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Evaluation of ciprofloxacin used as an intranasal antibiotic

E. AZIZLI[1], N. BAYAR MULUK[2], C. VEJSELOVA SEZER[3], H.M. KUTLU[3], C.C. CINGI[4]

[1]Otorhinolaryngology Private Practice, Istanbul, Turkey
[2]Department of Otorhinolaryngology, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkey
[3]Department of Biology, Faculty of Science, Eskisehir Technical University, Eskisehir, Turkey
[4]Department of Otorhinolaryngology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

Abstract. – OBJECTIVE: The objective of this research was to examine the effects of topical ciprofloxacin on cultured nasal epithelial cells of human origin.

MATERIALS AND METHODS: Human nasal epithelial cells were collected from patients who voluntarily donated tissue left over following septorhinoplasty. The samples were from individuals without any indication of rhinosinusitis. An assay that may be employed to investigate toxic effects at the cellular level is MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). This test reveals where DNA becomes fragmented, the nuclei condense, the outer cell membrane is altered, or the cytoskeleton appears disrupted. The present study employed this technique. Nasal epithelium in cell culture was exposed to ciprofloxacin for 24 hours at a temperature of 37°C, following which the MTT assay was undertaken before examining the cells by confocal microscopy to look for alterations indicating cytotoxicity. Another test of toxicity, the artificial scratch technique, was also used. Cells treated in this way were assessed using a light microscope.

RESULTS: The nasal epithelial cells in culture that were exposed to topical ciprofloxacin for 24 hours were less viable than controls and this result was statistically significant. For this length of exposure, the IC50 was calculated as 1.565 mg/mL. The peak reductions in cellular viability occurred with exposure at a concentration of 1.25 mg/mL and 0.625 mg/mL. These was only a mild decrease in viability at other concentrations, but these results were of statistical significance. The MTT assay and confocal microscopy confirmed this result. Cultured nasal epithelium not exposed to ciprofloxacin (i.e., controls) exhibited a compact morphological appearance when examined with confocal microscopy. The epithelial cells had a regular fusiform boundary, and the nuclei were intact. By contrast, the cultures with exposure to the antibiotic were of decreased size and their outline changed from fusiform to round.

The epithelial cells cultures where scratch injury was induced were examined by light microscopy, the extent of closure of the denuded area being assessed after 24 hours. It was noted that the area opened up by the experimental scratch was not closed completely by 24 hours later. This result shows that ciprofloxacin decreases the viability of nasal epithelial cells.

CONCLUSIONS: Topical application of ciprofloxacin to the nasal lining is not recommended, since this resulted in decreased cellular viability, cellular shrinking and alteration in outline from fusiform to round in cultured nasal epithelial cells. These changes indicate that topically applied ciprofloxacin is toxic to nasal epithelial cells. The outcomes of this study should be studied and correlated *in vivo* models.

Key Words:
Ciprofloxacin, Cultured nasal epithelial cells, Cellular viability, Topical application.

Introduction

The fluoroquinolone antibiotics are agents of high clinical efficacy and with several desirable pharmacokinetic characteristics, such as high bioavailability when administered by mouth, a large volume of distribution, and the ability to affect a wide range of different bacterial pathogens. However, as this group has been more widely used, resistant organisms have increased in frequency. Furthermore, there are a number of severe complications which may occur with fluoroquinolones, such as *Clostridium difficile* diarrhea, tendinopathy and neuropathy. There is also potential for drug-drug interactions with many different medications. These drawbacks mean that fluoroquinolones are usually only employed where the potential benefit evidently exceeds the potential risk[1].

Corresponding Author: Nuray Bayar Muluk, MD; e-mail: nbayarmuluk@yahoo.com; nurayb@hotmail.com

The mode of action of fluoroquinolones is direct inhibition of DNA synthesis by bacteria[2,3]. Every member of the fluoroquinolone group attaches to the DNA gyrase and DNA topoisomerase IV enzymes when they are acting on DNA, thereby causing DNA to break apart. For different bacterial organisms, a particular fluoroquinolone will more strongly inhibit one of these two enzymes. However, overall, the effect of DNA being broken apart by the antibiotic is that DNA stops being copied, the existing DNA undergoes further damage and the bacterium eventually dies[1].

