Steroid resistance in nasal polyposis: role of glucocorticoid receptor and TGF-β1*

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Summary

Background: Glucocorticoids (GCs) are considered drugs of choice for treating nasal polyps (NPs). However, a subset of patients shows a limited clinical response even to high doses of GCs. Altered expression of glucocorticoid receptors (GRs), namely GR-α and GR-β, is a potential mechanism underlying GC insensitivity. GCs modulate the expression of several cytokines, including transforming growth factor-β (TGF-β), which may contribute to cellular proliferation in NPs. The study investigates some biomolecular features of GC-resistant NPs, and examines possible differences from normal mucosa (NM).

Methodology: Radioligand binding assay (binding) was used to determine GR-α binding capacity; Western blotting was used to evaluate GR-α, GR-β, and TGF-β expression and GR-α subcellular distribution. NPs were sampled in 32 patients during ethmoidectomy; NM was taken from 15 healthy patients during rhinoplasty.

Results: GR-α was present in NPs and NM, with lower affinity for the ligand in NPs. GR-α was prevalent in the cytosol of NPs that were GR-α-negative to the binding assay. GR-β was expressed in NPs and absent in the majority of NM. TGF-β1 expression was higher in NPs than in NM.

Conclusions: GR-β and TGF-β1 might be involved in NP pathogenesis, but their role in modulating GC sensitivity is still unclear.

Key words: nasal polyposis, resistance, glucocorticoids, glucocorticoid receptor, transforming growth factor-β1

Introduction

Nasal polyposis is a multifactorial disease characterized by polymorphic histological findings and varied clinical manifestations. It is frequently associated with other pathological conditions or factors, including allergy, asthma, ASA intolerance, infections, genetic factors, muco-ciliary dysfunction and vasomotor imbalance. Its overall prevalence among adults is 1-4% and, in spite of considerable research, the underlying pathophysiological mechanisms are still not clear [1,2]. Bacterial and fungal infections are thought to play an important role in the genesis of polyp formation [3-5]. The pathogenesis appears to be related to chronic irritant stimuli, which lead to significant eosinophilic oedema in 85-90% of patients [6-8]. This is confirmed by the presence of an inflammation-rich tissue in nasal polyps (NPs) and in surrounding areas, promoted by overexpression of cytokines (namely FGF, IL-1β, TNF-α, GM-CSF and IL-5), chemokines (for example CCL24) and adhesion molecules (such as ICAM-1) [2,9,10].

As reported by the International Consensus on Nasal Polyposis and the European Position Paper on Rhinosinusitis and Nasal Polyps, topical and systemic glucocorticoids (GCs) are the first-line treatment for nasal polyposis, being the only pharmacological

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option effective against the signs and symptoms of the disease, thanks to their anti-inflammatory, immunosuppressive, and anti-allergic properties [11,12]. However, medical therapy only produces an improvement of nasal symptoms and a reduction in polyp size (as assessed by nasal endoscopy) in 60-80% of cases [13-15]. These results may be influenced by differences among studies in symptom evaluation, endoscopic scoring and follow-up.

Some factors may lead to the failure of GC therapy: polyp extension or histological aspect, etiologic factors, therapy protocol, as well as the presence of intracellular resistance mechanisms, as has been reported for steroid-resistant asthma [16-18]. An increased understanding of the molecular mechanisms involved in GC resistance in nasal polyposis may enable new strategies to be developed for treating this disease. Altered expression of the receptor that mediates GC actions, the glucocorticoid receptor (GR), and modifications in the signal cascade, has been found to be correlated to GC insensitivity. In particular, an imbalance between the two isoforms of GR, GRα and GRβ [9,10,19] has been suggested to be involved in failure to respond to treatment.

Among growth factors that might be involved in chronic inflammatory diseases of the airways, and thus in nasal polyposis, transforming growth factor-β (TGF-β) might play a key role. TGF-β mediates a broad spectrum of biological activities, and is important in regulating inflammation-promoting chemotaxis, and in activating inflammatory cells. Conversely, however, it also promotes the onset of tissue repair, leading to anti-inflammatory effects. It has been suggested that TGF-β1 levels may be up-regulated in NPs versus normal tissue [20-22], although other studies report the opposing trend, with lower levels of TGF-β1 activity in NPs than in normal mucosa [23-25].

