SCIENTIFIC REPORT

An in vitro study on the compatibility and precipitation of a combination of ciprofloxacin and vancomycin in human vitreous

M Hui, A K H Kwok, C P Pang, S W Cheung, R C Y Chan, D S C Lam, A F B Cheng

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Aims: To investigate the precipitation process of a mixture of vancomycin and ciprofloxacin by equilibrium dialysis and its subsequent effect on the level of available free antibiotics.

Methods: Concentrations of vancomycin and ciprofloxacin in an equilibrium dialysis chamber were measured during the equilibrium process by high performance liquid chromatography and fluorescence polarisation immunoassay. Normal saline (NS), balanced salt solution plus (BSS Plus), and vitreous were used separately as the medium of dialysis.

Results: Precipitation of ciprofloxacin occurred on incubation at 37°C. It formed precipitate on its own or when mixed with vancomycin in all the three media of NS, BSS Plus, and vitreous. There was more precipitation at higher initial ciprofloxacin concentrations; at 25.0 mg/l about 75% free drug in BSS Plus was lost after 72 hours. The extent of precipitation was similar in both NS and BSS Plus. In the dialysis chambers, 20 mg/l ciprofloxacin dialysed against 125 mg/l vancomycin was reduced to a concentration about 5.0 mg/l after 168 hours. Precipitation of vancomycin was negligible. Ciprofloxacin precipitated in vitreous at body temperature, irrespective of the presence of vancomycin. Even after precipitation, the resultant concentration of ciprofloxacin was still higher than the MIC_{90} of the drug against most Gram negative organisms.

Conclusions: Based on this in vitro study, ciprofloxacin precipitated in vitreous at body temperature, irrespective of the presence of vancomycin or the medium for intravitreal injection. The resultant amount of ciprofloxacin was still higher than the MIC_{90} of the drug against most Gram negative organisms after precipitation. The authors suggest ciprofloxacin in place of ceftazidime when used in combination with vancomycin for treatment of infective endophthalmitis.

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nfective endophthalmitis is an ophthalmic emergency with the potential of causing blindness.[1][2] The usual intravitreal antibiotic treatment includes a combination of vancomycin and aminoglycosides, such as amikacin or gentamicin.[3–5] Ceftazidime is also frequently administered instead of aminoglycosides, together with vancomycin with acceptable antibacterial efficacy and low toxicity.[6][7] However, vancomycin and ceftazidime are incompatible upon mixing, with precipitate formation.[8] This effect has recently been explored with checkerboard titration and equilibrium dialysis. We showed that ceftazidime precipitated to a significant extent, especially when prepared in balanced salt solution plus (BSS Plus), with resultant free antibiotic concentration being significantly reduced to a level of less than the MIC_{90} of the drug against many organisms.[9] Such antibiotic concentration would be inadequate for the treatment of a potentially blinding disease like infective endophthalmitis.

Ciprofloxacin is a fluorinated 4-quinolone; it inhibits the bacterial DNA gyrase activity and disrupts DNA supercoiling, resulting in inhibition of bacteria DNA synthesis and cell death.[10] Ciprofloxacin has a wide spectrum of anti-Gram negative activities,[11] and is widely used in various clinical infections, including urinary tract infections, abdominal infections, respiratory tract infections, soft tissue infections, and bone and joint infections.[12] In rabbit models, no retinal toxicity has been found when ciprofloxacin was injected intravitreally at the therapeutic doses of 0.1–0.5 mg.[13][14] The physiochemical compatibility of ciprofloxacin with several other intravenous agents has also been explored, including normal saline, gentamicin, netilmicin and teicoplanin. Only transient precipitation was observed with teicoplanin, which is also a glycopeptide, as is vancomycin, but not with other agents.[15]

In view of the potential usefulness of ciprofloxacin as an alternative to ceftazidime in the treatment of infective endophthalmitis, we investigated the precipitation process of a mixture of vancomycin and ciprofloxacin by checkerboard titration and equilibrium dialysis in various fluid media.