In most cases, patients tolerate fluoroquinolones well. The side effects occurring with highest frequency are of mild severity. Gastrointestinal symptoms, headache, mild vertigo and temporary mood or sleep disturbance may be noted. The precise frequency of side effects affecting the gut or central nervous system has not been established but appears to be around 3-fold higher than with other antibiotic medications[4]. It also seems that overgrowth of *C. difficile* is more common when fluoroquinolones are used than with many other antimicrobials[5,6].

Adverse effects of high severity are infrequent. Tendinopathy, tendon rupture, peripheral neuropathy, lengthening of the cardiac QT interval and even dissection or rupture of the aorta may occur, albeit not commonly. In a small number of cases, tendinopathy or neuropathy is irreversible and may cause disability. Fluoroquinolones should not normally be used if a patient has a history of tendinopathy or neuropathy or is at raised risk of such occurrences. Likewise, if a patient already has lengthening of the QT interval or is on a drug known to lengthen the interval, fluoroquinolone use is not recommended[1].

Unless there is no other antibiotic with a more favorable safety profile available, fluoroquinolones should not be prescribed to women during pregnancy or if nursing, or to pediatric patients. The reason for this advice is that fluoroquinolones may be toxic to the developing musculoskeletal system, both in utero and in childhood[1]. However, the side effects most often encountered are gastrointestinal disorders (leading to nausea, for example) or central nervous system symptoms, notably headache and light-headedness. As described above, the main concern is QT lengthening and tendinopathy, as well as formation of an aortic aneurysm or dissection. Blood glucose homeostasis may be disturbed and retinal detachment is also possible in some cases[1].

Medical treatment of chronic rhinosinusitis (CRS) aims to inhibit inflammation in the nose and sinuses, as a result of which the ostia become blocked and sinus clearance rendered ineffective. It is not routine practice to treat CRS with antimicrobials and there is a lack of rigorous evidence to justify their being employed in this way. Clinicians contemplating the administration of antibiotics to patients with CRS need to estimate the potential clinical benefit and compare it with the known side effects of these agents and the potential for inadvertently selecting resistant organisms. Antibiotics are generally only prescribed if a bacterial pathogen has been identified as the likely etiology. It is difficult in some cases to prove the bacterium.

For some clinicians with specialist expertise in CRS management, antibiotics only play a role if there is an abrupt deterioration in the clinical presentation. For others, however, antibiotic treatment appears justified where there is clinical suspicion of an infection, and this possibility cannot be excluded as a factor. An example of such a case would be where CRS not associated with nasal polyps does not improve despite topically applied steroid treatment for more than two months[7]. The author of this article concurs with the latter approach[7].

Given that the majority of cases of CRS are treated outside hospital, most pharmacotherapeutic regimens involve oral administration. Suitable antibiotics need to be effective against anaerobes (such as *Fusobacterium nucleatum*, or members of the genera *Prevotella, Porphyromonas* and *Peptostreptococcus*), as well as streptococcal organisms or *Staphylococcus aureus*. Whilst infection with *Haemophilusinfluenzae* or *Moraxella catarrhalis* seldom occurs in CRS, these pathogens may cause an acute clinical deterioration, therefore antibiotic cover for these organisms is also advisable[7].

For the majority of cases of chronic rhinosinusitis, co-amoxiclav is the treatment of choice where antimicrobial therapy is deemed necessary. The dose in children is 45 mg/kg body mass twice a day. For adults, a dose of 500 mg tds, 875 mg bd or 1,000 mg sustained release bd is suitable[7]. Depending on the circumstances of the case, a second line agent may be preferred[7]. Patients with an allergy to penicillin should be started on clindamycin at a dose of between 20 and 40 mg/kg body mass divided into 4 or 3 doses at regular intervals (for children), or 300 mg qds or 450 mg tds (for adults). Neither *Haemophilus influenzae* nor *Moraxella catarrhalis* are susceptible to clindamycin, however, nor is this agent effective against other Gram-negative bacilli. Treatment with clindamycin, therefore, is empirical and may need to be supplemented or substituted if there is

inadequate response. Moxifloxacin as monotherapy at a dose of 400 mg od is effective against both aerobic and anaerobic bacterial pathogens[7].