The aim of this study was to evaluate the expression of the two isoforms of GR, GR-α and GR-β, as well as TGF-β1 expression, in normal mucosa and in nasal polyps after failure of local or systemic corticosteroid treatment. The results confirm the involvement of GR-β and TGF-β1 in nasal polyp GC resistance, but also raise new questions, which will require further research.

Materials and methods

Study group

The series comprised 32 patients (24 male, 8 female, median age 51) with a diagnosis of nasal polyposis and failure of *medical treatment,* who had undergone naso-sinus surgery for polyps between 2007 and 2009 at the Otorhinolaryngological Unit of San Luigi Hospital, Italy. Patients underwent clinical examination, ear-nose-throat video-rhino-fibroscopy, nasal endoscopy, and maxillofacial computed tomography. Polyps were scored endoscopically (range 0-3) as proposed by Johansen et al., [26]. Patients underwent medical treatment with equiactive doses of local steroids: budesonide (200μg) or fluticasone or mometasone (100 μg) in each nostril once daily for at least 3 months. In cases of failure of local treatment, or of severe polyposis, systemic steroids were used: triamcinolone acetonide (40 mg i.m. every 10 days for 3 times) or betametasone (4 mg/day i.m. for 4 days). Patients were reassessed at the end of treatment; in the case of treatment failure (endoscopic score unchanged) surgery was scheduled. The minimum wash-out period from steroid therapy was 30 days. After obtaining informed consent authorizing surgery, all patients underwent FESS (Functional Endoscopic Sinus Surgery), in particular nasal polypectomy, anterior ethmoidectomy and, depending on which structures were involved, unilateral or bilateral posterior ethmoidectomy, medial maxillectomy, sphenoidotomy. After surgical removal, the polyps were sectioned; one half was used for histopathological diagnosis, while the other half was immediately frozen at -80°C until use for biological analyses.

Control group

Fifteen subjects (11 male, 4 female, median age 43) without sinus disease, suffering from nasal *septum deviation* and scheduled for septoplasty, were enrolled as control group. With subjects' informed consent, a small sample of medial wall of the inferior turbinate was taken and immediately frozen at -80°C.

Tissue analysis

If there was sufficient tissue, the GR isoforms were assessed by two different methods:

- "radioligand binding assay" (binding), which assesses GR binding capacity and binding affinity (Kd);
- "Western Blot assay" (WB), which determines the tissue level of receptor expression.

The GRβ isoform, which lacks the hormone binding domain, was detected by WB alone. WB was also utilized to determine GRα subcellular distribution (cytosol and nucleus) in a small number of polyps, and to analyze TGF-β expression.

Radioligand binding assay

Tissues were homogenized in TGMo buffer (10 mM Tris, 1 mM EDTA, 20 mM $MoNa_2O_4$, 10% glycerol, 5 mM dithiothreitol, pH 7.4) at 4°C using a teflon Potter Elvehjem tissue grinder, then ultracentrifuged (105,000*xg* for 1hr) to obtain cytosol. Cytosol aliquots were incubated for 18 hrs at 4°C with scalar concentrations (0.06-1 nM) of [1,2,4,6,7-^{3}H]-Dexamethasone (S.A. 23 Ci/mmol, GE Healthcare Europe, Milan, Italy). Binding in the presence of excess corticosterone (250nM) was used to determine non-specific binding. After incubation, unbound hormone was removed by treatment with dextran-coated charcoal and centrifugation at 3,000x*g*. The radioactivity present in supernatants was counted in a Beckman β-counter. Cytosol proteins were determined by the Lowry method [27], with bovine serum albumin as reference protein. The results of Scatchard analysis were expressed as femtomoles of hormone specifically bound per milligram of cytosol protein.

Western blotting

Cytosolic and nuclear extract preparation

Polyps of sufficient size were homogenized in a 50 mM Tris buffer (pH 7.2) containing 6 mM $MgCl_2$, 1 mM EDTA, 10% sucrose, 1 mM phenylmethyl–sulfonyl fluoride, 5 µg/mL leupeptin, 1 µg/mL pepstatin, 1 µg/mL aprotinin, using a teflon Potter Elvehjem tissue grinder at 4°C. The homogenates were centrifuged for 5 min at 2,000×g at 4°C. The resulting supernatant and pellet were further processed, generating cytosol and nuclear extract. For the cytosol preparation, the supernatant was ultracentrifuged (105,000×g) for 1 hr at 4°C and the final supernatant was used as cytosol tissue fraction. For nuclear extract preparation, the pellet was washed twice by resuspension in 0.5mL of homogenization buffer. The washed pellet was then resuspended in 0.25mL homogenization buffer containing 0.5 M NaCl. After incubation for 1 hr in an ice bath with frequent vortexing, the suspension was centrifuged (8,000×g) for 10 min at 4°C. The final supernatant was used as tissue nuclear extract. Protein concentrations for each cytosol and nuclear sample were determined following the Lowry method [27].

