



## Short Communication

## In-vitro adsorption and sieving coefficient of ticarcillin-clavulanate during continuous haemofiltration

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## ABSTRACT

There are very limited data on ticarcillin-clavulanate elimination by haemofiltration. We measured in vitro ticarcillin-clavulanate adsorption to polyacrylonitrile (PAN) filters and the sieving coefficient using a well-described bench model of haemofiltration. The dose of ticarcillin-clavulanate was 60/2 mg or 180/3 mg, and 0 or 12 g albumin was added to the 1 L of circulating blood-crystalloid mixture to produce four different experimental conditions. The experiment was repeated four times under each condition. Median (interquartile range [IQR]) ticarcillin adsorption varied from 28 (27–30) mg to 85 (78–90) mg. Adsorption was increased when the dose of ticarcillin was higher ( $P < 0.001$ ), but was not affected by the addition of albumin. Median (IQR) adsorption of clavulanate ranged from 0.67 (0.55–0.75) mg to 1.8 (0.33–3.5) mg and was neither dose dependent ( $P = 0.505$ ) nor significantly affected by the addition of albumin. Median (IQR) ticarcillin sieving coefficient ranged from 0.73 (0.67–0.75) to 0.99 (0.97–1.03). It was significantly higher with a higher dose of ticarcillin ( $P = 0.021$ ) and without addition of albumin ( $P = 0.015$ ). Median (IQR) clavulanate sieving coefficient ranged from 1.03 (1.00–2.24) to 2.0 (1.98–2.47). Clavulanate sieving coefficient was not significantly affected by dose or the addition of albumin.

These data indicate that significant adsorption of both ticarcillin and clavulanate occurs in vitro; however, this requires confirmation by clinical pharmacokinetic studies. The sieving coefficient data may help guide appropriate dosing of critically ill patients receiving haemofiltration until more extensive clinical pharmacokinetic data are available.

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## 1. Introduction

Ticarcillin-clavulanate is an intravenous antibacterial agent that consists of a beta-lactam, ticarcillin disodium, and a beta-lactamase inhibitor, clavulanate potassium. Approximately 60% to 70% of ticarcillin and 35% to 45% of clavulanic acid are excreted unchanged in urine during the first 6 h after administration of a

single dose of ticarcillin-clavulanate in a healthy individual with normal renal function. Dosage adjustment is recommended in severe renal failure [1]. At high serum concentrations ticarcillin is known to cause neurotoxicity, including seizures and changes in mental state [2]. Although ticarcillin can be removed by intermittent dialysis [1,3,4], renal replacement therapy in critically ill patients is commonly in the form of continuous therapy, such as haemofiltration, rather than intermittent dialysis [5]. Data on elimination of ticarcillin-clavulanate by haemofiltration are limited to a single study in three children [6,7].

There is considerable variability in the application of haemofiltration in critically ill patients. In particular, changes in effluent rate may result in marked changes in clearance [6]. As a result it may be more useful to measure sieving coefficient and calculate clearance from effluent rate, sieving coefficient and blood flow rate [6]. However, this does not take into account elimination that may

Abbreviations: PAN, polyacrylonitrile.

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**Table 1**  
Median (interquartile range) sieving concentration and adsorption by group.

Ticarcillin/clavulanate dose (mg)	Added albumin (g)	Ticarcillin sieving coefficient	Clavulanate sieving coefficient	Ticarcillin adsorption (mg)	Clavulanate adsorption (mg)
60/2	12	0.73 (0.67-0.75)	2.0 (1.57-2.79)	29 (28-30)	0.67 (0.55-0.75)
180/6	12	0.85 (0.82-0.87)	1.9 (1.05-2.97)	85 (78-90)	1.8 (0.33-3.5)
60/2	0	0.84 (0.81-0.88)	1.03 (1.00-2.24)	28 (27-30)	0.67 (0.53-0.88)
180/6	0	0.99 (0.97-1.03)	2.0 (1.98-2.47)	81 (74-87)	0.96 (0.60-1.3)

occur as a result of adsorption to the haemofilter or the extracorporeal circuit. In vitro data indicate that this may be important for some [8,9] but not all [10,11] antibiotics. Greater adsorption appears to occur to polyacrylonitrile (PAN) haemofilters than filters made of a different material [12].