This study examines how topically applied ciprofloxacin affects nasal epithelial cells in cell culture. The main focus of the study was to ascertain any evidence for toxicity of ciprofloxacin to the nasal epithelium when used in this manner.

Materials and Methods

This study was undertaken at the ENT Department of Eskişehir Osmangazi University, working alongside the Department of Biology within the Faculty of Science at Eskişehir Technical University. Nasal epithelial cells from healthy epithelium excised routinely during surgery (septorhinoplasty) from human patients was utilized. These patients all provided written consent for their tissue to be used in scientific research. Fragments of mucosa obtained in this way were immediately placed in a preservative medium to facilitate transfer to the Cell Culture Laboratory of Eskişehir Technical University.

Primary Cell Culture

Healthy epithelial cells were gathered from five volunteers, each of whom underwent septoplasty. None of the volunteers suffered from rhinosinusitis. First the strips of nasal epithelium were dissected on Petri dishes, which had been sterilized in advance. The epithelium was cut into small pieces. The epithelial cells were then placed into DMEM-F12 cell culture medium. The medium consisted of 10% fetal bovine serum with penicillin and streptomycin also included. These cell cultures were then maintained under specific conditions: a temperature of 37% and a moist atmosphere with 5% carbon dioxide. After 7 days, the excess tissue was washed away from the culture plate. Epithelium adhering to the plate was rinsed in phosphate-buffered saline (PBS), treated with trypsin and then passaged onto T25 cell culture plates. The cultures were judged sufficiently mature for the toxicity exposure section of the experiment once they were 85% confluent[8].

MTT Assay to Assess Cytotoxic Potential of Ciprofloxacin

Primary epithelial cells were separated from each other by trypsin treatment, after which they were transferred into 96-well containing plates. There were around 5,000 epithelial cells in each well. The concentration of different test reagents was altered for individual wells. The minimum concentration was 0.15 mg/mL, whilst the maximum was 5 mg/mL. The same culture conditions were used as in the initial phase, i.e., temperature held at 37°C and a moist atmosphere with 5% carbon dioxide.

For the assay, first ciprofloxacin was mixed with dimethylsulfoxide (DMSO) to a dilution of 5 mg/mL. It was arranged in such a way that the wells contained epithelial cells exposed to Ciprofloxacin at different concentrations. The lowest concentration was 0.15 mg/mL, whilst the highest concentration was 5 mg/mL. The incubation lasted 24 hours.

After the cells had been exposed to the ciprofloxacin for 24 hours, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in phosphate-buffered saline (PBS, Invitrogen) at a concentration of 5mg/mL was then placed in each well. The plates then underwent a further 4-hour period of incubation, without any variation in the culture conditions. When the final incubation period was over, the media were replaced with 200 µL DMSO. The three plates were then assessed using an ELISA plate reader set to a wavelength of 570nm. The absorbance was used to calculate viability percentages and the IC_{50} (half maximal inhibitory concentration)[8].

Confocal Microscopy

Epithelial cells were kept at a steady 37°C whilst exposed to ciprofloxacin for a period of 24 hours. The cells were cover slipped and placed into plates with 6 wells. There were 30,000 epithelial cells in each of the plate wells. The epithelial cells were exposed to ciprofloxacin at a concentration corresponding either to the 50% inhibitory concentration or to full strength. Control epithelial cells were not placed in contact with the antibiotic. Once the exposure period was over, the medium was washed off using PBS. A 15-minute fixation procedure was then undertaken at room temperature, using 2% glutaraldehyde. The fixated cells were then washed once more with PBS before being stained for confocal microscopy. The staining agents were Alexa Fluor-488 phalloidin and acridine orange.

The assessment of cytotoxic alterations was performed with a Leica SP5II confocal microscope (Wetzlar, Germany). The features the researchers were looking for were fragmented DNA, condensation of the nuclei, change to the cell outer plasma membrane and cytoskeletal distortion[8].