Total lysates

Polyps and control tissues were homogenized in RIPA buffer (20 mM Hepes, 150 mM NaCl, 5mM EDTA, 1mM DTT, 1% Triton X-100, 10% glycerol, 1 µg/mL leupeptin, 1 µg/mL aprotinin, 1 µg/mL phenylmethyl–sulfonyl fluoride, 1mM sodium orthovanadate) at 4°C using a teflon Potter Elvehjem tissue grinder. Subsequently samples were centrifuged at 16,000xg for 30 min. Protein concentration was assessed by the Lowry method [27].

Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis (SDS-PAGE)

Cytosol and nuclear fraction protein (30µg) and total lysate proteins (100µg) were resolved by SDS-PAGE through 8% polyacrylamide gel and transferred to nitrocellulose membrane (Hybond™ ECL™, GE Healthcare Europe, Milan, Italy) for immunoblotting. Ponceau S staining solution (Sigma-Aldrich) was used to detect proteins on nitrocellulose membranes, to confirm equal protein loading. Blots were probed with primary rabbit polyclonal antibodies: anti-GR-α (1:500, Santa Cruz sc-1002), anti-GR-β (1:1000, ABR PA3-514), anti TGF-β1 (1:500, Santa Cruz sc-146) and anti-actin (1:2000, Sigma-Aldrich). Blots were then probed with anti-rabbit secondary polyclonal antibody conjugated with horseradish peroxidase. Protein levels were detected using enhanced chemiluminescence ECL WB detection reagent (GE Healthcare Europe, Milan, Italy) and bands were visualized using a Kodak Image Station 440 CF (Eastman Kodak Company, Rochester, NY, USA). Image analyses were run on Kodak 1D 3.5 software (Eastman Kodak Company). GRs and TGF-β1 proteins were quantified by calculating the ratio between GRs or TGF-β1 and actin protein expression; the latter was selected as reference housekeeping protein.

Response to prednisone of cultured polyp tissue

After surgical removal, three nasal polyp specimens were each cut into three parts. The first was used for histopathologic diagnosis, the second was immediately frozen at -80°C until use for radioligand binding assay, and the third was subdivided into aliquots measuring approximately 3-4 mm^3 and maintained under organotypical culture conditions. To overcome the problem of biological heterogeneity, each experimental condition included between 6 and 10 randomly-selected tissue aliquots for each nasal polyp. Tissue aliquots were cultured for 24 hrs in 40 mm diameter Petri dishes containing RPMI-1640 medium enriched with fetal bovine serum (10%), 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin, and maintained at 37°C in a 95% air/5% CO_2 humidified atmosphere, before exposure to prednisone. Tissue aliquots were incubated with prednisone (25 µM) for 24 and 48hrs; after incubation they were washed three times in saline solution and then processed to obtain total lysates, in which GR-α, GR-β and TGF-β1 expression were evaluated by WB (see Western blotting section). Each lysate was further processed using the MILLIPLEX® Human Cytokine/Chemokine Kit (Millipore, Milan, Italy), following the manufacturer's instructions, to simultaneously quantify monocyte chemotactic protein 1 (MCP1/CCL2), tumor necrosis factor alpha (TNF-α), interferon-γ (INF-γ), interleukin-10 (IL-10) and granulocyte-macrophage colony-stimulating factor (GM-CSF). The intra-assay coefficient of variation was 4.6-13.8%; the inter-assay coefficient of variation was 3.7-17.2%. The detection limits were approximately 0.64–10.000 pg/mL.

Statistical analysis

The Mann Whitney test was used to compare TGF-β1 expression between nasal polyps and healthy mucosa. The Friedman test and Dunn post hoc comparisons were used to compare the effects of prednisone on GR-α, GR-β, TGF-β1 expression and cytokines/chemokines concentrations in cultured nasal polyp tissues.

