Potential interactions between radio contrast media and antibiotics and their effect on discography - an in vitro study

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Aims and objectives

Lindblom (1) described discography - an invasive diagnostic procedure - firstly in 1948. Iodine containing radio contrast medium (RCM) is injected into the nucleus pulposus of an intervertebral disc. Discography indications have significantly changed over time, due to further development of non-invasive cross-sectional imaging modalities, namely MRI. However, especially in the United States of America (USA), and in Australia provocative discography is again increasingly been used in patients with equivocal findings at MRI/CT, suffering from recurrent post-operative severe lower back, pre-operatively before spinal fusion, or before intradiscal (ID) injection of cortisone or anesthetics (2-10).

The most serious, but rare complication is *iatrogenic bacterial discitis*. Several studies have shown that prophylactic intravenous (IV) injection of antibiotics can significantly reduce the incidence of bacterial discitis. Therefore in 1989 Fraser and collaborators (11, 12) conducted animal experiments, administering intravenous cefazolin, exactly 30 minutes before inoculation of bacteria into animals’ discs, in order to prevent bacterial discitis. Further animal studies demonstrated that after pre-interventional systemic antibiotics administration the applied antimicrobials could be detected in the intervertebral discs (with higher concentrations in the annulus fibrosus than in the nucleus pulposus). In all animal studies it was emphasized that timing of systemic antibiotic prophylaxis was critical, and that the time frame was limited (13-17). Post-interventional systemic injection of antibiotics was never deemed beneficial. The most serious disadvantage of antibiotic prophylaxis is the increase of bacterial resistance.

An attractive alternative is intradiscal (ID) injection of an antibiotic during discography. One preliminary study on the efficacy of antibiotics, combined with one RCM, i.e. iohexol, was conducted by Klessig in 2003 (18). To our knowledge, data of potential efficacy of different RCM alone on bacterial strains have not been published.

ID injection of an antibiotic provides high local concentration in the disc, whereas bacterial resistance is highly unlikely because of sole local - and not systemic application.

Therefore the aim of the present in vitro study was to test the efficacy of three commonly used antibiotics on laboratory strains of five different bacteria. Furthermore the antimicrobial effect of two commercially available RCM, in use for discography, and one new RCM, on those five bacteria was evaluated in order to detect potential cross reactions between RCM and antibiotics.
Methods and materials

Bacterial strains

In our study the following bacterial strains were used: Staphylococcus (S) aureus (ATCC 29213), Staphylococcus (S) epidermidis (ATCC 12228), Klebsiella (K) pneumoniae (ATCC 700324), Escherichia (E) coli (ATCC 25922), and Pseudomonas (P) aeruginosa (ATCC 27853) (American Type Culture Collection, Manassas, VA, USA).

Culture methods

All bacterial strains were plated on blood agar for overnight growth at 37°C (well plates from Nunc Comp., Roskilde, Denmark). Freshly grown isolated colonies from blood agar plates were picked up, and inoculated overnight in Trypton Soy Broth (TSB, OXOID ltd, Basingstoke, Hampshire, UK) at 37°C for all assays.

Bacterial dose

On the next day the bacterial concentrations were measured for optical density (OD) at 600 nm. Bacterial concentrations were diluted to 1x10^7/mL, from which 50 µl each were dispensed per well.

Radio contrast media and antibiotic

Three nonionic iodinated radio contrast media were used, i.e. iohexol and iodixanol, both in use for discography, and one new dimeric nonionic substance, iosimenol. The iodine (I) concentrations of those compounds are as follows: iohexol (Amersham Health, Carringtonhill, Ireland) 652 mg compound/mL stock solution, iodixanol (Amersham Health, Carringtonhill, Ireland) 648 mg compound/mL stock solution, and iosimenol (Koehler Chemie, Alsbach-Hahnlein, Germany) 660 mg compound/mL stock solution. Eight dilutions of each compound were prepared (table 1).

Table 1

Concentration of iodinated nonionic RCM tested

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Iohexol</th>
<th>Iosimenol</th>
<th>Iodixanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>324 mg/ml</td>
<td>330 mg/ml</td>
<td>326 mg/ml</td>
</tr>
</tbody>
</table>
Six serial dilutions of the three antibiotics ampicillin (Asia Pharmaceutical Ind., Syria), gentamicin (Sandoz, Cairo, Egypt), and ceftriaxone (Gulf Pharmaceutical Industries, RAK, UAE) were prepared (table 2), and dispensed on the wells inoculated with the five above listed bacterial strains.

### Table 2

**Concentration of antibiotics tested**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Ampicillin</th>
<th>Gentamicin</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>256 µg/ml</td>
<td>256 µg/ml</td>
<td>256 µg/ml</td>
</tr>
<tr>
<td>2</td>
<td>128 µg/ml</td>
<td>128 µg/ml</td>
<td>128 µg/ml</td>
</tr>
<tr>
<td>3</td>
<td>64 µg/ml</td>
<td>64 µg/ml</td>
<td>64 µg/ml</td>
</tr>
<tr>
<td>4</td>
<td>32 µg/ml</td>
<td>32 µg/ml</td>
<td>32 µg/ml</td>
</tr>
<tr>
<td>5</td>
<td>16 µg/ml</td>
<td>16 µg/ml</td>
<td>16 µg/ml</td>
</tr>
<tr>
<td>6</td>
<td>8 µg/ml</td>
<td>8 µg/ml</td>
<td>8 µg/ml</td>
</tr>
</tbody>
</table>