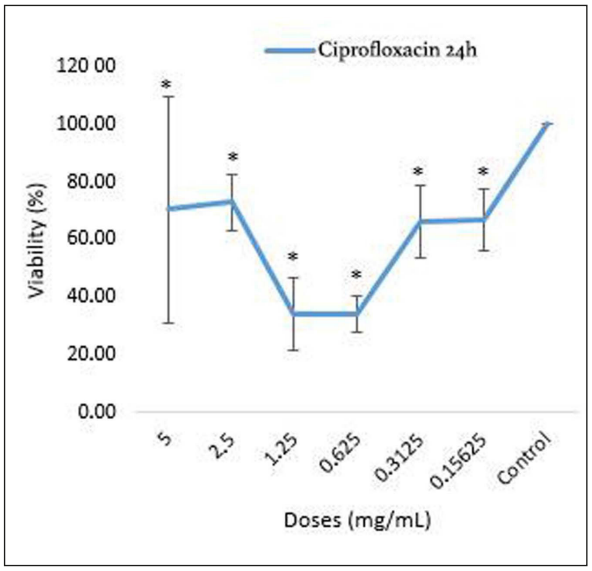

Figure 1. Viability curve of primary nasal cells treated with different ciprofloxacin concentrations for 24 hours.

Wound Healing Assay

Cells of nasal epithelial origin underwent an incubation for 24 hours. The epithelial cells were cultured in plates with 6 wells, each of which held 3 x 10^5 cells. A sterile pipette tip of size 20-200 µL was used to scratch a region of the plate where the cells were confluent. This was then followed by rinsing the plates with PBS. The initial appearances at light microscopy were recorded, just after the medium was replaced with either fresh medium alone (for the controls) or fresh medium plus ciprofloxacin. The extent of scratch closure was assessed microscopically after 24 hours Epithelial cells that were not treated in this way formed the control group[9].

Statistical Analysis

The data from the experiments described above were analyzed statistically using the GraphPad Prism 6.0 programme (GraphPad Software Inc., San Diego, CA, USA) running on Windows. ANOVA (one-way analysis of variance) was performed on multiple comparisons.

$p<0.05$ was considered statistically significant.

Results

MTT Assay Results

Primary nasal epithelial cells in cell culture were significantly less viable following exposure for 24 hours to ciprofloxacin. For an exposure lasting 24 hours, the IC_{50} was found to be 1.565 mg/mL. The maximum loss of cellular viability occurred with exposure to the antibiotic at a concentration of 1.25 mg/mL and 0.625 mg/mL. The cells were also slightly less viable at the other doses, as can be seen in Figure 1. Despite the small decrease, the result did reach statistical significance.

Results from Confocal Microscopy

The observations made using confocal microscopy are in accord with the results of the MTT assay. The nasal epithelial cells in the control wells had a compact morphological appearance. There was preservation of the spindle-shaped cell outline and the nucleus remained intact (Figure 2A). However, in the wells containing ciprofloxacin, the epithelial cells had decreased in size and the spindle-shaped outline was lost, the cells appearing round instead (Figure 2B).

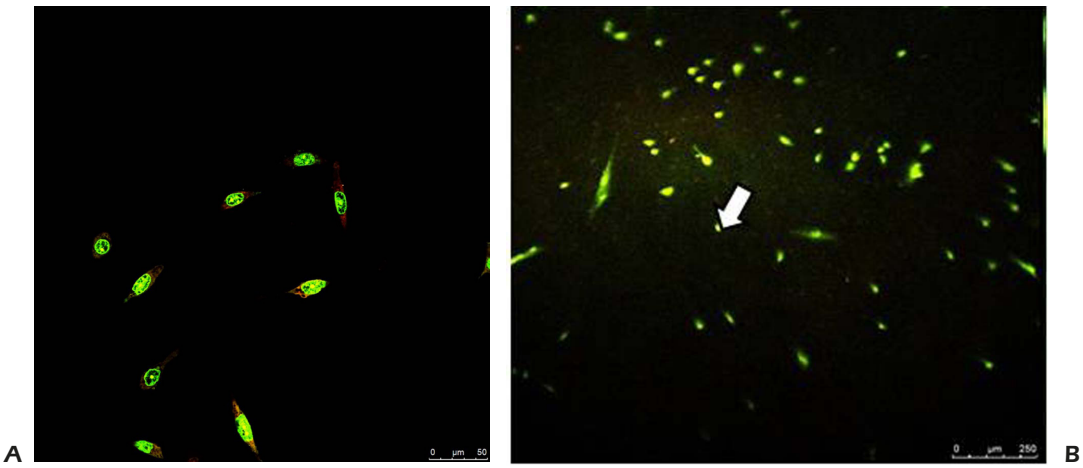

Figure 2. Confocal microscopy images of primary cultured nasal cells. **A**, Control group and test cells exposed to **(B)** Ciprofloxacin. Arrow: Nucleus. Magnification: 40X.