Results

GR binding capacity and binding affinity

All 32 polyp samples were examined with the binding assay. Conversely, only 8 of the 15 samples of normal mucosa were examined with this technique, the remaining seven being too small to carry out this assay; they were thus analyzed by WB. 43.75% of polyps (14 of 32 polyps) were negative at the binding assay (GRα-). The GR-α positive (GRα+) samples contained variable GR-α concentrations, ranging from 9 to 57 fmol/mg, with a median value of 20 fmol/mg of cytosol protein. The value of Kd, which expresses binding receptor affinity, ranged from a minimum of 0.18 nM to a maximum of 3.49 nM, with a median value of 0.81 nM.

With regard to the eight control specimens, four were negative at the binding assay, with concentrations ranging from 15 fmol/

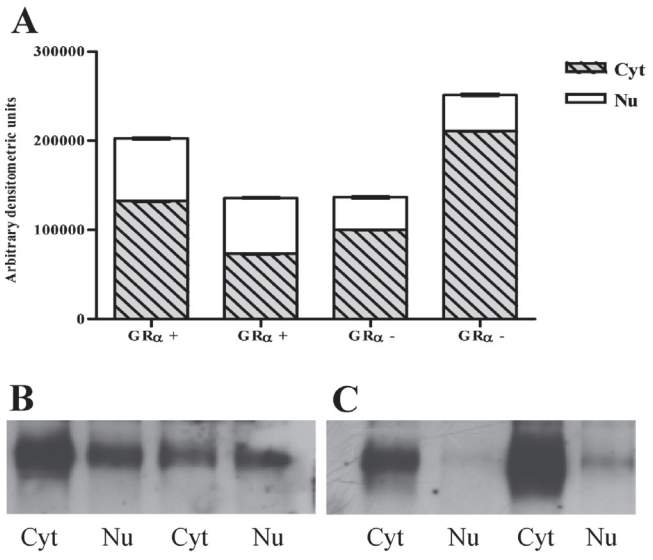

Figure 1. Intracellular GR-α distribution in nasal polyps (NPs) positive (GRα+) or negative (GRα-) to the binding assay. (A) cytosolic and nuclear GR-α levels in two GRα+NPs and two GRα-NPs; results are expressed as arbitrary densitometric units; Cyt= cytosolic fraction; Nu = nuclear fraction. (B) and (C) representative Western blots of GR-α distribution in two GRα+ NPs and two GRα- NPs.

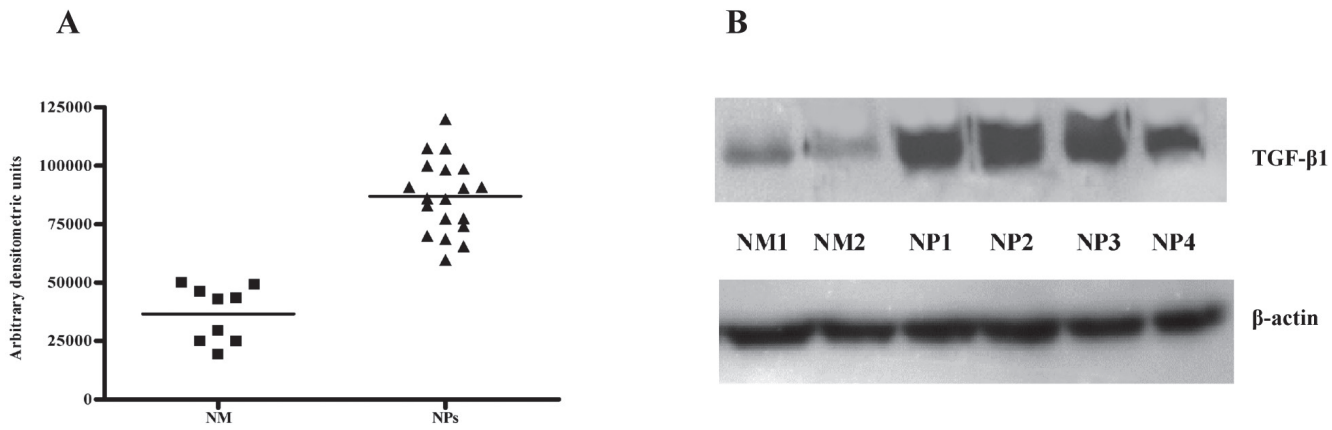

Figure 2. TGF-β1 expression in nasal polyps (NPs) and normal mucosa (NM). (A) symbols represent TGF-β1 levels in each individual patient; bars represent the mean value in each group. Mann Whitney test: $p < 0.0001$ NPs vs NM. (B) representative Western blot.

mg to 27 fmol/mg (median: 22 fmol /mg). Receptor affinity, K_d, ranged from 0.24 nM to 0.89 nM; the median value was 0.56 nM.