METHODS

All experiments were conducted in duplicate, and the mean values taken for analysis. Vitreous was obtained from donor cadaver eyes with prior informed consent. The age of the donors ranged from 65 to 75 years, and the time of preparation was within 1 hour of the death of the donors and stored at –80°C immediately after collection before experimental use as a pool. The degree of syneresis of the pooled vitreous was visually low. Dosages of vancomycin (Abbott, Chicago, IL, USA) and ciprofloxacin (Bayer, Germany) were 1.0 mg and 0.2 mg respectively, prepared in 0.1 ml of 0.9% normal saline (Otsuka, Guangdong, China) or balance salt solution plus (BSS Plus, Alcon, Fort Worth, TX, USA). The vitreous volume of an adult emmetropic human eye is about 4 ml, giving a respective empirical concentration of 0.25 mg/ml and 0.05 mg/ml for vancomycin and ciprofloxacin when injected into the vitreous. The actual concentrations would be different, depending on the extent of precipitation and the actual vitreous volume.

Assay of antibiotics

Ciprofloxacin was assayed by high performance liquid chromatography (HPLC)[16] and vancomycin by a fluorescence polarisation immunoassay (Abbott TDx, Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA).

Table 1 Checkerboard study

A Initial concentrations (mg/l) of ciprofloxacin/vancomycin prepared in microtitre plate (amount in µg)

0/0	0/250	0/125	0/62.5	0/31.25
(0/0)	(0/125)	(0/62.5)	(0/31.25)	(0/15.625)
50/0	50/250	50/125	50/62.5	50/31.25
(25/0)	(25/125)	(25/62.5)	(25/31.25)	(25/15.625)
25/0	25/250	25/125	25/62.5	25/31.25
(12.5/0)	(12.5/125)	(12.5/62.5)	(12.5/31.25)	(12.5/15.625)
12.5/0	12.5/250	12.5/125	12.5/62.5	12.5/31.25
(6.25/0)	(6.25/125)	(6.25/62.5)	(6.25/31.25)	(6.25/15.625)
6.25/0	6.25/250	6.25/125	6.25/62.5	6.25/31.25
(3.125/0)	(3.125/125)	(3.125/62.5)	(3.125/31.25)	(3.125/15.625)

B Measurable concentrations (mg/l) of ciprofloxacin/vancomycin after 24 hours of incubation at 37°C in NS (calculated amount in µg)

0/0	0/356	0/189	0/105	0/54
(0/0)	(0/107)	(0/57)	(0/31)	(0/16)
67/0	62/376	64/184	66/78	68/56
(20/0)	(19/113)	(19/55)	(20/23)	(20/17)
36/0	33/376	36/209	33/99	29/50
(11/0)	(10/113)	(11/63)	(10/30)	(9/15)
15/0	13/352	13/179	14/92	14/46
(4/0)	(4/105)	(4/54)	(4/28)	(4/14)
7/0	8/570	8/261	7/131	6/52
(2/0)	(2/171)	(2/78)	(2/40)	(2/16)

C Measurable concentrations (mg/l) of ciprofloxacin/vancomycin after 72 hours of incubation at 37°C in NS (calculated amount in µg)

0/0	0/1288	0/637	0/249	0/42
(0/0)	(0/116)	(0/57)	(0/25)	(0/17)
100/0	174/1180	100/345	151/238	57/50
(17/0)	(17/118)	(15/52)	(18/29)	(23/20)
29/0	62/841	51/311	92/301	29/46
(12/0)	(9/126)	(8/47)	(9/30)	(12/18)
31/0	43/1086	67/825	13/90	15/43
(6/0)	(5/130)	(4/50)	(5/36)	(6/17)
5/0	5/320	6/160	6/91	6/39
(2/0)	(2/128)	(2/64)	(2/37)	(2/16)

D Percentage loss of antibiotics after 24 hours of incubation at 37°C in NS based on the amount calculated (ciprofloxacin/vancomycin in %)

