of MMPs and COX-2 in stimulated RASFs. As illustrated, GW282974 strongly reduced the mRNA expression levels of MMP-1 in stimulated synovial cells in a dose-dependent manner, reaching significance when applying 10 µM of the compound (p<0.05). Thus, MMP-1 was...
reduced from 42 (18) to 24 (11) for 5 μM and to 16 (4)-fold when 10 μM of GW282974 were used (fig 1A). Similarly, MMP-3 was reduced from 1884 (1984) to 861 (666) (5 μM), and to 722 (692)-fold (10 μM; fig 1B). MMP-13 was reduced from 53 (22) to 28 (7) (5 μM) or to 12 (4)-fold (10 μM, p, 0.05; fig 1C).

The mRNA expression levels of IL-6 were reduced from 247 (125) to 161 (84) (5 μM) or to 123 (70)-fold (10 μM, fig 1D) and COX-2 finally was significantly reduced from 1070 (212) to 408 (70) (5 μM, p, 0.05) or to 246 (35)-fold (10 μM, p, 0.05), respectively (fig 1E). On protein levels, GW282974 reduced the expression of MMP-1 from 20 (14) ng/ml to 15 (11) (5 μM) and 11 (9) ng/ml (10 μM, fig 2).

Since COX-2 metabolises arachidonic acid into eicosanoids and inflammatory mediators such as PG, the protein levels of IL-6 and the level of the biolipid PGE2 were measured in the supernatants of RASFs (fig 3). GW282974 reduced the levels of IL-6 from 114 (42) ng/ml to 59 (25) ng/ml, (fig 3A) and PGE2 reduced from 42 (18) to 24 (11) for 5 μM and to 16 (4)-fold when 10 μM of GW282974 were used (fig 1A). Similarly, MMP-3 was reduced from 1884 (1984) to 861 (666) (5 μM), and to 722 (692)-fold (10 μM; fig 1B). MMP-13 was reduced from 53 (22) to 28 (7) (5 μM) or to 12 (4)-fold (10 μM, p, 0.05; fig 1C). The mRNA expression levels of IL-6 were reduced from 247 (125) to 161 (84) (5 μM) or to 123 (70)-fold (10 μM, fig 1D) and COX-2 finally was significantly reduced from 1070 (212) to 408 (70) (5 μM, p, 0.05) or to 246 (35)-fold (10 μM, p, 0.05), respectively (fig 1E). On protein levels, GW282974 reduced the expression of MMP-1 from 20 (14) ng/ml to 15 (11) (5 μM) and 11 (9) ng/ml (10 μM).

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from 107 (24) ng/ml to 12 (6) ng/ml, (fig 3B). The effects observed on the protein expression levels of MMP-1 were strongly dose-dependent. With respect to PGE\(_2\) and IL-6, only a marginal dose-dependent effect was seen. These data suggest that maximal reduction of COX-2 dependent mediators might already be achieved by using low micromolecular concentrations of GW282974.

**Effects of GW282974 on proliferation and apoptosis**

Previous work has demonstrated that the inhibition of EGFR affects viability of malignant cells\(^\text{25-29}\) and might inhibit growth and proliferation of RA synovial cells.\(^\text{7}\) Figure 4 shows the effects of GW282974 on the proliferation rate of RASFs (n = 4). The proliferation rate of RASFs stimulated with IL-1\(\beta\) and TNF\(\alpha\) was arbitrarily set as 100%. Single administration of GW282974 reduced the rate of proliferating fibroblasts in a dose-dependent manner. Maximal reduction to 87.4 (20%) was seen by adding 10 \(\mu\)M of GW282974 as analysed by the ViaLight proliferation assay after 7 days. These findings are consistent with data obtained from fibroblasts treated with Herceptin (a monoclonal antibody against EGFR), which reduced the proliferation of fibroblasts by about 20% (data not shown).

By contrast, no induction of apoptosis could be detected. When GW282974 was applied in the highest dose used (10 \(\mu\)M), no differences in the amount of apoptotic cells could be seen after 48 h when compared to untreated control cells, indicating that GW282974 has no cytotoxic effects within effective concentrations.

**DISCUSSION**

Specific inhibitors of tyrosine kinase activity have emerged as molecular-targeted agents for the treatment of various diseases, including chronic myelogenic leukaemia,\(^\text{24}\) pulmonary hypertension,\(^\text{25}\) and lung cancer.\(^\text{26}\) Here, we report the results of experiments, in which we tested an inhibitor of tyrosine kinase activity (GW282974) in RA. GW282974 is a small-molecule inhibitor directed against the receptor tyrosine kinase activity of ErbB2 with a claimed 100-fold selectivity at 50 nM.\(^\text{4}\) For cell proliferation experiments, in which we tested an inhibitor of tyrosine kinase activity (GW282974) in RA. GW282974 is a small-molecule inhibitor directed against the receptor tyrosine kinase activity of ErbB2 with a claimed 100-fold selectivity at 50 nM.\(^\text{4}\) For cell culture, synovial fibroblasts were used representing key players involved in inflammation and joint destruction. This synergistic effect did not reach statistical significance when compared to the stimulation achieved by inflammatory mediators alone. Since EGF was added in a very low concentration, the synergism observed in the present study might still be of biological relevance underpinning the potential role of EGF in the pathogenesis of RA.