GR-α subcellular distribution

The results obtained from evaluating GR-α subcellular distribution showed that in GRα- polyp tissues the receptor was prevalently localized in the cytosol, whereas in GRα+ polyp tissues it was almost evenly distributed between cytosol and nucleus (Figure 1).

GR-α, GR-β and TGF-β1 expression

WB revealed the presence of GR-α in all polyps examined (14 polyps), including in cases that were negative to the binding assay (5 of 14). GR-β was also expressed in all polyps (Table 1). Similarly, all normal mucosa specimens examined by WB were positive for GR-α, whereas only 3 of 7 normal mucosa specimens expressed GR-β in appreciable amounts (Table 2). WB detected TGF-β1 in 19 polyps and 7 normal mucosa specimens. TGF-β1 was expressed in all polyp and normal tissues tested, but at higher expression levels in NPs (Figure 2).

Response to prednisone of polyp tissues in culture

Figures 3-5 show the results obtained after incubating NPs (3 different polyps) in culture with prednisone for 24 or for 48 hrs. GR-α, GR-β and TGF-β1 expression decreased, achieving statistical significance after 48 hrs of treatment. Prednisone was also effective against pro-inflammatory cytokines, reducing their tissue concentrations in a time-dependent manner that was specific for each cytokine (Figure 6). The three polyp specimens

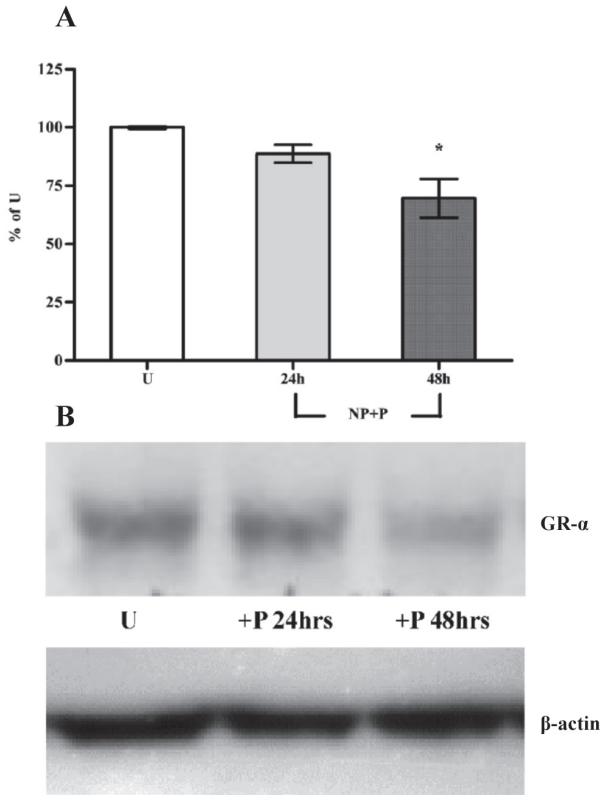

Figure 3. Effect of prednisone on GR-α expression in cultured nasal polyp tissues. (A) GR-α levels in cultured nasal polyp tissues expressed as percentage of untreated polyps. U = untreated nasal polyps; NP+P = nasal polyps in presence of prednisone 25μM for 24 and 48h. Friedman test and Dunn post hoc comparison test: *p < 0.001 vs U; 3 evaluations for each sample. (B) representative Western blot.