0/0	0/15	0/9	0/0†	0/0†
20/0	26/10	23/11	21/25	18/0†
15/0	21/10	17/0†	21/45	30/3
30/0	38/16	38/14	33/11	38/12
33/0	25/0†	22/0†	30/0†	45/0

E Percentage loss of antibiotics after 72 hours of incubation at 37°C in NS based on the amount calculated (ciprofloxacin/vancomycin in %)

0/0	0/8	0/8	0/20	0/0†
32/0	31/6	40/17	28/9	8/0†
8/0	25/0†	39/25	26/4	7/0†
11/0	17/0	36/21	15/0†	7/0†
30/0	32/0†	26/0†	25/0†	28/1

F Measurable concentrations (mg/l) of ciprofloxacin/vancomycin after 24 hours of incubation at 37°C in BSS Plus (calculated amount in µg)

0/0	0/270	0/138	0/63	0/33
(0/0)	(0/122)	(0/62)	(0/28)	(0/15)
51/0	52/274	51/120	43/64	41/32
(23/0)	(24/124)	(23/54)	(19/29)	(19/15)
28/0	27/264	28/141	27/65	28/34
(13/0)	(12/119)	(13/63)	(12/29)	(13/15)
13/0	13/269	14/147	13/64	13/33
(6/0)	(6/121)	(6/66)	(6/29)	(6/15)
7/0	7/254	7/140	7/65	7/33
(3/0)	(3/114)	(3/63)	(3/29)	(3/15)

G Measurable concentrations (mg/l) of ciprofloxacin/vancomycin after 72 hours of incubation at 37°C in BSS Plus (calculated amount in µg)

0/0	0/225	0/135	0/62	0/35
(0/0)	(0/101)	(0/61)	(0/28)	(0/16)
14/0	17/245	15/137	13/67	14/34
(6/0)	(8/110)	(7/62)	(6/30)	(6/15)
25/0	26/257	24/136	25/63	22/33
(11/0)	(12/116)	(11/61)	(11/29)	(10/15)
14/0	13/270	13/137	13/62	13/33
(6/0)	(6/121)	(6/62)	(6/28)	(6/15)
7/0	6/256	7/135	7/62	7/33
(3/0)	(3/115)	(3/61)	(3/28)	(3/15)

Table 1 Continued

H Percentage loss of antibiotics after 24 hours of incubation at 37°C in BSS Plus based on the amount calculated (ciprofloxacin/vancomycin in %)				
0/0	0/3	0/1	0/9	0/6
8/0	6/1	9/14	22/7	26/7
0†/0	1/5	0†/0†	4/7	2/4
4/0	4/3	1/0†	4/7	4/4
2/0	6/9	1/0†	0/6	0†/4

I Percentage loss of antibiotics after 72 hours of incubation at 37°C in BSS Plus based on the amount calculated (ciprofloxacin/vancomycin in %)				
0/0	0/19	0/3	0/10	0/0
75/0	69/12	73/1	78/4	76/2
9/0	7/7	15/2	13/9	20/5
2/0	7/3	9/1	7/11	7/6
5/0	8/8	0†/3	4/12	5/ 5

*Various concentrations of ciprofloxacin and vancomycin were mixed in normal saline and balanced salt solution plus and incubated at 37°C. The medium was aliquot after 24 hours and 72 hours for antibiotics assay.
†No measurable loss.

Study 1: Visual and pH test
Standard mixture solutions of 1.0 mg vancomycin and 0.2 mg ciprofloxacin in 0.1 ml of 0.9% normal saline (NS) or balanced salt solution plus (BSS Plus) were mixed separately with 4.0 ml of NS, BSS Plus, and vitreous for incubation at 37°C.

Study 2: Checkerboard analysis
Mixture samples with various concentrations of ciprofloxacin and vancomycin prepared in NS or BSS Plus were incubated at 37°C in microtitre plates (table 1) with cover by paraffin foil. Aliquots were taken at 24 hours and 72 hours for assays by HPLC (ciprofloxacin) and TDx (vancomycin) to determine the concentration and amount of free drugs.

