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Research Article

Bacteriostatic and Bactericidal Effects of DISCOGEL®

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Abstract

Intradiscal DISCOGEL® injection is an accepted interventional method for the treatment of intervertebral disc herniation. This injectable implant, also known as Radiopaque Gelified Ethanol (RGE), consists of jellified ethanol in combination with a radiopaque agent, tungsten, and has various mechanisms of action. Studies have reported Discogel to be the most effective and safest therapeutic method with long-term benefits, a success rate of 91.4% and a risk of complications of less than 0.5%. In this study, we investigated the antibacterial effects of Discogel against seven common bacterial strains involved in post-intervention and post-surgical infections, namely *P. aeruginosa, S. aureus, E. faecalis, S. pyogenes, C. acnes, A. baumannii* and *K. aerogenes*. In-vitro experiments and statistical analysis revealed that different concentrations of DISCOGEL® have significant activity against all tested bacterial strains. No significant relationship between the Gram classification of bacteria and the antibacterial effects of DISCOGEL® was observed. this injectable implant is responsible for these additional benefits.

Keywords: DiscoGel; Intervertebral Disc Herniation; Antibacterial Effects; Antimicrobial Resistance; Nosocomial Infections; Radiopaque Gelified Ethanol; RGE; DISCOGEL®; Antibacterial Effects

Introduction

Low back pain is a common cause patient visits to the physician's office worldwide. The spinal column, which is constituted of vertebrae and intervertebral discs, is a flexible structure extending from the base of the skull to the tail. This structure plays several critical roles in the body. It protects the spinal cord against external pressure and traumas, allows trunk movements in different directions, and assists in maintaining an upright posture. Spinal degeneration and disc herniation are among the most common organic causes of spinal and radicular pain.

Discs are cartilaginous cushions present between adjacent vertebrae that act as a shock absorber and provide flexibility to the spinal column. The disc structure can be divided into the outer Annulus Fibrosus and the central gelatinous Nucleus Pulposus. Asymmetrical and excessive pressure on Nucleus Pulposus may damage the Annulus Fibrosus resulting in various degrees of disc herniation. Depending on the severity of the herniation, symptoms may include local or radicular pain, paresthesia, muscle weakness, and sphincter disturbances. The treatment could be conservative, interventional, minimally invasive, or surgical and the choice depends on the severity of the signs and symptoms.

Several studies show that Discogel, jellified ethyl alcohol, therapy is the most effective therapeutic option for disc herniation with the best safety profile and associated with fewest complications compared to other interventional techniques [1]. Discogel therapy is currently used in many countries worldwide, including in most

European countries. Radiopaque Gelified Ethanol (RGE) is a sterile viscous solution with 96% ethyl alcohol, a cellulose derivative, and tungsten, a radiopaque element that produces a chemonuclear lysis when injected into the disc. Other mechanisms of action have been reported. For instance, Discogel exerts a mechanical action by dehydrating the turgescent protruding disk [2].

In practice, post-procedure infections are of great concern following any open-surgery or intervention as they may lead to complications and have a negative impact on the therapeutic outcome. From a theoretical perspective, the presence of ethyl alcohol in Discogel would reduce the risk of post-procedure infections. The purpose of this investigation is to determine whether microbiologic studies in culture medium support this theory. To date, various studies have investigated the mechanism of action and effectiveness of Discogel therapy; however, no studies have been performed to address the bactericidal and bacteriostatic effects of this injectable implant.

Therefore, we have investigated the antibacterial effects of DISCOGEL® on Gram-positive bacteria such as *S. aureus*, *E. faecalis*, *S. pyogenes*, *C. acnes* and Gram-negative bacteria such as *A. baumannii, P. aeruginosa* and *K. aerogenes* as they are the most common causes of post-surgical infections, osteomyelitis, and arthritis.

Materials and Methods

DISCOGEL®

DiscoGel® was purchased from the local distributor of Gelscom SAS, France.

Source of bacterial strains

Standard bacterial strains (Persian Type Culture Collection (PTCC)) were provided by the Iranian Biological Resource center. Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes* ATCC 19615 and *Cutibacterium acnes* ATCC 6919 were used. Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella aerogenes* ATCC 13048, and *Acinetobacter baumannii* ATCC 19606 were used.

Bacterial first cultures

The standard strains of *S. aureus* were smeared, stained with Gram's Method and analyzed under a microscope. Staphs were purple and clustered. The bacteria were cultured on Blood Agar (BA) and Mannitol Salt Agar (MSA) medium for 24 hours at 37°C. *Staphylococcus aureus* produces beta hemolysis in the BA medium, creating a clear halo around its colonies [3]. *Staphylococcus aureus* in MSA creates golden colonies. This step is to affirm the genera and the species of bacteria.