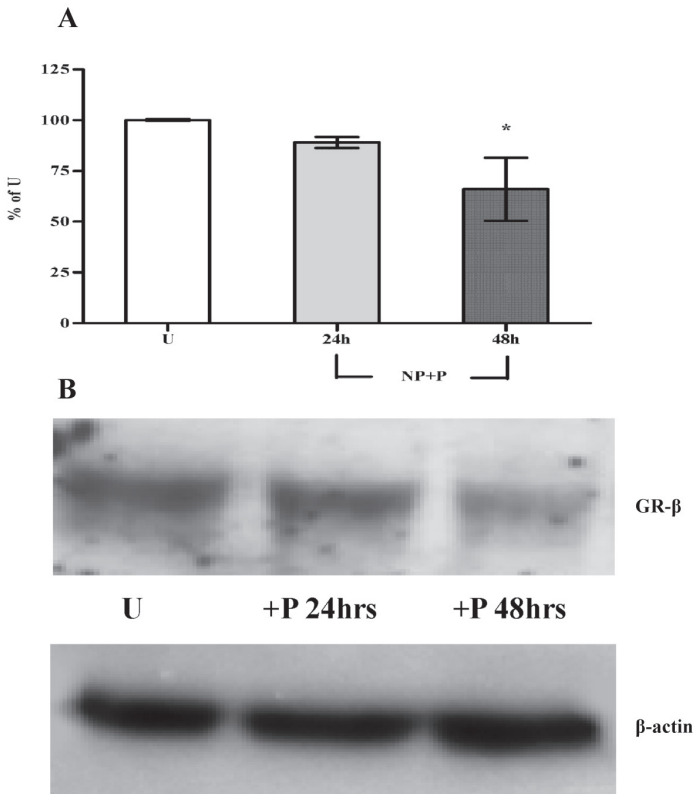

Figure 4. Effect of prednisone on GR-β expression in cultured nasal polyp tissues. (A) GR-β expression in cultured nasal polyp tissues expressed as percentage of untreated polyps. U = untreated nasal polyps; NP+P = nasal polyps in presence of prednisone 25μM for 24 and 48h. Friedman test and Dunn post hoc comparison test: *p < 0.05 vs U; 3 evaluations for each sample. (B) representative Western blot.

were found to be GR-α positive when evaluated by the radioligand binding assay.

Discussion

Nasal polyposis is a complex condition related to specific and non-specific nasal hyper-reactivity. Its etiopathological mechanisms are linked to different factors, in particular to inflammation and oedema caused by an alteration of membrane ionic transport in the ethmoidal mucosa. Topical and systemic GCs are the first-line treatment for nasal polyposis, in particular in mild to moderate forms of the disease. Surgical treatment is reserved for cases in which steroids are contraindicated, medical therapy has failed, compliance is lacking, or complications have occurred. Thanks to their anti-inflammatory and anti-allergic properties, GCs are effective in reducing nasal symptoms: they improve nasal respiration and reduce the size of polyps and the frequency of relapses [28].

Some patients affected by nasal polyposis fail to respond to steroid therapy. However, only recently attention has been paid to the molecular mechanisms underlying resistance to GCs [29].

The most reliable hypothesis points to alterations of receptor or signal cascades, in particular to an imbalance between the two isoforms of GR: GR-α, a ubiquitous receptor that mediates most actions of GCs, and GR-β, which differs from GR-α in that it lacks a hormone binding domain and is thus unable to bind steroids [30-32].

This study evaluated, in healthy mucosa and in polyp specimens, the expression, binding capacity and binding affinity of GR-α, and the expression of GR-β and TGF-β. The radioligand binding assay revealed great variability in GR-α binding capacity within the group of patients affected by nasal polyposis (absence of free receptor in 43.75% of cases) and also in the normal mucosa specimens (absence of free receptor in 50% of cases). These results appear to suggest that there is no difference between healthy and polyp tissues in terms of the density of available receptors, nor of their ability to bind hormones.

Evaluation of receptor binding affinity showed great variability of K_d values in both groups, with a trend toward higher values in polyps (0.95 nM) than in normal mucosa (0.56 nM), suggesting that the affinity of GR-α for its ligand is lower in NPs. When