Study 3: Equilibrium dialysis in vitreous
Equilibrium dialysis studies were performed in an equilibrium dialyser (Spectrum Medical Industries, Los Angeles, CA, USA) with a half cell working volume of 1.0 ml. Vancomycin (125 µg) and ciprofloxacin (20 µg) were prepared in NS and added into half cell chamber A which was separated from half cell chamber B by a semipermeable Spectrapor dialysis membrane with a molecular weight cut off at 6000–8000 D (Spectrum Medical Industries). Both chambers were filled with 1.0 ml of vitreous. The whole system was incubated at 37°C. Aliquots were taken from half cell chamber B at appropriate time intervals up to 168 hours for antibiotic assays. The experiment was then repeated with the antibiotics prepared in BSS Plus and incubated to 192 hours.

RESULTS
Study 1: Visual and pH test
Precipitate was visually detected in NS, BSS Plus, and vitreous after incubation for 1 day at 37°C. There was no change in pH before or after precipitation.

Study 2: Checkerboard analysis in microtitre plates
Vancomycin alone in NS showed no visual precipitation. No measurable decrease was detected at an initial concentration of 31.25 mg/ after 24 and 72 hours of incubation. At higher initial concentrations, the measurable loss ranged from 0%–20%, median 9% (first rows, table 1B–E). Ciprofloxacin alone showed visible precipitation in all wells. Its concentration decreased by 15%–33%, median 25%, after 24 hours of incubation, and 8%–32%, median 20%, after 72 hours (first columns, table 1D and E).

In mixtures of ciprofloxacin and vancomycin there were similar losses in vancomycin by 0%–25%, median 7%, after 24 hours and 0%–25%, median 0%, after 72 hours of incubation, suggesting the loss of vancomycin was minimal and independent of ciprofloxacin. Ciprofloxacin loss was 14%–45%, median 25%, after 24 hours, and 7%–40%, median 26%, by 72 hours, indicating persistent precipitation (table 1B–E).

When prepared in BSS Plus, vancomycin alone precipitated from 1%–9%, median 4%, after 24 hours. After 72 hours, the loss was 0.03%–19%, median 7% (first row, table 1H and I), suggesting minimal vancomycin loss. It was independent of whether NS or BSS was used, since the difference in the amount of vancomycin in NS or BSS Plus was insignificant before and after incubation (Student's paired t test). For ciprofloxacin alone, the loss was 0%–8%, median 3%, after 24 hours of incubation. After 72 hours, the decrease was up to 75% where the initial ciprofloxacin concentration was highest at 25.0 mg/l. At lower initial concentrations (12.5 mg/l–3.125 mg/l), the ciprofloxacin loss was 2%–9%, median 5%, (first column, table 1I).

In mixture with vancomycin, ciprofloxacin precipitation was 0%–26%, median 4%, after 24 hours (table 1H). After 72 hours, great loss of ciprofloxacin was observed at an initial high concentration of 25.0 mg/l (range: 69%–78%, median: 74%). At lower concentrations, the loss was 0%–20%, median 7% (table 1I).

Study 3: Equilibrium dialysis in vitreous
First the antibiotics were prepared in NS and injected into dialysis chamber A which was filled with vitreous. The vancomycin concentration in chamber B rose to about 50% of its original concentration in chamber A after about 24 hours and then plateaued off, showing no loss up to the end of the experiment at 168 hours. For ciprofloxacin, its concentration rose to about 41% in chamber B by 5 hours, then there was a further small rise in concentration to about 44% at 24 hours, afterwards it slowly declined to 36% by the end of the experiment at 168 hours (fig 1A). This suggested a small initial loss of ciprofloxacin by 10%, which slowly increased to about 30% by 168 hours. The concentrations of ciprofloxacin during equilibrium dialysis were 7.97 mg/l by 5 hours and 4.94 mg/l by 168 hours (fig 1B). The duplicate experiment with the antibiotics prepared in BSS Plus showed similar results (fig 2).