Assay procedure

50 µl of each RCM alone, 50 µl of each antibiotic alone, and 50 µl of combinations (of each RCM with each antibiotic) were dispensed in the wells, starting with the highest concentrations of the respective RCM and antibiotics according to table 1. 50 µl of TSB and 50 µl of RCM, antibiotics, and RCM and antibiotics in combination were added and mixed. Then serial dilutions were obtained, and from the last dilution 50 µl were discarded leaving 50 µl in each well. Subsequently 50 µl of bacterial culture from freshly grown bacterial culture (1 x 10^7/mL) were added. Positive and negative control wells were maintained without RCM or antibiotics. Plates were incubated at 37°C overnight, and read by ELISA (Elisa Reader Magellan from Tecan Austria GmbH, 5082 Grodig, Austria) at 450 nm. All experiments were repeated ten times for the RCM alone, and five times,
respectively, for the experiments using the three antibiotics, and the three antibiotics in combination with the three different RCM. All results were recorded for calculation and statistical analysis.

Calculation and statistical analysis

Bacterial growth in all treated groups was calculated as percentage of control growth, applying the following formula:

\[
\frac{(\text{Experimental OD} - \text{sterility OD})}{(\text{growth control OD} - \text{sterility OD})} \times 100
\]

To test statistical significance the Mann-Whitney test was performed. SPSS 15.0 (SPSS Inc. Chicago, Illinois) software package was used for all statistical evaluations, and Microsoft Excel 2007 (Microsoft Coop, Redmond, WA, USA) was used for graphical presentation. P < 0.05 was set for statistical significance.
Results

As ubiquitous microbes S aureus, S epidermidis und E coli were chosen; K pneumoniae and P aeruginosa were used as organisms responsible for hospital born infections.

For each bacterium tested the inhibition of growth was identical for each antibiotic within one dilution for all five repetitions. The best overall inhibition of bacterial growth was recorded for gentamicin (fig. 1) for all five bacterial strains tested. Inhibition of bacterial growth was significant for all bacterial strains tested, and for all dilutions compared with the controls. All three antibiotics inhibited the growth of S aureus best. Ampicillin also significantly inhibited the growth of all bacterial strains (fig. 1). Growth of S aureus, S epidermidis and E coli was inhibited at all dilutions; however, significant growth inhibition of K pneumoniae and P aeruginosa was only observed at high concentrations (low dilutions) (K pneumoniae at dilutions 1-4, P aeruginosa at dilutions 1-2). Inhibition of bacterial growth by ceftriaxone, compared with control groups, was also significant for all bacterial strains (fig. 1). Significant growth inhibition of S aureus, S epidermidis, K pneumoniae and E coli by ceftriaxone was detected at all dilutions, whereas growth of P aeruginosa pneumoniae was only significantly inhibited at high concentrations (dilutions 1-3).

When applied together with the three antibiotics, iohexol, iodixanol, or iosimenol did not change the inhibition of bacterial growth of the particular antibiotic. Neither a statistically significant inhibition, nor an enhancement of the antibiotic activity was observed. Moreover we could not detect a synergism between any antibiotic combined with any RCM with regard to inhibition of growth of all bacterial strains. Fig. 2, 3, and 4 demonstrate the inhibition of bacterial growth by gentamicin (fig. 2), ampicillin (fig 3), and ceftriaxone (fig. 4) alone, and when combined with the three RCM.

The concentrations for each antibiotic in combination with iohexol, iodixanol, or the new compound iosimenol, sufficient to inhibit all five bacteria were as follows: gentamicin 64 µg/ mL and 128 µg/ mL for E coli, respectively, ampicillin 256 µg/ mL (undiluted), ceftriaxone 128 µg/ mL, with some limitations for inhibition of growth of P aeruginosa.

Consequently each antibiotic tested maintained its efficacy in the presence of the three RCM against all five organisms. There was no obvious enhancement or antagonism between the three antibiotics and the three RCM tested.

When investigated alone iohexol, iodixanol, and iosimenol showed evidence of mild antibiotic activity as well, but only at high concentrations (fig. 5). Statistically significant
inhibition of bacterial growth for all laboratory strains was only observed at dilutions 1 and 2 for the three RCM.
Fig. 1: Bacterial growth after treatment with six dilutions of three antibiotics in percent of positive controls

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Fig. 2: Bacterial growth after treatment with six dilutions of gentamicin +/- three contrast media in percent of positive controls

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Fig. 3: Bacterial growth after treatment with six dilutions of ampicillin +/- three contrast media in percent of positive controls

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**Fig. 4:** Bacterial growth after treatment with dilutions of ceftriaxone +/- contrast media in percent of positive controls

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**Fig. 5:** Bacterial growth after treatment with eight dilutions of three contrast media in percent of positive controls

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Conclusion

- The antibiotic which inhibited best bacterial growth of all five laboratory strains was gentamicin.

- Inhibition of growth of K pneumoniae and P aeruginosa was limited after administration of ampicillin and ceftriaxone at high dilutions (i.e. low concentrations).

- All three antibiotics, ampicillin, gentamicin, and ceftriaxone, retained their efficacy in the presence of the RCM iohexol, iodixanol, and the new substance iosimenol.

- Iohexol, iodixanol, and iosimenol alone showed only minor antibiotic effects at high concentrations (low dilutions).

- Intradiscal injection of the three antibiotics at discography may offer an adequate antibiotic prophylaxis against post-interventional bacterial discitis.

- Intradiscal injection of antibiotics could replace systemic injection before discography, with its inherent risk of generating antibacterial resistance, and its limited effect caused by the short time frame of peak intradiscal antibiotic concentration.

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Fig. 6: Prof. Dr. Ruth D Langer

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References