This in-vitro study was conducted to measure adsorption of ticarcillin and clavulanate to PAN and the sieving coefficient of both drugs, using published methodology [12].

## 2. Material and Methods

The study consisted of two parts. The first part was designed to measure adsorption and the second to measure sieving coefficient.

Both parts of the study used a previously described bench model of haemofiltration [12]. This consisted of a reservoir of a blood-crystalloid mixture to which ticarcillin-clavulanate was added. This mixture was then pumped through a standard haemofiltration circuit with a 0.6 m<sup>2</sup> PAN haemofilter (Multiflow 60; Hospal, Meyzieu, France) at a rate of 200 mL/min. Ultrafiltrate was removed at a rate of 1000 mL/h. In the first part, the ultrafiltrate was returned to the reservoir creating a closed system from which drugs could only be eliminated by adsorption. In the second part, ultrafiltrate was replaced by a bicarbonate-buffered replacement solution (Haemosol BO, Gambro Dasco S.p.A, Sondalo, Italy). The blood-crystalloid mixture consisted of 1 unit of expired packed red blood cells, with and without 12 g albumin, made up to a total volume of 780 mL with heparinized lactated Ringer's solution (5000 unit heparin/L). The haemofilter and circuit were primed with 220 mL 0.9% saline to make a total circulating volume of 1000 mL.

Ticarcillin-clavulanate doses were chosen to reflect clinically relevant concentrations [13]. The doses used in our study were 60 mg and 180 mg of ticarcillin equivalent, resulting in ticarcillin concentrations of 60 mg/L and 180 mg/L and clavulanate concentrations of 2 mg/L and 6 mg/L. The experiment was conducted four times under four different conditions: 60 mg/2 mg ticarcillin/clavulanate without albumin; 60 mg/2 mg ticarcillin/clavulanate with albumin; 180 mg/6 mg ticarcillin/clavulanate without albumin and 180 mg/6 mg ticarcillin/clavulanate with albumin.

During the first part of the study, blood samples were taken from the reservoir immediately before adding ticarcillin-clavulanate and then 20, 50 and 80 min later. Adsorption was estimated by subtracting the measured concentration of ticarcillin and clavulanate from the original dosed concentration and converting this to an amount.

The second part of the study consisted of six 15-min periods between which the point of dilution was alternated between pre-dilution and post-dilution. Each 15-min period consisted of a 9-min equilibration and then a 6-min sampling period when the ultrafiltrate was collected. Blood samples were taken from the filter inlet and filter outlet 3 min after the beginning of the sampling period.

Sieving coefficient was calculated from:

$$\text{Sieving coefficient} = (2 \times [\text{Drug}]_u) / ([\text{Drug}]_a + [\text{Drug}]_v) [6]$$

Where [Drug]<sub>u</sub> is the ultrafiltrate drug concentration, [Drug]<sub>a</sub> is the filter inlet drug concentration and [Drug]<sub>v</sub> is the filter outlet concentration

Blood samples were centrifuged immediately after sampling. The serum and ultrafiltrate samples were stored at -80°C until assayed.

Ticarcillin and clavulanate in plasma were measured by a validated Liquid Chromatography-Mass Spectrometry method using a Nexera2 UHPLC system coupled to a 8030+ triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). The linear range when assaying ticarcillin was 1 to 200 mg/L with intra-batch accuracy of 11.2, 0.3 and 1.0% and precision of 2.6, 10.6 and 0.9% at 3, 30 and 150 mg/L, respectively. The linear range when assaying clavulanate in plasma was 0.33 to 6.67 mg/L, with intra-batch accuracy of 1.4 and 2.3% and precision of 14.3 and 3.6% at 1 and 5 mg/L, respectively.

