***In vitro* assessment of the antibiotic efficacy of contrast media and antibiotics and their combinations at various dilutions**

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ABSTRACT. Discography is a controversial diagnostic procedure involving the injection of radiographic contrast medium (RCM) into the intervertebral disc. Iatrogenic bacterial discitis is a rare but serious complication. The intervention has been increasingly performed in our patients here in the United Arab Emirates. Prophylactic intravenous antibiotic administration can reduce post-interventional discitis; however, this may favour the development of bacterial resistance. Direct intradiscal injection of an antibiotic together with the RCM is a potential alternative. To date, there has been only one study on the efficacy of antibiotics added to an RCM. Equally, there are only limited data regarding the potential direct effect of RCM on bacterial growth. The purpose of this study was to determine whether the efficacy of antibiotics is affected when RCM are added. In an *in vitro* study, the effect of non-ionic RCM on the growth of five laboratory bacterial strains, alone and in combination with three broad-spectrum antimicrobials, was tested. Bacterial growth was assessed in the absence and the presence of RCM, antibiotics and their combinations. All three RCM alone demonstrated some inhibition of bacterial growth at high concentrations. In the presence of the RCM, all three antibiotics retained their inhibitory effect on bacterial growth. In conclusion, our *in vitro* experiments did not reveal any changes in the antimicrobial efficacy of the three antibiotics in the presence of the three tested RCM. Subsequent clinical trials will need to assess whether intradiscal antibiotic administration may be a suitable substitute for, or a supplement to, prophylactic systemic antibiotics before discography.

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Lindblom [1] was the first author to describe discography, which is performed to outline the morphology of the intervertebral disc. Radiographic contrast media (RCM) are injected into the nucleus pulposus of a disc [1–3]. Owing to the further development in cross-sectional imaging procedures, especially in MRI, indications for discography or CT discography have been substantially changed. At present, *provocative discography* is increasingly being carried out, especially in the USA and Australia, for disc stimulation in order to provoke or to reproduce discogenic pain [4–7]; special indications are lower back pain with equivocal findings on MRI, post-surgical failed lower back pain, status prior to spinal fusion or the injection of cortisone or anaesthetics into an intervertebral disc [3–5, 7–15]. The most serious complication is post-interventional bacterial discitis owing to the invasiveness of the procedure [2, 4, 5, 16]; as such, many publications deal with prophylactic intravenous (iv) administration of antibiotics. Several animal experiments in sheep, lambs and rabbits, conducted in the 1980s, 1990s and in 2006, demonstrated antibiotics in the intervertebral discs after systemic iv injection (with higher concentrations in the annulus fibrosus than in the nucleus pulposus) [17–23].

However, it was emphasised that the timing of the systemic antibiotic prophylaxis was critical [17, 21, 23]. Conversely, post-interventional systemic administration of antibiotics was *not* considered beneficial [21, 22]. A study in humans before lumbar spinal fusion showed that cefazolin was detectable in disc samples of these patients after iv antibiotic prophylaxis, with a peak concentration between 37 min and 53 min after iv injection [19].

In 1990, Osti et al [23] conducted animal experiments and, subsequently, a clinical study in 127 patients, examining 337 discs, in whom an antibiotic was added to the intradiscally administered RCM, in addition to earlier iv prophylaxis [23]. Post-interventional discitis was not detected in either the animals or the patients.

To date, different recommendations for the prevention of post-interventional discitis are in place, incorporating either systemic intravenous injection of antibiotics [16], a combination of iv and intradiscally injected antibiotics [24], or even no antibiotics at all [25]. In order to avoid increasing bacterial resistance to systemically administered antibiotics [14, 26], intradiscally injected antibiotics might be an alternative owing to the direct application of the antibiotic into the disc. One study on the efficacy of antibiotics in combination with iohexol has already been conducted by Klessig et al [26].

Discography is a constantly increasing intervention in the United Arab Emirates (UAE) and, as hospital-borne

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infections are also a major problem in this country, the aim of our study was to investigate the effect of three different non-ionic RCM (including one new dimeric compound that is still in clinical trials), alone and in combination with three broad-spectrum antibiotics, on different laboratory bacterial strains to detect any potential effect of the RCM on antibiotic efficacy.

