■ RESEARCH

In vitro study of the antimicrobial effects of radiological contrast agents used in arthrography

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Aspiration arthrography using an iodinated contrast medium is a useful tool for the investigation of septic or aseptic loosening of arthroplasties and of septic arthritis. Previously, the contrast media have been thought to cause false negative results in cultures when present in aspirated samples of synovial fluid, probably because free iodine is bactericidal, but reports have been inconclusive.

We examined the influence of the older, high osmolar contrast agents and the low osmolar media used currently on the growth of ten different micro-organisms capable of causing deep infection around a prosthesis. Five media were tested, using a disc diffusion technique and a time-killing curve method in which high and low inocula of micro-organisms were incubated in undiluted media. The only bactericidal effects were found with low inocula of *Escherichia coli* and *Pseudomonas aeruginosa* in ioxithalamate, one of the older ionic media.

The low and iso-osmolar iodinated contrast media used currently do not impede culture. Future study must assess other causes of false negative cultures of synovial fluid and new developments in enhancing microbial recovery from aspirated samples.

Contrast enhanced arthrography, during which a tissue biopsy or aspiration of synovial fluid can be performed, is a useful diagnostic tool to assess loosening of hip and knee arthroplasties with or without infection. It is occasionally used in the diagnosis of septic arthritis.¹⁻⁶ Although arthrography is not necessary in order to perform joint aspiration, it helps to confirm the position of the needle and may make the radiological signs of loosening of the prosthesis more apparent.^{2,7} In these procedures iodinated contrast agents are often used. Originally these were high osmolar molecules with ionic, monomeric structures containing three iodine atoms, such as diatrizoate and metrizoate. In the last decades, iodinated contrast media have been developed further because of reports of adverse reactions in patients.⁸ Newer non-ionic media have a lower osmolarity and are less toxic.⁹ A modern agent such as iodixanol consists of hexa-iodinated dimeric molecules, is isotonic with blood and is rapidly excreted from the body in an unchanged form.¹⁰ The intrinsic stability of the molecules prevents the bound iodine from becoming free and chemically active.¹¹

Iodinated contrast media used for arthrography have been presumed to be a major cause of the frequent false negative cultures from

intra-articular samples, probably because free iodine is bactericidal and used as a disinfectant. Several studies have been performed to assess this potentially bactericidal effect,¹²⁻²⁰ but the available evidence is inconsistent and relatively old (Table I), and the newest iso-osmolar media have not been assessed. Because of the serious clinical implications of false negative cultures we have investigated the antimicrobial effects of selected older and modern iodinated contrast agents.

Materials and Methods

Contrast agents. The iodinated contrast media tested were sodium meglumine ioxithalamate (Telebrix 35, ionic high osmolar monomer, 350 mg I/ml), sodium meglumine ioxaglate (Hexabrix 320, ionic low osmolar dimer, 320 mg I/ml) and iobitridol (Xenetix 300, non-ionic low osmolar monomer, 300 mg I/ml), all manufactured by Guerbet (Gorinchem, The Netherlands). Iohexol (Omnipaque, non-ionic low osmolar monomer, 300 mg I/ml) and iodixanol (Visipaque, non-ionic iso-osmolar dimer, 320 mg I/ml), both produced by GE Healthcare (Eindhoven, The Netherlands), were also tested.

Micro-organisms. The choice of micro-organisms was based on species that have been

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Table I. Bactericidal effect of iodinated contrast media in previous studies