DISCUSSION
Intravitreal injection of combinations of vancomycin and ceftazidime has been advocated and successfully used in the treatment of infective endophthalmitis.[5] [6] [17] [18] However, in a recent study utilising similar experimental models of check-
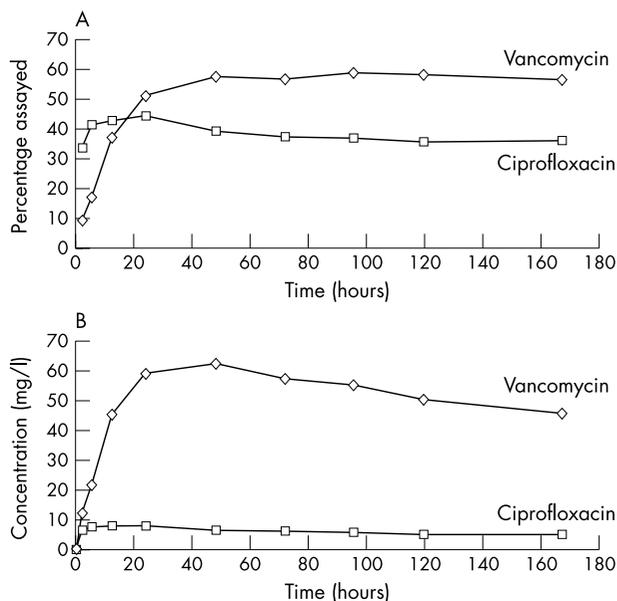
Figure 1 Equilibrium dialysis in vitreous medium with vancomycin and ciprofloxacin initially prepared in normal saline. (A) Percentage change in concentration in vitreous of chamber B. (B) Actual change in concentration in vitreous of chamber B.

erboard analysis and equilibrium dialysis as in this present study, we have shown that ceftazidime precipitated on its own or when mixed with vancomycin in any of the three media of NS, BSS Plus, and vitreous. More precipitation was formed if ceftazidime was initially prepared in BSS Plus than in NS, with up to 88% loss in concentrations of the measurable free antibiotics.[9]

In this present study, we explored the precipitation process of a mixture of vancomycin and ciprofloxacin. In the checkerboard experiments, vancomycin showed negligible to slight loss when prepared alone or in combination with ciprofloxacin. In equilibrium dialysis, the vancomycin origin-
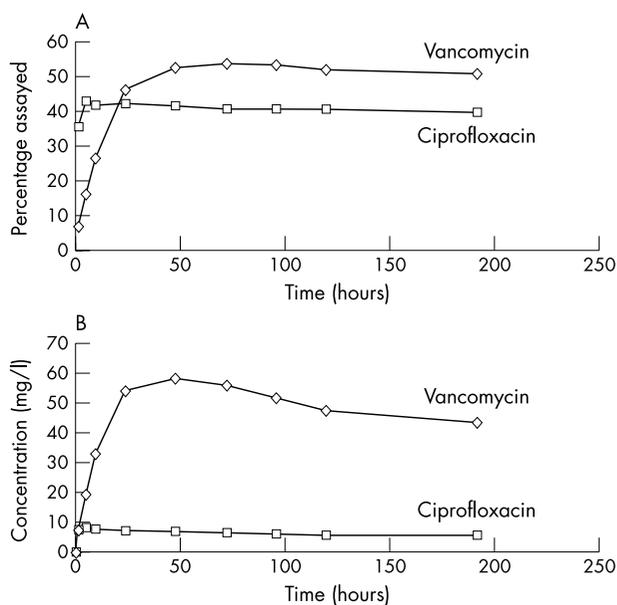

Figure 2 Equilibrium dialysis in vitreous medium with vancomycin and ciprofloxacin initially prepared in balanced salt solution plus. (A) Percentage change in concentration in vitreous of chamber B. (B) Actual change in concentration in vitreous of chamber B.