Methods and materials

Bacterial strains

Five bacterial strains were used in this study: *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), *Klebsiella pneumoniae* (ATCC 700324), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853; American type Culture Collection, Manassas, VA).

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 tryptone soy broth (TSB) (Oxoid Ltd, Basingstoke, UK) at 37°C for all assays.

Bacterial dose

On the following day, the bacterial concentrations were measured for optical density (OD) at 600 nm. Bacterial concentrations were diluted to 1×10^7 ml⁻¹, from which 50 µl of each was dispensed per well.

RCM and antibiotics

Three different non-ionic iodinated radiographic contrast media were used: iohexol and iodixanol, both approved for discography, and one new non-ionic dimeric substance, iosimenol, which is not yet available on the market. The concentrations of those compounds are as follows: iohexol (Amersham Health, Carringtonhill, Ireland), 652 mg ml⁻¹ stock solution; iodixanol (Amersham Health), 648 mg ml⁻¹ stock solution; and iosimenol (Koehler Chemie, Alsbach-Hahnlein, Germany), 660 mg ml⁻¹ stock solution. Eight dilutions of each compound were prepared (Table 1).

Table 1. Dilutions of the iodinated radiographic contrast media

Dilution	Iohexol (652 mg ml ⁻¹)	Iodixanol (648 mg ml ⁻¹)	Iosimenol (660 mg ml ⁻¹)
1	324 mg ml ⁻¹	326 mg ml ⁻¹	330 mg ml ⁻¹
2	295 mg ml ⁻¹	260 mg ml ⁻¹	264 mg ml ⁻¹
3	194 mg ml ⁻¹	195 mg ml ⁻¹	198 mg ml ⁻¹
4	162 mg ml ⁻¹	163 mg ml ⁻¹	165 mg ml ⁻¹
5	81 mg ml ⁻¹	81.5 mg ml ⁻¹	82.5 mg ml ⁻¹
6	40.5 mg ml ⁻¹	40.7 mg ml ⁻¹	41.25 mg ml ⁻¹
7	20.25 mg ml ⁻¹	20.35 mg ml ⁻¹	20.6 mg ml ⁻¹
8	10.12 mg ml ⁻¹	10.17 mg ml ⁻¹	10.3 mg ml ⁻¹

Table 2. Dilutions of the antibiotics

Dilution	Ampicillin	Gentamicin	Ceftriaxone
1	256 µg ml ⁻¹	256 µg ml ⁻¹	256 µg ml ⁻¹
2	128 µg ml ⁻¹	128 µg ml ⁻¹	128 µg ml ⁻¹
3	64 µg ml ⁻¹	64 µg ml ⁻¹	64 µg ml ⁻¹
4	32 µg ml ⁻¹	32 µg ml ⁻¹	32 µg ml ⁻¹
5	16 µg ml ⁻¹	16 µg ml ⁻¹	16 µg ml ⁻¹
6	8 µg ml ⁻¹	8 µg ml ⁻¹	8 µg ml ⁻¹

Six serial dilutions of three antibiotics — ampicillin (Asia Pharmaceutical Ind., Syria), gentamicin (Sandoz, Cairo, Egypt) and ceftriaxone (Gulf Pharmaceutical Industries, RAK, UAE) — were prepared (Table 2) and dispensed into the wells inoculated with all five bacterial strains.

Assay procedure

50 µl of each RCM alone, 50 µl of each antibiotic alone and 50 µl of combinations (each RCM with each antibiotic) were dispensed into the wells, starting with the highest concentrations of each RCM and antibiotics according to Table 1. 50 µl of TSB and 50 µl of RCM, antibiotics or RCM and antibiotics in combination were added and mixed. Serial dilutions were then obtained, and from the last dilution 50 µl was discarded, leaving 50 µl in each well. Subsequently, 50 µl of freshly grown bacterial culture (1×10^7 ml⁻¹) was added. Positive and negative control wells were maintained without RCM or antibiotics. Plates were incubated at 37°C overnight and read by ELISA (Magellan; Tecan Austria GmbH, Grodig, Austria) at 450 nm. All experiments were repeated 10 times for the RCM alone, and 5 times for the antibiotics alone and the antibiotics in combination with the different RCM. All results were recorded for calculation and statistical analysis.