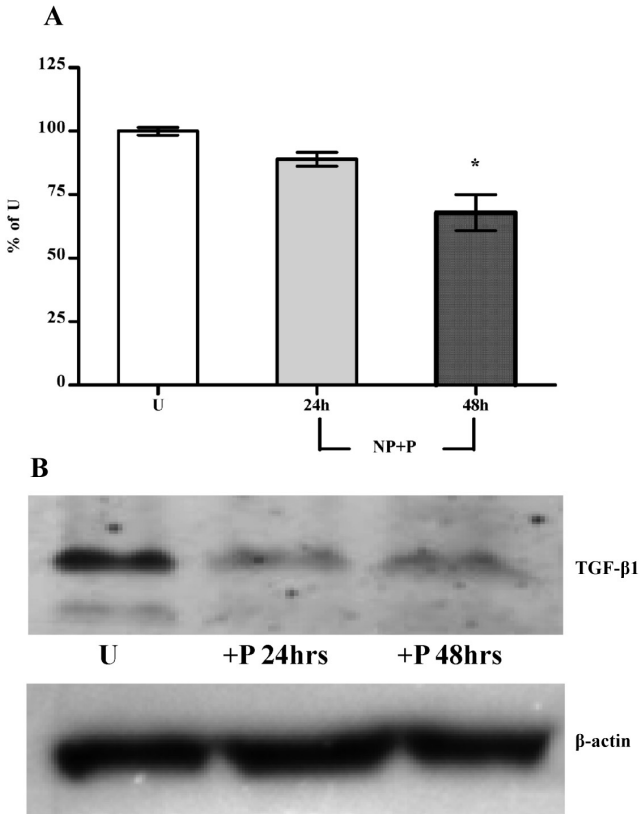

Figure 5. Effect of prednisone on TGF-β1 expression in cultured nasal polyp tissues. (A) TGF-β1 expression in cultured nasal polyp tissues expressed as percentage of untreated polyps. U = untreated nasal polyps; NP+P = nasal polyps in presence of prednisone 25μM for 24 and 48h. Friedman test and Dunn post hoc comparison test: *p < 0.05 vs U; 3 evaluations for each sample. (B) representative Western blot.

Table 1. GR-a and GR-β expression in nasal polyps.

Patient	GR-α (fmol/mg)	Kd (nM)	WB GR-α	WB GR-β
AM	18	0,314	+	+
AS	ND	ND	+	+
BAL	ND	ND	+	+
BAR	14	0,345	+	+
BAS	23	0,613	+	+
BE	ND	ND	+	+
CA	9	0,432	+	+
CE	11	0,185	+	+
CH	ND	ND	+	+
CHI	ND	ND	+	+
CI	46	0,849	+	+
NO	27	1,11	+	+
MA	9	0,655	+	+
PAL	11	0,818	+	+

ND: not determined; + : expressed

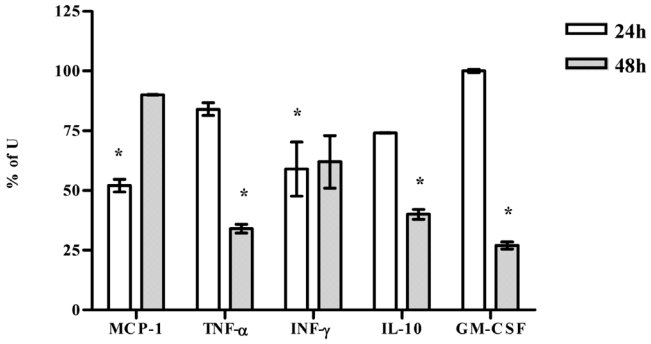

Figure 6. Effects of prednisone on cytokine concentrations in nasal polyps maintained in culture. Results are expressed as percentage of untreated polyps. Friedman test and Dunn post hoc comparison test: *p < 0.05 vs U; 3 evaluations for each sample.

Table 2. GR-a and GR-β expression in normal mucosa.

Patient	WB GR-α	WB GR-β
CA	+	-
DS	+	+
FI	+	-
NI	+	+
PO	+	-
RO	+	+
SE	+	-

+ : expressed; - : not expressed

GR-α was assessed by WB, all examined tissues were found to be positive, both in normal mucosa and in polyp tissue, including the cases that were negative to the binding assay.
Several conditions might give rise to these differences in the results obtained by the binding assay and by WB. The binding assay detects free receptor levels in tissue cytosol. A negative result with this technique might be due to: 1) absence of the receptor in the tissue examined; 2) massive translocation of the receptor from cytosol to nucleus after its activation by the hormone; 3) inability of the receptor present in the tissue to bind to the hormone. The results achieved by WB enable us to exclude the first hypothesis, but not the others. However, assessment of the GR-α subcellular distribution points to a different cellular receptor distribution in GRα- and GRα+ polyps to binding, with a prevalent localization of the receptor in the cytosol of GRα- polyps. The high receptor concentration in the cytosol fraction, with negligible positivity in the nucleus, may be related to a reduction in ligand-receptor interaction. We may thus assume that resistance to GCs is not attributable to reduced GR-α expression, but that it might be due to a reduction in receptor affinity for steroids, or in receptor ability to bind hormones.