ally in chamber A only, became almost evenly distributed in both chambers A and B after incubation to 192 hours. These findings were similar to the results of the equilibrium dialysis process of combinations of vancomycin and ceftazidime.[9] On the other hand, ciprofloxacin precipitated out irrespective of the presence of vancomycin (range: 20.25%–26.3%) and the medium of preparation (whether NS or BSS Plus). The precipitation process was stable till 72 hours of incubation. However, the precipitation was greatly enhanced when the initial concentration was at the highest level of 25.0 mg/l after 72 hours of incubation, suggesting that the ciprofloxacin precipitation was related to high concentrations, and the precipitation process continued on prolonged incubation. The findings of this experimental model have an impact on the design of the dosing regimen in clinical practice. On the one hand, the dosage administered must be high enough to reliably eradicate the offending bacteria; on the other hand, too high a concentration will accelerate its precipitation process, leading to a loss of antibiotic content up to about 74%. Meanwhile, the therapeutic level recommended gives an intravitreal level that is not too high and is at the "supra-precipitation" level.[13 14]

Further, in the dialysis experiments, the ciprofloxacin level was decreased from its initial concentration of 20 mg/l to 7.97 mg/l by 5 hours and 4.94 mg/l by 168 hours, no matter whether the antibiotic was prepared in NS or BSS Plus. The antibiotic distributed itself readily in the two dialysis chambers. If there is no loss, the concentration should be 10 mg/l; therefore its level has been lowered by 30% after 168 hours of dialysis. This is in contrast with ceftazidime in a previous study, in which a loss of 80% when prepared in NS and of 90% when prepared in BSS Plus was recorded.[9] In addition, the remaining ciprofloxacin concentration was many times above the MIC_{90} of the drug against the common Gram negative bacteria encountered (table 2).[19] Benz et al have shown that 92% of Gram negative organisms in culture proved endophthalmitis were susceptible to ciprofloxacin.[20] In postoperative endophthalmitis, Kunimoto et al have shown that Gram negative isolates were most susceptible to ciprofloxacin (87.5%) but were poorly susceptible to ceftazidime (60.9%).[21] In post-traumatic endophthalmitis, they have also shown that 100% and 66.7% of Gram negative organisms were susceptible to ciprofloxacin and ceftazidime, respectively.[22]

In summary, ciprofloxacin precipitates in vitreous, but to a much lesser extent than ceftazidime. This process is independent of the medium, so there is no need to avoid the use of BSS Plus during preparation of the antibiotics nor during intraocular surgery like pars plana vitrectomy.[23 24] Vancomycin and ciprofloxacin in a mixture is stable since precipitation is mild. Such stability not only provides an alternative treatment regimen for infective endophthalmitis, but also may avoid frequently repeated intravitreal injections

Table 2 Minimal inhibitory concentration (MIC) of ciprofloxacin against common Gram negative bacteria isolated from the bloodstream in a teaching hospital[19]

Bacterial species	MIC_{90} (mg/l)
Escherichia coli	≤0.03
Klebsiella spp	0.12
Indole positive Proteus spp	0.25
Proteus mirabilis	0.06
Salmonella spp	0.03
Enterobacter, Citrobacter	0.25
Acinetobacter spp	1
Pseudomonas aeruginosa	0.25
Other non-fermenters	2

compared with less stable agents. Dosages of vancomycin and ciprofloxacin at 1.0 mg and 0.2 mg respectively, prepared in 0.1 ml of 0.9% normal saline or BSS Plus, are recommended. Meanwhile, a toxicity study should be conducted to ascertain whether ciprofloxacin poses toxicity effects to the human eye.

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Authors' affiliations
M Hui, S W Cheung, R C Y Chan, A F B Cheng, Department of Microbiology, The Chinese University of Hong Kong, Hong Kong
A K H Kwok, C P Pang, D S C Lam, Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong
A K H Kwok, Department of Ophthalmology, The Hong Kong Sanatorium and Hospital, The Chinese University of Hong Kong, Hong Kong

Correspondence to: Dr Alvin K H Kwok, Department of Ophthalmology, Hong Kong Sanatorium and Hospital, 2 Village Road, Happy Valley, Hong Kong; alvinkwok@hksh.com

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