Results from the Wound Healing Assay

The main outcome examined in the wound healing assay was the ability of cells to reform a confluent layer. This was measured as the width of denuded area remaining 24 hours after the scratch, compared with immediately afterwards. It was noted that the clear area resulting from the scratch injury visible at the start of incubation (at time zero) (Figure 3) was not completely closed over by proliferation of primary nasal epithelial cells 24 hours later. At the end of the incubation period (at the 24th hour), it was evident that ciprofloxacin caused decrease in viability of cells (Figure 4).

Discussion

The fluoroquinolone antibiotics are agents of high clinical efficacy and with several desirable pharmacokinetic characteristics, such as high bioavailability when administered by mouth, a large volume of distribution and the ability to destroy a wide range of different bacterial pathogens[1]. They are effective against Gram-negative enteric anaerobes (such as *Escherichia coli*) and numerous bacteria responsible for respiratory infections. They have activity against atypical bacterial species, certain Gram-positive species, anaerobic bacteria and Mycobacteria[1].

– Ciprofloxacin is especially effective against Gram-negative bacilli, such as *Pseudomonas aeruginosa*.

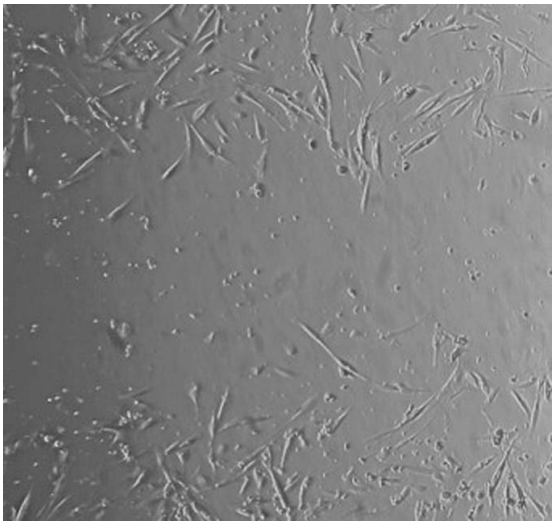

Figure 3. Wound healing images of primary nasal cells at the initial stage (0th hour). Untreated (Control) cells. Magnification: 40X.

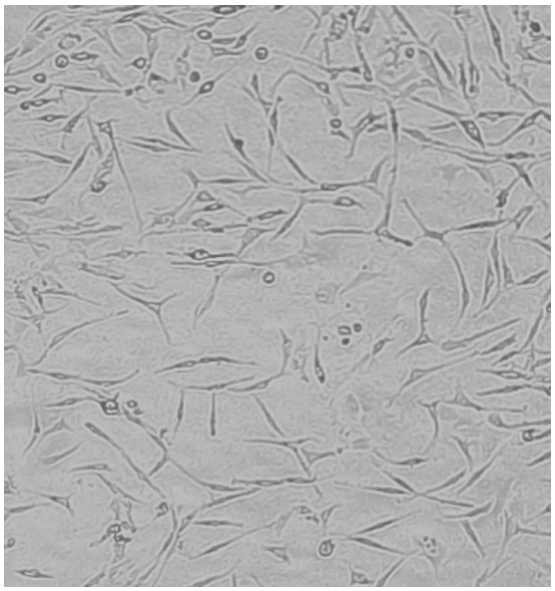

Figure 4. Wound healing images of primary nasal cells at the end of incubation period, i.e., at 24th hour. Untreated (Control) cells. Magnification: 40X.