Calculation and statistical analysis

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

$$\frac{(\text{Experimental OD} - \text{sterility OD}) / \text{growth}}{\text{control OD} - \text{sterility OD}} \times 100 \text{ (1)}$$

To test for statistical significance, the Mann-Whitney test was performed. The SPSS 15.0 (SPSS Inc. Chicago, IL)

Figure 1. Bacterial growth for gentamicin, ampicillin and ceftriaxone as a percentage of control growth. Inhibition of bacterial growth is statistically significant for all bacterial strains and for all dilutions of gentamicin. Inhibition of bacterial growth is statistically significant for ampicillin, except for *K. pneumoniae* at dilutions 5 and 6 and for *P. aeruginosa* at dilutions 3–6. Inhibition of bacterial growth is statistically significant for ceftriaxone, except for *P. aeruginosa* at dilutions 3–6.

software package was used for all statistical evaluations, and Microsoft Excel 2007 (Microsoft Corp, Redmond, WA) was used for graphical presentation. $p \leq 0.05$ was set for statistical significance.

Results

For each bacterium tested, the inhibition of growth was identical for each antibiotic within one dilution for all five repetitions. *S. aureus*, *S. epidermidis* and *E. coli* were selected as ubiquitous organisms; *K. pneumoniae* and *P. aeruginosa* were chosen as they are mainly responsible for hospital-borne infections. The best overall inhibition of bacterial growth was recorded for gentamicin (Figure 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 positive controls. Ampicillin also significantly inhibited the growth of all bacterial strains tested (Figure 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 observed only at high concentrations (low dilutions) (*K. pneumoniae* at dilutions 1–4; *P. aeruginosa* at dilutions 1 and 2). Inhibition of bacterial growth by ceftriaxone, compared with the control groups, was also significant for all bacterial strains tested (Figure 1). Significant growth inhibition of *S. aureus*, *S. epidermidis*, *K. pneumoniae* and *E. coli* by ceftriaxone was detected at all dilutions. Growth of *P. aeruginosa* was significantly inhibited only at high concentrations (dilutions 1–3).

All three antibiotics inhibited the growth of *S. aureus* most effectively. Ampicillin and ceftriaxone were almost equally effective against *E. coli*.

When combined with the three antibiotics, iohexol, iodixanol and iosimenol all failed to substantially change the inhibition of bacterial growth of each antibiotic. Neither a statistically significant inhibition nor a statistically significant enhancement of the particular antibiotic activity was observed (Figures 2–4). Furthermore, we could not detect a synergism between any antibiotic combined with any RCM with regard to the inhibition of growth of whichever bacterial strain was tested.

The concentrations for each antibiotic in combination with either iohexol, iodixanol or iosimenol that were sufficient to inhibit all of the five bacteria tested were: gentamicin, $64 \mu\text{g ml}^{-1}$ is sufficient for the four other tested bacterial strains $128 \mu\text{g ml}^{-1}$ applies only to *E. coli*; ampicillin, $256 \mu\text{g ml}^{-1}$ (undiluted); ceftriaxone, $128 \mu\text{g ml}^{-1}$, but with some limitations for inhibition of growth of *P. aeruginosa*.

Hence, each antibiotic tested maintained its efficacy in the presence of iohexol, iodixanol and iosimenol against all five organisms. There was no apparent enhancement or antagonism between the three antibiotics and the three RCM tested.

When investigated alone, iohexol, iodixanol and iosimenol were also found to show evidence of mild antibacterial activity, but only at high concentrations (Figure 5). Statistically significant inhibition of bacterial growth of all bacterial strains by the three RCM alone was observed only at dilution 1, and in a few instances at dilution 2.

Figure 2. Bacterial growth for gentamicin plus the three radiographic contrast media as a percentage of control growth. No statistically significant difference was observed compared with the experiments using gentamicin alone.