The smeared standard strains of *E. faecalis* were stained with Gram's method and analyzed under a light microscope. The cultures appeared as purple diplococcic or short chains. Then, to confirm the presence of these bacteria among the group D beta-hemolytic streptococci, the bacteria were incubated in the bile Esculin medium for 24 hours at 37°C [4]. When the bacteria produced a black precipitate, the test was considered positive. In the next step, the salt tolerance test was performed to differentiate *enterococcal and non-enterococcal streptococci*. For this purpose, the bacteria were inoculated with medium containing high salt and incubated for 24 hours [5]. Ambient clouding was a sign of the growth of these bacteria and a positive test.

The smeared and stained standard strains of *S. pyogenes* were analyzed under the microscope. The bacteria were purple and filamentous. They were then cultured in Blood Agar medium for 24 h at 37°C. *Streptococcus pyogenes* induces beta hemolysis in the BA medium, creating a clear halo around the colonies that indicates complete hemolysis of RBCs. Then, we performed the bacitracin susceptibility test. The bacterial growth inhibition indicates that the bacterium is *Streptococcus pyogenes*.

The smeared and stained standard strains of *C. acnes* were seen in purple and bacilliform under the microscope. The bacteria were then cultured in Blood Agar with McIntosh and Filde's anaerobic jar [6].

Observation of thin Gram-negative bacilli that are arranged separately or in pairs indicates *P. aeruginosa*. After aerobic incubation, the bacteria were cultured in Blood Agar or MacConkey agar for 24 hours at 37 - 42°C (growth at 42°C helps differentiate other Pseudomonas species in the fluorescence group) [7]. This step is to confirm the genus and species of the bacterium examined.

The standard strains of *K. aerogenes* were smeared and stained with Gram's method then analyzed under a microscope. We found short Gram-negative bacilli with small capsules, convex, surrounded colonies with clear, mucoid edges. Bacteria were cultured for 24 hours at 37°C in EMB and MacConkey agar media.

A. baumannii is a coccobacillus similar to Neisseria, however, it is oxidase negative. Bacteria were cultured for 24 hours at 37°C in MacConkey agar and BA.

E-test

We used the Epsilometer test (E-test) method [8] to determine the Minimum Inhibitory Concentration (MIC) and ascertain bacterial susceptibility to the selected antibiotics. Plates (100 × 15 mm) containing the Müller-Hinton agar medium were inoculated by standardized inoculum of each bacterial strain from the first culture (0.5 McFarland) with sterile cotton swabs. Then a strip was placed in the midline of each plate and the plates were incubated at 37°C for 24 to 48 hours. After the incubation period, the results were read and compared with CLSI guidelines.

Kirby-Bauer test

The antibacterial test was performed using the Kirby–Bauer disk diffusion method [9] to investigate the antibacterial effects of DISCOGEL®. A 100 × 15 mm plate containing Müller-Hinton agar medium (at a uniformed depth of 4 mm) was prepared for each bacterial strain. When the media was first prepared, we tested the pH of the MH agar to affirm that it is between 7.2 and 7.4 at room temperature. According to McFarland standards, a standardized inoculum of each bacterial strain from the first culture was inoculated using a sterile cotton swab on the surface of the solidified medium. Plates were allowed to dry for approximately 5 minutes. Then we carefully placed the disks we previously prepared using different concentrations of DISCOGEL® on the surface of the medium at equal intervals (more than 24 mm, center to center). Moreover, due to the chemical structure of DISCOGEL®, it was necessary to use an ethanol disk as a negative control and a blank negative control disk, along with the others.

Plates were incubated at an incubation temperature of 37°C (98.6°F) in a non-CO_2 incubator for 24 to 48 hours. For a more definitive detection of methicillin-resistant Staphylococcus, we incubated the plate at 35°C for 24 hours. Finally, we measured the inhibition zone sizes and determined the susceptibility or resistance of the organism tested using the CLSI guidelines [10-12].

Statistical analysis

The results were presented as mean ± standard deviation (SD). Then we used SPSS software to compare the effects of DISCOGEL® on the growth of different bacterial strains; a *p*-value less than 5% indicates a significant difference in growth. This difference indicates whether DISCOGEL® has reduced bacterial growth or not.