– By contrast, levofloxacin, moxifloxacin and delafloxacin are less effective against *P. aeruginosa* than ciprofloxacin but can eliminate Gram-positive pathogens.
– Some anaerobic species are also susceptible to moxifloxacin. This antibiotic is also more effective in targeting Mycobacteria than the other fluoroquinolone agents.
– So far, delafloxacin has been used by few clinicians since it is the latest fluoroquinolone to enter the market. Anaerobic bacteria are susceptible to it, as are methicillin resistant strains of *S. aureus* (i.e., MRSA).

The effect of treating CRS with antibiotics has previously been examined in one trial[10]. This study employed double blinding and a double placebo design and was carried out at several separate locations. The antibiotics chosen were ciprofloxacin and co-amoxiclav, both for a short course[10]. The diagnosis of CRS was given to patients with inflammation lasting a minimum of 3 months, either unilaterally or bilaterally and without accompanying nasal polyps. The two antimicrobials were similarly effective at curing CRS (58.6% and 51.2%), with rates of pathogenic eradication of 88.9% or 90.5%. The number of cases where the pathogen had still not reappeared at day 40 was highest in the group administered ciprofloxacin after a positive culture (83.3% *vs.* 67.6%, p=0.043). The group administered ciprofloxacin also had the lowest rate of middle ear canal dis-

charge of pus (p=0.050), whilst the group taking co-amoxiclav had the most resolution of inflammation (p=0.040). Both these results were obtained by performing an endoscopic examination. Whilst 25% of those given co-amoxiclav complained of one or more side effects, the figure for ciprofloxacin was lower, at 12.4%, indicating that ciprofloxacin has greater tolerability (p=0.012). In both groups receiving active agent, all side effects were absent by the time the treatment finished. Both groups complained most about gastrointestinal disturbance. Placebo control was not used.

Antimicrobials used to treat CRS generally need to be administered for lengthy durations, so that the agent can reach a level adequate for pathogenic eradication[11,12]. If the course is not completed, the minimal inhibitory concentration may not be reached, and selection of drug-resistant organisms may occur[13]. The use of continuous release formulations allows dose frequency to be decreased, as well as providing a more sustained, steady plasma level of the agent. They are also associated with greater patient concordance with therapy[14]. Another possible choice is a drug-eluting implant. This device ensures long term antibiotic release at the site where the drug needs to act[13,15]. Although the prescription of fluoroquinolones for sinusitis was common in the past, the USA FDA has now provided extra warning about the risk of tendinitis and ruptured tendon with these drugs when given systemically[16]. The availability of a topical preparation of antibiotic would mean that the antibiotic could be present locally at a level exceeding the MIC, whilst the low systemic level would reduce the burden of adverse effects. In our study, the antibiotic we chose to investigate was ciprofloxacin, as this agent is active against a broad range of bacterial pathogens and is effective in eradicating *P. aeruginosa*. This agent has already been employed topically at other anatomical sites, namely on the skin, the eyes and the ears[17-19].

This study examined how topically applied ciprofloxacin affects nasal epithelial cells in cell culture. The main focus of the study was to ascertain any evidence for toxicity of ciprofloxacin to the nasal epithelium when used in this manner. The nasal epithelial cells in culture that were exposed to topical ciprofloxacin for 24 hours were less viable than controls and this result was statistically significant. For this duration of exposure, the IC_{50} was calculated as 1.565 mg/mL. The peak reductions in cellular viability occurred with exposure at a concentration of 1.25 mg/mL and 0.625 mg/mL. There was only a mild decrease in viability at other concentrations, but these results were also statistically significant. The MTT assay and confocal microscopy confirm this result. Cultured nasal epithelium not exposed to ciprofloxacin (i.e., controls) exhibited a compact morphological appearance when examined with confocal microscopy. The epithelial cells had a regular fusiform boundary, and the nuclei were intact. By contrast, the cultures with exposure to the antibiotic were of decreased size and their outline changed from fusiform to round.

The epithelial cells cultures where scratch injury was induced were examined by light microscopy, the extent of closure of the denuded area being assessed after 24 hours. In the cultures exposed to ciprofloxacin, the area opened up by the experimental scratch was not closed completely by 24 hours later. This result shows that ciprofloxacin decreases the viability of nasal epithelial cells.