With regard to GR-β, recent research shows that it is functionally related to a failure to respond to GCs [33,34]. Unlike GR-α in the ligand-binding domain, GR-β does not bind to GCs, and functions as a dominant-negative inhibitor of GR-α. The hormone-activated-GR-α, interacting with GR-β, forms a heterodimer that has little action on transcription, and that is thus responsible for a reduced anti-inflammatory response to steroids.
In addition, in some diseases characterized by an alteration of the immune system, overexpression of GR-β has been reported, leading to an increased release of pro-inflammatory cytokines, namely IL-1, 2, 4, 7, 8, and 18, TNF-α and IFN-α and γ [35]. In particular, overexpression of GR-β has been detected in T cells and in bronchial epithelial cells, and has been suggested as a potential mechanism of resistance to GCs in asthma patients [36-38]. Specifically, increased expression of GR-β is reported in approximately 30% of macrophages in polyps. These cells are important producers of IL-1 and TNF-α, cytokines that allow inflammation to protract [38]. It has been shown that polyps that do not respond to treatment with intranasal fluticasone propionate have a higher expression of IL-5 and GR-β. This suggests that the increased expression of GR-β may modulate the response to local steroids [39].

In our study, GR-β was expressed in all 14 polyp specimens, but was absent in 57% of samples of normal mucosa (4 of 7). This confirms that GR-β is up-regulated, and supports the hypothesis that, in GC-resistant polyps, the action of GR-α might be inhibited by GR-β. TGF-β is a ubiquitous growth factor that, on one hand promotes chemotaxis and activation of inflammatory cells, while on the other hand it promotes the onset of tissue repair, resulting not only in a significant anti-inflammatory effect, but also in fibrotic induction [22,41,42]. Modulation of TGF-β expression is complex, and is important in the regulation of crucial processes of inflammation and immune response, as well as in the formation of extracellular matrix and chemotaxis of inflammatory cells [22,43-46].

Increased levels of TGF-β1 have been reported in polyp tissue, suggesting the cytokine may play a role in epithelial differentiation and tissue remodeling in nasal polyposis [21,47]. In our study, evaluation of TGF-β1 expression showed it to be higher expressed in polyps than in normal tissue. This stresses the importance of inflammation as an etiopathogenic mechanism of NPs: on one hand inflammation elevates expression of GR-β, resulting in resistance to steroids, while on the other hand it leads to increased expression of TGF-β1, promoting the early shutdown of the inflammatory process and the beginning of tissue repair.

Although all NPs examined in this study were from patients who had shown clinical resistance to GCs, the preliminary results obtained after treatment with prednisone of some specimens in culture show that, when the tissue can bind the hormone, as in our three cases that were all GRα+ to the binding assay, exposure to a GC produces the typical effects of steroid treatment, for example decreasing both isoforms of GR and the pro-inflammatory cytokines, suggesting the integrity of GR-signaling. Although the number of tissue specimens examined is small, and although it remains difficult to explain why tissues excised after a lack of clinical response to steroid treatment were responsive to "*in vitro*" treatment, in our view these data are interesting and deserve further investigation. In light of the above results, it may be said that resistance to GCs is not attributable to low levels of GR-α expression, but rather appears to be due to a reduction in GC affinity for the ligand: receptor affinity was lower in polyps than in healthy mucosa. With regard to GR-β, numerous and contradictory studies have analyzed its function, expression, and contribution to GCs insensitivity. Our study, in agreement with numerous reports showing an association between GCs insensitivity and increased GR-β expression in NPs, revealed a higher expression of the GR-β in GC-resistant polyps compared to normal tissue, giving further evidence of the cytokine's up-regulation in this scenario, caused by inflammation.

As reported above, TGF-β1 was strongly expressed in NPs versus normal mucosa, suggesting that it might be involved in the pathophysiological development of nasal polyposis, where it could participate in the complex regulation of local inflammation.

In conclusion, better understanding of the mechanisms un

derlying resistance to GCs and of the roles played by TGF-β1 and other cytokines in nasal polyposis could have future clinical implications and direct research toward the development of more appropriate therapeutic strategies.

Authorship contribution

SA, CO and SR designed research; GA, VC, FP performed research; AL, AF and SDF contributed analytic tools; GA, VC SR analyzed data; SR and CO wrote the paper.

Conflict of interest

All authors declare that they have no conflict of interest.

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