Authors	Micro-organisms	Contrast agents	Bactericidal effect on
Narins and Chase ¹³	<i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Proteus</i> spp., <i>Pseudomonas</i> spp., <i>Enterococcus</i> spp.	Diatrizoate*	<i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Proteus</i> spp., <i>Enterococcus</i> spp.
Kuhns et al ¹⁴	<i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Proteus mirabilis</i> , <i>Proteus vulgaris</i> , <i>Pseudomonas aeruginosa</i>	Acetrizoate* Diatrizoate	<i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Proteus</i> spp., <i>Pseudomonas aeruginosa</i> <i>Escherichia coli</i>
Melson et al ¹⁵	<i>Staphylococcus aureus</i> , coagulase negative staphylococci, <i>Streptococcus pyogenes</i> , <i>Streptococcus pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Haemophilus influenzae</i> , <i>Neisseria gonorrhoeae</i>	Diatrizoate Iothalamate*	– –
Johansen and Clausen ¹⁶	<i>Escherichia coli</i> , <i>Proteus vulgaris</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i>	Metrizamide† Metrizoate*	– –
Kim and Lachman ¹⁷	<i>Staphylococcus aureus</i>	Diatrizoate Iothalamate	<i>Staphylococcus aureus</i> –
Dawson et al ¹⁸	<i>Escherichia coli</i> , <i>Enterobacter aerogenes</i> , <i>Morganella morganii</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus saprophyticus</i> , <i>Enterococcus faecalis</i>	Diatrizoate Ioglycamate‡ Iohexol† Iopamidol† Iothalamate Ioxaglate‡ Metrizamide	<i>Escherichia coli</i> , <i>Enterobacter aerogenes</i> – – <i>Escherichia coli</i> , <i>Enterobacter aerogenes</i> <i>Escherichia coli</i> – –
Dory and Wautelet ¹⁹	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus milleri</i> , <i>Mycobacterium tuberculosis</i>	Diatrizoate Metrizamide	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> <i>Escherichia coli</i>
Lee and MacEwen ²⁰	<i>Staphylococcus aureus</i> , <i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i>	Iothalamate	–

* ionic high osmolar monomer

† non-ionic low osmolar monomer

‡ ionic low osmolar dimer

isolated from infected hip prostheses.^{4,5,7} The strains used were *Staphylococcus aureus* ATCC 29213 (*Staph. aureus*), *Staphylococcus epidermidis* ATCC 12228 (*S. epidermidis*), *Enterococcus faecalis* ATCC 29212 (*E. faecalis*), *Streptococcus pyogenes* ATCC 19615 (*S. pyogenes*), *Bacillus cereus* ATCC 14579 (*B. cereus*), *Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Candida albicans* ATCC 90028 (*C. albicans*), and *Corynebacterium jeikeium* and *Propionibacterium acnes*, both from external microbiology quality assurance samples. All strains were stored frozen at -85°C. For this study, isolates were thawed, plated onto sheep blood agar and incubated overnight at 35°C. After subculturing twice, fresh overnight cultures were used.

Qualitative growth inhibition testing. Qualitative, preliminary tests with two ionic and one non-ionic contrast agent were performed to determine whether any growth-inhibiting effect would occur. A disc diffusion technique, as applied for classic antimicrobial susceptibility testing, was used.²¹ A saline suspension of each test strain with turbidity equivalent to a 0.5 McFarland standard suspension (approximately 1.5×10^8 colony forming units (cfu)/ml) was plated onto an appropriate agar medium. A

standardised paper disc impregnated with 20 ml of contrast medium was placed on the agar surface. The test was carried out for each strain in combination with ioxithalamate (undiluted and diluted 1:2, 1:5 and 1:10), ioxaglate (undiluted) and iobitridol (undiluted). After 24 and 48 hours of incubation at 35°C, any growth inhibiting effect was read by measuring the zones of inhibition around the discs.

Quantitative growth inhibition testing. Suspensions were made of each test strain in the undiluted contrast media ioxithalamate, ioxaglate, iobitridol, iohexol and iodoxanol with both a high and a low inoculum. For the high inoculum, a suspension with a concentration of approximately 5×10^8 cfu/ml was prepared. Colony counts were carried out on the freshly prepared suspension (T0) and after two (T2) and 24 (T24) hours of incubation at 35°C. The time points were chosen to simulate actual transport times. At each time point serial dilutions were made in undiluted contrast medium. From this, several fixed aliquots were spread onto appropriate agar media. After incubation the series of plates in which the T0 plate contained 30 to 300 colonies were counted.

For the low inoculum, serial dilutions were made from a 0.5 McFarland suspension to a final concentration of approximately 5×10^3 cfu/ml in undiluted contrast media

Table II. High inocula of micro-organisms in five contrast media. Mean concentrations (10^7 cfu/ml) of freshly prepared suspensions (T0), and after two (T2) and 24 (T24) hours of incubation