Results

The MIC values determined for amoxicillin against *S. pyogenes* and *E. faecalis* were 1.8 and 2 mcg/mL, respectively, confirming that *S. pyogenes* was resistant and *E. faecalis* was sensitive to this antibiotic. MIC values of cephalosporin against *K. aerogenes* and *C. acnes* were 6 and 0.6 mcg/mL, respectively, showing that *K. aerogenes* and *C. acnes* are sensitive to the antibiotic. We also tested standard imipenem strip on *A. baumannii* and *P. aeruginosa* and the MIC values were determined as 16 and 32 mcg/mL, respectively, indicating that both bacterial strains are resistant to imipenem. Finally, the antimicrobial susceptibility test was performed on S. aureus using standard methicillin strip and the MIC value observed was 4 mcg/mL, confirming that it should be a methicillin-resistant strain.

Bacterial susceptibility test (KB-test)

In vitro antibacterial activity of DISCOGEL® against the bacterial strains studied was assessed by measuring their corresponding inhibition zones (Table 1).

Bacterial strain	Tested antibiotic	MIC (mcg/mL)	AR	Antibiotic disk	100% DiscoGel	50% DiscoGel	25% DiscoGel	Ethanol (20%)	Neg. control (blank)
P. aeruginosa	Imipenem	32	Resistant	19.6 ± 2.43	16.05 ± 1.11	13.65 ± 1.24	6.00 ± 3.75	0	0
S. aureus	Methicillin	4	Resistant	20.09 ± 0.93	17.15 ± 0.85	12.70 ± 0.94	0.09 ± 0.02	0	0
E. faecalis	Amoxicillin	2	Sensitive	13.5 ± 1.81	15.10 ± 1.17	10.13 ± 1.07	0.58 ± 0.11	0	0
S. pyogenes	Amoxicillin	1.8	Resistant	12.05 ± 2.50	14.00 ± 1.56	11.30 ± 0.94	6.00 ± 1.22	0	0
C. acnes	Cephalosporin	0.6	-	19.00 ± 1.37	17.95 ± 1.14	11.00 ± 2.70	0.58 ± 0.11	0	0
A. baunnii	Imipenem	16	Resistant	13.65 ± 1.84	16.89 ± 0.51	15.32 ± 1.74	9.90 ± 1.07	0	0
K. aerogenes	Cephalosporin	6	Sensitive	15.75 ± 2.20	15.97 ± 1.31	12.55 ± 0.84	0.49 ± 0.07	0.46 ± 0.09	0

Table 1: Antibacterial resistance test and zone of inhibition of different concentration of DISCOGEL®*

*: Values of inhibition zones are represented as mean ± standard deviation (SD) and in millimeter unit.

P. aeruginosa was resistant to imipenem (19.6 ± 2.43 mm), but it was shown to be inhibited by DISCOGEL® at three different concentrations of 1, 0.5 and 0.25. The size of the inhibition zone (mean ± SD) was 16.05 ± 1.11 mm, 13.65 ± 1.24 mm and 6.00 ± 3.75 mm, respectively; in the presence of two negative control disks (Figure 1A).

S. aureus was resistant to methicillin (20.09 ± 0.93 mm), but it was shown to be inhibited by DISCOGEL® at three different concentrations of 1, 0.5 and 0.25. The size of the inhibition zone (mean ± SD) was 17.15 ± 0.85 mm, 12.70 ± 0.94 mm and 0.09 ± 0.02 mm, respectively; in the presence of two negative control disks (Figure 1B).

E. faecalis was sensitive to amoxicillin (13.50 ± 1.81 mm), and it was shown to be inhibited by DISCOGEL® at three different concentrations of 1, 0.5 and 0.25. The size of the inhibition zone (mean ± SD) was 15.10 ± 1.17 mm, 10.13 ± 1.07 mm and 0.58 ± 0.11 mm, respectively; in the presence of two negative control disks.

S. pyogenes was resistant to amoxicillin (12.05 ± 2.50 mm), but it was shown to be inhibited by DISCOGEL® at three different concentrations of 1, 0.5 and 0.25. The size of the inhibition zone (mean ± SD) was 14.00 ± 1.56 mm, 11.30 ± 0.94 mm and 6.00 ± 1.22 mm, respectively; in the presence of two negative control disks.

C. acnes was sensitive to cephalosporin (19.00 ± 1.37 mm), and it was shown to be inhibited by DISCOGEL® at three different concentrations of 1, 0.5 and 0.25. The size of the inhibition zone (mean ± SD) was 17.95 ± 1.14 mm, 11.00 ± 2.70 mm and 0.58 ± 0.11 mm, respectively; in the presence of two negative control disks (Figure 1C).