Figure 3. Bacterial growth as a percentage of control growth for ampicillin plus the three radiographic contrast media. No statistically significant difference was observed compared with the experiments using ampicillin alone.

Discussion

In our *in vitro* study, the three antibiotics, ampicillin, gentamicin and ceftriaxone, retained their efficacy in the presence of all three iodinated RCM (iohexol, iodixanol and iosimenol) against laboratory strains of *S. aureus*, *S. epidermidis*, *K. pneumoniae*, *E. coli* and *P. aeruginosa*. A synergistic effect between gentamicin and iohexol against *E. coli*, as described in the experiments of Klessig et al [26], could not be confirmed in our study. In addition, two more non-ionic RCM were tested, including one new dimeric compound, iosimenol, which is not yet commercially available. Whereas Klessig tested gentamicin, cefazolin and clindamycin, we examined gentamicin, ampicillin and ceftriaxone, which are frequently administered in our local population.

To reach bactericidal concentrations, iv antibiotics have to be administered at a much higher dose than in the *in vitro* studies. Dosages required depend on the bacterial strain inoculated into the disc. As recommended by Osti et al [23] and subsequently by Endres and Bogduk [24], intradiscal antibiotic dosages for discography range from 1 to 10 mg ml⁻¹ [23, 24]. Taking into account the results of our experiments, dosages for gentamicin of 1 mg ml⁻¹ would inhibit growth of all five bacterial strains examined by over 10-fold. Ampicillin and ceftriaxone, however, require higher dosages to inhibit bacterial growth; dosages of >2.5 mg ml⁻¹ for ampicillin and ceftriaxone are necessary to exceed the *in vitro* inhibition of bacterial growth by 10-fold.

Several older studies [27–29] have already demonstrated bacteriostatic and even bactericidal effects of commercially available ionic and non-ionic RCM when

different laboratory bacterial strains were inoculated into the RCM solutions. Our *in vitro* experiments also showed the inhibition of bacterial growth by the three non-ionic RCM alone. In addition to the mild antimicrobial effect of iohexol found by Klessig et al [26], iodixanol and the new dimeric contrast medium iosimenol alone inhibited bacterial growth at high concentrations (low dilutions), mainly at dilution 1, which is equivalent to 150 mg iodine ml⁻¹, and also for some bacterial strains at dilution 2. This indicates that the concentrations of RCM containing approximately 300 mg iodine ml⁻¹ should be employed. This is the highest approved concentration for neuroaxial use, and is at least double the *in vitro* dose (dilution 1) required for inhibition of bacterial growth. Moreover, the new dimeric contrast medium shows similar results to the two established compounds so that, from this point of view, iosimenol also appears to be suitable for intradiscal injection (if this indication is sought for approval). Furthermore, after adding the three non-ionic RCM to the solution, the efficacy of the three antibiotics tested remained unchanged, and so the mixing of both compounds should not show any negative effect.

Fraser et al [20] performed comprehensive animal experiments in sheep, inoculating *S. epidermidis* into three adjacent intervertebral discs under the hypothesis that discitis after discography is caused by infection rather than by a chemical reaction to the injected RCM [20]. Chemical discitis is assumed to be one of the mechanisms by which there is an exacerbation of back pain following discography; however, so far, no chemical discitis after intradiscal RCM (or antibiotic) injection has

Figure 4. Bacterial growth for ceftriaxone plus the three radiographic contrast media as a percentage of control growth. No statistically significant difference was observed compared with the experiments with ceftriaxone alone.

Figure 5. Bacterial growth as a percentage of control growth after treatment with dilutions of iohexol, iodixanol and iosimenol. A statistically significant inhibition of bacterial growth for all laboratory strains is only evident at dilutions 1 and 2.

been proven histologically in animal studies or in patients. In further human studies, Fraser's group examined patients with discitis after discography. Bacteria were isolated from the affected discs in three out of four patients undergoing discectomy and fusion within 6 weeks of the intervention. Taking into account the results of these animal and human studies, Fraser et al concluded that discitis after discography is "due to infection introduced by the needle tip" [20].