With respect to the destruction of articular cartilage, we found a strong reduction of the expression levels of major MMPs involved in joint destruction (MMP-1, -3, -13) when we administered GW282974 to stimulated fibroblasts. Since these enzymes are pivotal agents in the destruction of articular cartilage and bone, GW282974 might have joint-protective properties when administered early in the course of RA.

Moreover, increasing evidence indicate a tight connection between the EGFR and COX-2 pathways, which form a positive feedback loop.\(^\text{15, 29}\) Thus, activation of EGFR induced the transcriptional activation of COX-2, probably through activator protein (AP)-1 and mitogen-activated protein kinases (MAPK),\(^\text{10}\) for which binding sites have been identified in the COX-2 promoter region.\(^\text{27}\) In addition, both transcription factors are highly involved in the pathogenesis of RA (for reviews see Huber et al and Ospelt et al).\(^\text{31, 32}\) COX-2, by contrast, increases the expression of EGFR by PGE\(_2\), whose production is enhanced.\(^\text{15, 29, 30, 34}\)

Our current data are in line with these hypotheses. When GW282974 was administered to RASFs, the expression levels of MMPs, ILs, COX-2 and, subsequently, the production of bioactive lipids and pro-inflammatory mediators such as PGE\(_2\) and IL-6 were strongly reduced.

It has been previously reported that EGFR blocking agents affect cell growth and proliferation.\(^\text{30}\) We also found such effects when using GW282974, suggesting that therapeutic use of this compound might indeed reduce the synovial hypercellularity of RA. In contrast to other studies however, we found no alterations in the rate of apoptosis of synovial fibroblasts. This finding is probably due to the low drug concentrations used in our studies. Another explanation for the differences in the rate of apoptosis upon inhibition of by GW282974 might be the high resistance of RASFs against apoptosis-inducing factors as it was demonstrated repeatedly.\(^\text{17, 30, 31}\)

TNF\(\alpha\) blocking agents achieved great therapeutic success in the treatment of inflammatory rheumatologic disorders within recent years and, thus, were supplemented by various novel cytokine targets such as anti-IL-6-receptor antibodies as well as cytokine-independent targets including the use of rituximab (monoclonal antibodies against CD20) that was originally used in non-Hodgkin’s lymphomas.\(^\text{38}\) However, a substantial number of RA patients under TNF-blocking biologicals shows progressive destruction of joint and bone even when combined with immunosuppressive drugs. By contrast, the development of non-selective MMP-blocking agents with potential joint-protective properties was strongly set back by severe side effects of the drugs, in particular facilitating the formation of metastasis in animal models and some humans.\(^\text{3}\)

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**Figure 3** Effect of GW282974 on prostaglandin E\(_2\) (PGE\(_2\)) and interleukin (IL)-6 production in rheumatoid arthritis synovial fibroblasts (RASFs) stimulated with IL-1beta/tumour necrosis factor (TNF)alpha (n = 3). Levels of IL-6 (A) and of PGE\(_2\) (B) were determined by Luminex (“in house” GlaxoSmithKline assay). Results are expressed as the average percent inhibition of protein or lipid released with the compound (5 \(\mu\)M or 10 \(\mu\)M) as compared to an untreated control.

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**Figure 2** Effect of GW282974 on prostaglandin E\(_2\) (PGE\(_2\)) and interleukin (IL)-6 production in rheumatoid arthritis synovial fibroblasts (RASFs) stimulated with IL-1beta/tumour necrosis factor (TNF)alpha (n = 3). Levels of IL-6 (A) and of PGE\(_2\) (B) were determined by Luminex (“in house” GlaxoSmithKline assay). Results are expressed as the average percent inhibition of protein or lipid released with the compound (5 \(\mu\)M or 10 \(\mu\)M) as compared to an untreated control.
The tyrosine kinase inhibitor GW282974 shows substantial therapeutic impact on the expression levels of matrix degrading enzymes and pro-inflammatory mediators alike. GW282974 thus appears to act directly on the two interfaces of inflammation and destruction. Of interest, EGF used as single stimulatory agent showed virtually no effect on the gene expression profile RASFs in the experimental settings of the study presented. This might be due to the low concentration applied or due to low protein expression of the respective ErbB receptors in the cells used. By contrast, GW282974 strongly affected the TNF- and IL-1-mediated expression of disease-relevant genes such as matrix-degrading enzymes and pro-inflammatory factors. Moreover, as shown by the results of the proliferation assay, GW282974 reduced the rate of cytokine-induced proliferation in RASFs even in the complete absence of EGF. Based on these findings, we propose a novel, yet undescribed mechanism of action of the tyrosine kinase inhibitor GW282974 that is probably unrelated to EGFR activity.

Whether this mechanism is based on the inhibition of a functional crosstalk between the common molecular pathways involving TNFα, IL-1 and EGF or, alternatively, whether it is due to general off-target activities of the compound used, has to be investigated by further studies.

Regardless, our data emphasise the potential role of GW282974 as future therapeutic agent in RA.

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REFERENCES