Micro-organism	Contrast medium														
	ioxithalamate			loxaglate			lobitridol			lohexol			lodixanol		
	T0	T2	T24	T0	T2	T24	T0	T2	T24	T0	T2	T24	T0	T2	T24
<i>Bacillus cereus</i>	2.0	2.0	0.45	2.4	2.4	0.25	1.7	4.0	0.20	1.9	5.5	0.80	4.6	8.5	0.70
<i>Candida albicans</i>	3.2	2.4	2.3	1.7	1.7	0.9	0.80	1.3	0.75	2.0	1.4	1.4	0.70	2.0	0.70
<i>Corynebacterium jeikeium</i>	120	99	74	43	63	55	19	17	51	2.3	6.3	4.5	6.5	12	11
<i>Enterococcus faecalis</i>	94	51	47	30	32	25	29	27	23	22	23	20	11	7.8	7.0
<i>Escherichia coli</i>	98	73	13	80	68	30	120	140	63	110	100	52	8.3	13	15
<i>Propionibacterium acnes</i>	180	170	190	68	72	53	18	20	17	39	49	33	26	28	33
<i>Pseudomonas aeruginosa</i>	100	24	0.20	94	46	57	190	160	99	130	150	110	44	85	44
<i>Staphylococcus aureus</i>	160	150	160	130	140	150	87	88	97	46	39	40	33	36	40
<i>Staphylococcus epidermis</i>	61	39	63	69	75	45	61	71	38	86	80	38	29	25	18
<i>Streptococcus pyogenes</i>	150	150	100	25	19	4.8	34	24	12	22	14	5.3	11	8.8	7.0

Table III. Mean percentages (%) of surviving cells of low inocula of micro-organisms in five contrast media and saline after two (T2) and 24 (T24) hours of incubation

Micro-organism	Contrast medium											
	ioxithalamate		loxaglate		lobitridol		lohexol		lodixanol		Saline	
	T2	T24	T2	T24	T2	T24	T2	T24	T2	T24	T2	T24
<i>Bacillus cereus</i>	68	38	98	63	129	77	86	95	97	87	66	2
<i>Candida albicans</i>	115	34	92	31	87	135	101	163	84	27	83	101
<i>Corynebacterium jeikeium</i>	107	42	103	45	100	63	69	16	99	37	61	1
<i>Enterococcus faecalis</i>	127	36	81	83	66	47	98	56	73	61	97	78
<i>Escherichia coli</i>	61	6	89	42	78	35	111	24	79	70	82	17
<i>Propionibacterium acnes</i>	71	60	93	14	89	72	88	70	98	53	85	3
<i>Pseudomonas aeruginosa</i>	26	0	61	142	84	155	77	330	87	441	56	23
<i>Staphylococcus aureus</i>	109	89	95	55	91	40	99	37	80	37	84	11
<i>Staphylococcus epidermis</i>	79	29	75	54	74	27	66	51	53	42	92	0
<i>Streptococcus pyogenes</i>	89	23	79	17	109	60	91	60	99	58	63	0

and in saline control samples. From the three lowest dilutions, colony counts were carried out at T0, T2 and T24 by spreading fixed aliquots onto appropriate agar media. After incubation, the colonies were counted and the percentages of the surviving cells were calculated. All experiments were performed in duplicate and the mean values obtained were used to determine any bactericidal effect with diminished recovery of viable cells, which might lead to negative culture results *in vivo*.

Results

We found no inhibiting effect with disc diffusion testing with any of the three tested contrast media (ioxithalamate, ioxaglate, lobitridol) on any of the strains.

Table II shows the mean concentrations of the micro-organisms counted at T0, T2 and T24 in the high inoculum tests. The inocula at T0 varied between 1.7×10^7 and 1.9×10^9 cfu/ml. No complete bactericidal effect occurred and after 24 hours of incubation all species were still cultured. At T2, the colony counts of *P. aeruginosa* in ioxithalamate were markedly lower than those at T0 and more so at T24. After 24 hours some bactericidal effect was also seen for

E. coli in ioxithalamate and for *B. cereus* and *S. pyogenes* in several different agents.

In the low inoculum tests, at T2 and T24 the percentages of surviving cells of both *E. coli* and *P. aeruginosa* in ioxithalamate were significantly lower than those in the saline control (Table III). After 24 hours *P. aeruginosa* could not be cultured from ioxithalamate. No bactericidal effects occurred for *E. coli* and *P. aeruginosa* with any other contrast medium. The latter showed growth in all contrast media but ioxithalamate. For all other combinations no bactericidal effect was found compared with the saline control except after 24 hours of incubation for some contrast media and *E. faecalis* and *C. albicans*. In these cases, however, there was not a complete loss of viable cells (Table III).