A. baumannii was resistant to imipenem (13.65 ± 1.84 mm), but it was shown to be inhibited by DISCOGEL® at three different concentrations of 1, 0.5 and 0.25. The size of the inhibition zone (mean ± SD) was 16.89 ± 0.51 mm, 15.32 ± 1.74 mm and 9.90 ± 1.07 mm, respectively; in the presence of two negative control disks.

K. aerogenes was sensitive to cephalosporin (15.75 ± 2.20 mm), and it was shown to be inhibited by DISCOGEL® at three different concentrations of 1, 0.5 and 0.25. The size of the inhibition zone (mean ± SD) was 15.97 ± 1.31 mm, 12.55 ± 0.84 mm and 0.49 ± 0.07 mm, respectively. In this case, some sensitivity to ethanol was also observed (0.46 ± 0.09 mm) but there was no inhibition zone around the blank disk (Figure 2).

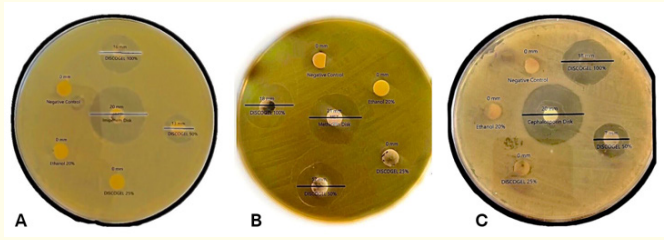

Figure 1: Inhibition zones of (A) *P. aeruginosa* (B) *S. aureus* (C) *C. acnes* using the Kirby–Bauer disk diffusion.

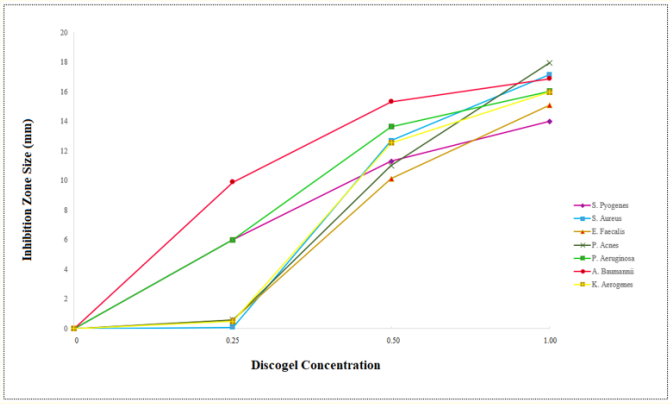

Figure 2: Bacterial inhibition zone curve with increasing concentration of the DISCOGEL®. (Each point represents an average of ten sample measurements).

Statistical results

The specific correlation between the inhibition zone and the concentration of DISCOGEL® in P. aeruginosa was significant (PCC = 0.892, $p < 0.001$). In the case of S. aureus, there was also a significant correlation between the inhibition zone and the concentration of DISCOGEL® (PCC = 0.924, $p < 0.001$). Statistical results using Pearson's r also shows significant correlation between the concentration of DISCOGEL® and the inhibition zone of *E. faecalis* (PCC = 0.944, $p < 0.001$), *S. pyogenes* (PCC = 0.924, $p < 0.001$), *C. acnes* (PCC = 0.945, $p < 0.001$), *A. baumannii* (PCC = 0.870, $p < 0.001$), and *K. aerogenes* (PCC = 0.915, $p < 0.001$).

We also analyzed the results using Kendall's tau_b correlation coefficient to determine if there is a relationship between Gram classification of bacteria and growth inhibition. However, statistical data showed no significant correlation (p value = 0.091 and $\tau b = -0.086$).

Discussion

Discitis is a catastrophic complication of any spinal intervention or surgery and considering the increasing popularity and shown effectiveness of DISCOGEL®, its potential antibacterial effects would be impressively advantageous. This study investigated the inhibitory effects of this intradiscal injectable implant on the growth of several strains of the most common bacteria among hospitalized and non-hospitalized patients for the first time.