In subsequent animal experiments, Fraser et al [21] examined the efficacy of antibiotic prophylaxis prior to discography in relation to the time of iv antibiotic injection, and found that timing was crucial. The antibiotic has to be injected 30–45 min before the intervention to provide ~1% of the antibiotic serum level in the disc. 60 min after iv injection, no antibiotic could be detected in the harvested discs [17, 21, 23]. The results of Fraser's animal studies elucidated that, in the clinical environment, the timing of an iv antibiotic is apparently most essential in order to receive an adequate antibiotic level in the disc at the time of the intervention.

Osti et al [23] also conducted animal experiments in sheep, injecting a mixture of an ionic contrast medium and a bacterial suspension plus cefazolin into adjacent intervertebral discs after an earlier iv antibiotic injection. No discitis was seen in any sheep. Subsequently, a prospective clinical trial in >120 patients undergoing lumbar discography was carried out using the ionic contrast medium diatrizoate mixed with 1 mg cefazolin per 1 ml of the medium. None of the patients showed any signs of discitis during follow-up examinations. Those results led Osti et al [23] to recommend adding a broad-spectrum antibiotic to the RCM injected at discography; this was subsequently emphasised by Endres and Bogduk [24], who recommended iv plus intradiscal antibiotics to prevent bacterial discitis.

Diatrizoate, used by Osti et al [23], is an ionic contrast medium no longer approved for neuroaxial injection, whereas iohexol and iodixanol are non-ionic RCM used

currently for neuroaxial administration. Several concentrations are commercially available, based on the iodine content of the particular compound. No publications have reported on potential chemical discitis while using approved non-ionic RCM at varying dilutions for discography. However, in the past, chemical discitis was described as a potential complication after disc surgery [30].

Conversely, as indicated by several authors [2, 4, 8, 21, 23], any type of antibiotic prophylaxis cannot be a substitute for correct indications, patient selection and accurate technique. Already in 1987, Fraser underlined how technical improvements, such as a "two needle technique", and the usage of "stilette needles" had already reduced the incidence of discitis from 2.7% to 0.7% [21]. In 2006, a publication showed that the incidence of bacterial discitis after discography was as low as 0.016% (per examination) [4].

In summary, our study clearly demonstrates that adding different broad-spectrum antibiotics to RCM does not change the efficacy of gentamicin, ampicillin or ceftriaxone. Therefore, the previous experiments by Klessig et al [26] are confirmed, in principle, by our *in vitro* tests. The occurrence of a chemical discitis after intradiscal injection of antibiotics has so far not been proven in animal studies or in patients [17, 18, 20, 21, 23, 31].

Prospective, preferably controlled, double-blinded, clinical trials for intradiscal antibiotic injection would be highly recommended in order to provide evidence that this regimen is appropriate in patients undergoing discography. In the UAE, recurrent lower back pain has become a severe and increasing clinical problem, and so more interventional procedures, such as provocative discography, are used to a greater extent in our local patient population. Moreover, hospital-borne infections are also increasingly being seen, and so recommendations are sought in order to reduce the incidence of bacterial resistance. Thus, for our environment, a protocol for discography without systemic administration of a broad-spectrum antibiotic would be desirable.

Conclusions

- Inhibition of bacterial growth of all five laboratory bacterial strains was best achieved by gentamicin.
- Ampicillin and ceftriaxone were limited at inhibiting bacterial growth of *K. pneumoniae* and *P. aeruginosa* at high dilutions.
- Ampicillin, gentamicin and ceftriaxone retained their efficacy in the presence of all three RCM — iohexol, iodixanol and the new non-ionic dimeric substance iosimenol.
- Iohexol, iodixanol and iosimenol alone revealed mild antibiotic effects at high concentrations.
- A benefit of intradiscal antibiotic injection would be the simplicity of one single injection without the uncertainty of the exact timing of systemic antibiotic administration, as demonstrated in several animal studies.
- Controlled clinical trials are recommended to investigate whether intradiscal injections of a mixture of RCM with a broad spectrum antibiotic could be employed to prevent post-interventional bacterial discitis.

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