Discussion

Disc diffusion testing showed no influence on the growth of the tested micro-organisms. Because of this, subsequent testing was performed using undiluted contrast media. Quantitative testing showed only a bactericidal effect of ioxithalamate on *E. coli* and on *P. aeruginosa*, which was most evident when low inocula were used. This agrees with

earlier findings, where severe bactericidal effects were mostly found for Gram-negative bacteria (Table I). The obvious growth of *P. aeruginosa* in low osmolar contrast agents has also been observed by others and is probably explained by the ability of this organism to thrive in aqueous solutions.¹⁹

Iodinated contrast media have been considered to be the cause of false negative cultures of joint fluid. The evidence from previous studies in favour of this is mixed and sometimes contradictory (Table I). In 1960 Svoboda and Ježková¹² studied the influence of different types of iodinated contrast media used at that time in radiography on a panel of 16 different microbial species. The growth-inhibiting effect caused by some media was called 'slight and inconstant', but was found to increase with higher concentrations of contrast medium and longer exposure times. Several studies have described the influence of iodinated cystographic contrast media on urinary pathogens.^{13,14,16} Bactericidal effects were seen *in vitro*, but *in vivo* the recovery of pathogens from urine cultures performed after cystography was never affected.

Melson et al¹⁵ exposed bacterial strains known to cause septic arthritis and infection of prostheses *in vitro* to arthrographic contrast media containing diatrizoate or iohalamate. Colony counts of bacterial suspensions were carried out with final concentrations of $\geq 10^5$ cfu/ml in contrast medium and in saline controls, after being left at room temperature for 30 minutes to imitate the transport time between collection of the sample and arrival at the laboratory. No diminished recovery was observed. After a further exposure of up to 24 hours all bacteria could still be cultured.

Based on their predecessors' work, Kim and Lachman¹⁷ exposed *Staph. aureus* to several dilutions of different arthrographic contrast media during incubation at 37°C for up to 48 hours, using bacterial inocula between 10^1 and 10^8 cfu/ml. The bactericidal effect exerted by some of the diatrizoate-containing media proved to be higher for lower inocula of *Staph. aureus* exposed to less diluted medium for a longer time.¹⁷ In agreement with this, Dory and Wautelet¹⁹ found that the growth inhibiting effect of meglumine amidotrizoate (diatrizoate) on *E. coli* and *P. aeruginosa* progressed from partly to completely bactericidal if the sizes of the inoculum were lowered to $\leq 10^4$ cfu/ml.

These results show that the inconsistencies between the previous studies may be explained by differences in the sizes of the bacterial inoculum and dilutions of the medium. In our study we tested an extensive panel of micro-organisms with five different contrast media in undiluted form, using high and low inocula, because of the influence of the size of the inoculum on any growth-inhibiting effect, but also because of the low bacterial counts found in infections of orthopaedic prostheses.^{22,23} Dawson et al¹⁸ tested seven contrast media and concluded that the antibacterial effects were the strongest, but not exclusively, with the older high-osmolality contrast media. Since an iso-osmolar medium had not been tested before, we included iodixanol.

The only complete bactericidal effect we found was with *P. aeruginosa* in ioxithalamate, one of the older, high osmolar, allegedly more toxic media. Hence, the false negative results found in cultures currently are still not explained, but are not caused by the tested modern contrast agents. The reduced survival in normal saline after 24 hours of incubation suggests that they might have a protective effect (Table III). Other causes of negative cultures may include autolysis of the micro-organisms or delayed arrival of the sample in the microbiology laboratory with suboptimal transport conditions. Antibiotic treatment of the patient before aspiration can play a role.⁵ A local anaesthetic injected prior to aspiration can have a bactericidal effect.^{19,24} Synovial fluid itself may have bactericidal properties.^{25,26}

Attempts have been made to improve techniques of culture by using different broths and solid culture media, yet still many cultures have false negative results.²⁷ Modern recovery techniques such as 16S rDNA PCR (universal detection of bacterial presence based on conserved ribosomal DNA sequences) might enhance recovery, but are not yet capable of detecting patterns of resistance and will not be within reach of every microbiology laboratory.²⁸ The influence of contrast agents on this delicate analysis is not yet known.

We conclude that the iodinated contrast media currently used do not impede the detection of micro-organisms in cultures of synovial fluid and can therefore be safely used in aspiration arthrography.

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