A review on common pathogen leading to post-surgical (mostly neurosurgical) infection follows: *Pseudomonas aeruginosa* is a Gram-negative non-fermented bacillus that spreads throughout the hospital environment in wet containers such as food and fruits, tubs, toilets, respiratory devices, dialyzers, and even disinfectants [13,14]. This organism is generally not considered as part of the normal human microbial flora with the exception of patients that have been hospitalized [15]. The comprehensive environmental release of *Pseudomonas spp*. is probably due to its facile growth needs and nutritional versatility [16]. Fortunately, *pseudomonas* infections are mostly opportunistic and limited to patients receiving broad-spectrum antibiotics due to normal flora suppression or immunocompromised patients [17]. *Pseudomonas aeruginosa* has numerous virulence factors including adhesins, toxins, and type III secretory system [18]. *Pseudomonas* is inherently resistant to a variety of antibiotics. Additionally, it can develop resistance to other antibacterial agents as a result of horizontal transfer of existing resistance genes and new mutations [18]. *Pseudomonas* is a common cause of respiratory infections, skin and soft tissue infections, osteochondritis, osteomyelitis, urinary tract infections (UTIs), ear and eye infections, sepsis, and endocarditis [19]. It is associated with long clinical courses, long incubation periods, recurrences, and serious complications and therefore exerts a high economic burden [19].

Staphylococcus aureus is a Gram-positive coccus and a facultative anaerobic member of the firmicutes [20]. This bacterium is also referred to as Golden Staph. *S. aureus* is a normal colonizer of the skin and mucous membranes present in up to half of the adults, 15% of which are persistent carriers [21]. This bacterium produces golden colonies. This pigment has an important role in pathogenicity because it acts as an antioxidant and protects the bacterium against reactive oxygen species, produced by the immune system [22]. *Staphylococcus aureus* causes a wide range of infections from mild skin infections to life-threatening conditions such as TSS, endocarditis, and sepsis [23].

Enterococcus faecalis is a Gram-positive bacterium present in the human gastrointestinal flora [24]. It can cause UTIs and life-threatening conditions such as endocarditis, septicemia, meningitis, especially nosocomial infections due to the high load of antibiotic-resistant strains in the hospital environment [25].

Streptococcus pyogenes is a Gram-positive round-shaped bacterium that is often called group A Streptococcus (GAS) because of its ability to produce beta-hemolysis zones on blood agar (typically, 2–3 mm) [23]. It is estimated to be responsible for about 2 million infections each year. These bacteria can cause streptococcal pharyngitis, strep throat, meningitis, pneumonia, endocarditis, Erysipelas, and necrotizing fasciitis [26].

Cutibacterium acnes, heretofore *Propionibacterium acnes* [27,28] is a Gram-positive rod-shaped bacterium that is part of the human microbiota [29]. It can cause acne [27], blepharitis as well as chronic conditions such as endophthalmitis, particularly following ophthalmic surgeries.

Acinetobacter baumannii is a Gram-negative short coccobacillus that is considered as an opportunistic human pathogen [30]. Infection with *A. baumannii* is an important nosocomial infection, especially in immunocompromised people. Although other Acinetobacter species are found in soil, *Acinetobacter baumannii* is mainly confined to hospital environments [31].

Klebsiella aerogenes, (formerly *Enterobacter aerogenes*) [32] is a Gram-negative rod-shaped bacterium [33]. It is motile due to the presence of peritrichous flagella. *K. aerogenes* is found in human gastrointestinal mucosa as a commensal bacterium and rarely causes disease in healthy patients. K. aerogenes is usually considered as a nosocomial pathogen responsible for some opportunistic human infections [34].

The findings reported in this study clearly supported the theory that DISCOGEL® is able to inhibit the growth of several bacterial strains *in vitro*. Its antibacterial effects are concentration-dependent and will be more significant with increasing concentration and local accumulation of this intradiscal injectable implant. Notably, these features are not due to the presence of the ethanol in its pharmaceutical formulation.

Our investigation supports the findings of previous studies on antibacterial effects of tungsten [35] and proposes that such properties of Discogel could be due to the tungsten contained therein.

Statistical analysis of the results showed that there was a significant correlation between concentration of DISCOGEL® and the inhibition zone, regardless of the species of bacteria tested using the Pearson correlation coefficient (PCC = 0.897, $p < 0.001$).

Conclusion

This study examined and established an additional feature of DISCOGEL® in regards to its antibacterial properties. In-vitro analysis of DISCOGEL® therapy showed that the antibacterial effects of this injectable implant reduce the risk of intradiscal infections following interventional discal procedures. As mentioned, previous studies have shown the anti-inflammatory role of DISCOGEL® and have yielded significant results [33]. The results of the current study also strengthen these findings and emphasize on the antibacterial benefits of DISCOGEL® in addition to its clinical effects. However, it is necessary to further study these properties in-vitro and in-vivo to broaden our understanding.

Conflict of Interests

The authors declare that they have no competing interests.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors. The approved Grant Number is IR.ABZUMS.REC.1397.099.

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