It is generally assumed that antibiotic agents applied topically can ensure targeted delivery of the agent to the lining of the sinuses and nose. However, generally there is little supporting evidence for efficacy of antibiotics applied topically, albeit they do appear efficacious in short term use in particular cases[20-25]. A review article[20] cited 3 trials where treatment was randomized to either antibiotic or placebo and where no superiority of the active agent was demonstrated. There was also a single trial involving topical mupirocin, where symptomatic relief with the antibiotic was noted in patients with sinus culture-proven staphylococcal infection[20]. Nevertheless, this benefit was short term, and no benefit was demonstrable either clinically or microbiologically two months later. There is, furthermore, an association between topically applied mupirocin and raised levels of harmful bacterial species, in particular lower levels of Gram-positive species but raised levels of Gram-negative species that may cause infection, as well as *Corynebacterium* spp[26]. A further systematic review drew attention to some evidence indicating that topical antibiotic treatment guided by culture results may be beneficial in patients with CRS who have had sinus surgical interventions following the failure of medical treatment. This evidence was, however, of low quality[25].

Implants of drug-eluting type can remain in long term proximity to mucosae and keep releasing steady quantities of antibiotic. This makes them a viable therapeutic option for treatment of biofilms secreted by bacteria which resist systemic treatment. The implant delivers a raised local

concentration where it is needed without the patient incurring systemic adverse effects, including toxicity[13,15,27,28].

The ability of antibiotics to penetrate biofilms is a key element in treating drug-resistant cases of CRS. Scanning electron microscopy is a suitable technique for confirming the existence of a biofilm adherent to the sinus epithelial surface when biopsies have been obtained[29].

A drug-eluting stent for use in CRS has been investigated by Cho et al[30]. This stent was used to eradicate *P. aeruginosa* in rabbits with sinusitis. A biofilm secreted by a specific strain of *P. aeruginosa*, namely PAO-1, was less extensive with the ciprofloxacin-containing stent present than when absent, in an *in vitro* model. This reduction was statistically significant ($p<0.0001$). When this device was implanted in rabbits with sinusitis secondary to PAO-1 infection, the condition improved significantly, as assessed by endoscopic criteria ($p<0.0001$) or computed tomography ($p<0.002$). Histopathological assessment, including scanning electron microscopy, showed a more healthy-appearing mucosal and submucosal layer, with biofilm noticeably absent. Electron microscopy did reveal biofilms in rabbit mucosa taken from animals in which a sham stent was inserted, but no evidence of biofilms was present for the animals implanted with the ciprofloxacin-eluting device. Although the examination of the sinuses was restricted to areas not exceeding 20mm^2, which might not be representative of the entire sinus, the probability of this finding occurring by chance was reduced by sampling identical regions of the sinus in each animal[30].

Delivery of antimicrobial agents by nebulization is typically only used in cases following surgery on the sinuses, after which the sinusal ostia are unusually patent[31]. However, even for this group of patients, the evidence base for this practice consists of at most a few studies of observational design involving limited numbers. One study[32] retrospectively looked at 42 cases of CRS, finding that patients who opted for antibiotic delivery *via* nebulizer remained free of infection for longer than individuals opting for oral (systemic) medication. A different study[33] with an observational design concluded that antibiotic therapy *via* nebulizer was safe and some 83% (34 out of 41) of patients suffering from CRS complicated by recurring infections following surgery reported symptomatic improvement. The use of a nebulizer may be more beneficial than a nasal spray for treating CRS, since nebulized particles spread more diffusely over the nasal and sinusal lining[34,35]. Despite these encouraging signs so far, there remains a need for more research to delineate exactly where delivery of antibiotics by nebulizer fits into the treatment path for CRS[7].

A newly reported Brazilian study[36] compared the use of Nasopore containing betamethasone or ciprofloxacin with Nasopore containing only saline. When examined after 90 days, the degree of swelling of the mucosa was lowest in the patients supplied with betamethasone-impregnated Nasopore ($p=0.007$). This finding can be used to justify soaking nasal dressings in a steroid solution following endoscopic sinus surgical procedures. Another study published recently by Bing et al[37] involved 31 cases of CRS. Patients were randomly assigned to two groups: one group were given a non-impregnated gelatin sponge (i.e., control), the other the same type of sponge but impregnated with acellular dermal matrix components. The two groups were then scored using Lund-Kennedy criteria. The findings were that the matric-impregnated sponge was superior to the plain sponge when weeks 2 and 8 were compared ($p<0.05$). Furthermore, the group with the impregnated gelatin had a more rapid regrowth of epithelium than the group with the plain sponge.

A study undertaken by Sahin-Yilmaz et al[38] examined topical treatment of acute bacterial rhinosinusitis in a murine model. The researchers compared topical (nasal) application of dexamethasone alone or dexamethasone combined with ciprofloxacin. Some 30 mice of C57B1/6 type were separated into 3 equal-sized groups. The animals were infected with *Streptococcus pneumoniae* and one day later allocated at random to either placebo, dexamethasone, or the combined steroid plus antibiotic. The animals were then sacrificed either 3 or 10 days after starting treatment. An infection developed in the mice receiving placebo, with a cellular inflammatory infiltrate noted. However, the animals with the two active treatments fared neither better nor worse than those administered placebo. The researchers explained this surprising result as potentially due to several factors, namely an inadequately concentrated dose, overly swift clearance from the nose or non-penetration of the lesion by the drops used. This seemed more likely than the competing explanation that neither steroid nor antibiotic alter the course of infection.

In Kurt et al[39] experimental study, Topical nasal drops of indomethacin were reported to decrease the inflammatory features in acute si-

nusitis. They concluded the need of undertaking human trials to evaluate its effects on humans.

According to the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020[40], amoxicillin/penicillin (beta-lactams) are effective; and moxifloxacin (fluoroquinone) is not effective in acute bacterial rhinosinusitis. In chronic rhinosinusitis, it is reported as using long-term antibiotics and its impact on patients is uncertain. Moreover, topical antibacterial therapy for improving symptoms is also reported as not effective in patients with chronic rhinosinusitis[40].

This is the first study to be published on how ciprofloxacin applied intranasally affects the nasal epithelial lining. The nasal epithelial cells in culture that were exposed to topical ciprofloxacin for 24 hours were less viable than controls and this result was statistically significant. The peak reductions in cellular viability occurred with exposure at a concentration of 1.25 mg/mL and 0.625 mg/mL. Cultured nasal epithelial cells with exposure to the antibiotic were of decreased size and their outline changed from fusiform to round. For wound healing assay, the area opened up by the experimental scratch was not closed completely by 24 hours later. Ciprofloxacin exposure evidently results in lower cellular viability. The authors therefore advise against using ciprofloxacin topically on nasal mucosa for rhinosinusitis (acute or chronic). Toxicity on nasal epithelium has been demonstrated.

Conclusions

Given the cellular alterations observed in nasal epithelial cells exposed to ciprofloxacin (i.e., lower viability, decrease in cell magnitude and rounding), which all indicate toxicity, it is advised that intranasal ciprofloxacin is unsuitable for use in rhinosinusitis of acute or chronic type. The outcomes of this study should be studied and correlated *in vivo* models.

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding

There were no funds for this article.

Ethics Approval

This is a cell-culture study. Ethics committee approval was not needed.

Informed Consent

Human primary nasal epithelium was obtained from healthy tissue removed routinely as part of surgery (septorhinoplasty) from individuals who gave written consent for their tissue to be used in scientific research.

Authors' Contributions

Elad Azizli: Planning, designing, literature survey, interpretation of the results, active intellectual support.
Nuray Bayar Muluk: Planning, designing, literature survey, interpretation of the results, active intellectual support, writing, submission.
Canan Vejselova Sezer: Planning, designing, data collection, literature survey, interpretation of the results, active intellectual support.
Hatice Mehtap Kutlu: Planning, designing, data collection, performing the study, literature survey, interpretation of the results, active intellectual support, writing.
Cemal Cingi: Planning, designing, literature survey, performing the study, interpretation of the results, active intellectual support, English editing.

ORCID ID

Elad Azizli: 0000-0002-6494-8664
Nuray Bayar Muluk: 0000-0003-3602-9289
Canan Vejselova Sezer: 0000-0002-3792-5993
Hatice Mehtap Kutlu: 0000-0002-8816-1487
Cemal Cingi: 0000-0003-3934-5092

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