Ticarcillin and clavulanate concentrations in ultrafiltrate were determined by a validated high performance liquid chromatography method with ultraviolet detection on a Nexera-PDA instrument (Shimadzu, Kyoto, Japan). Ticarcillin was measured in ultrafiltrate from 1 to 200 mg/L with intra-batch accuracy of 2.3, 1.3 and 0.9% and a precision of 0.1, 0.1 and 0.2% at 6, 30 and 150 mg/L, respectively. Clavulanate in ultrafiltrate was measured from 0.167 to 6.67 mg/L with intra-batch accuracy of 19.1, 5.5 and 2.4% and a precision of 6.2, 0.6 and 0.1% at 0.2, 1 and 5 mg/L, respectively. The effect of dose and the addition of albumin on adsorption was analysed using the Mann-Whitney U test.

## 3. Results

The magnitude of adsorption to the filter and sieving coefficients in the different experimental settings are given in Table 1.

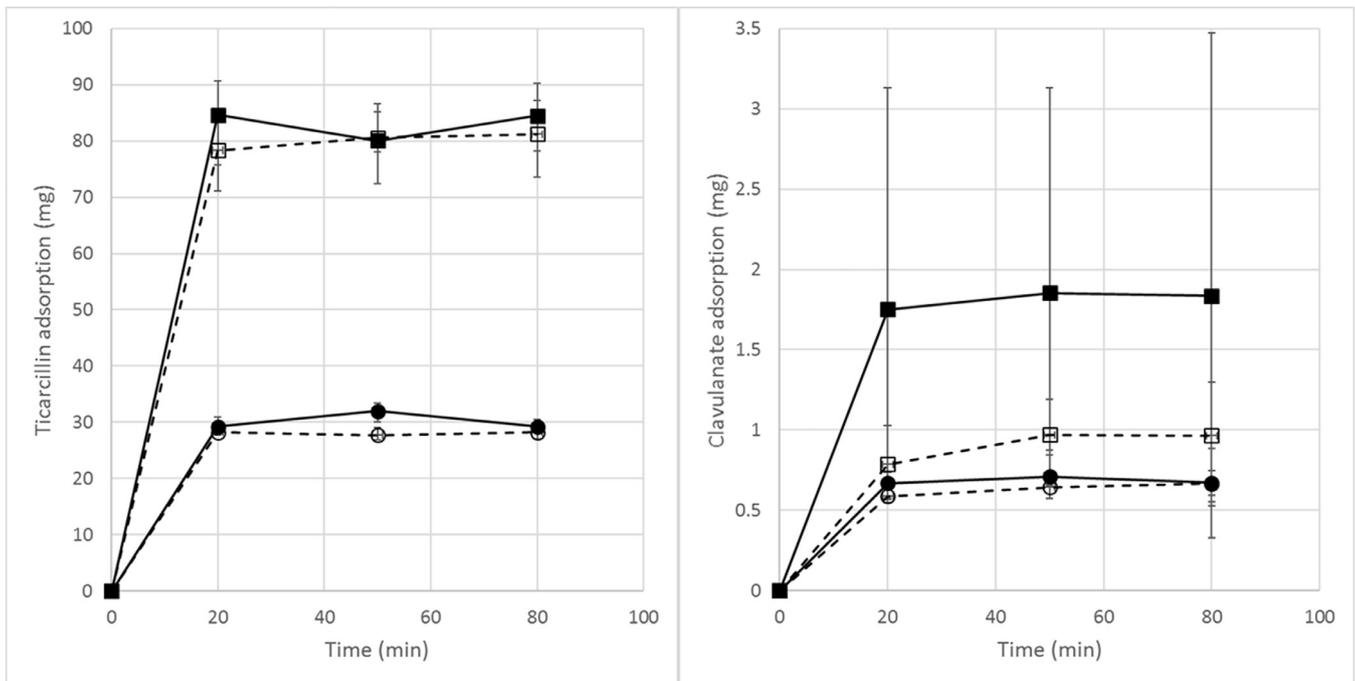
Absolute ticarcillin adsorption was increased when the dose of ticarcillin was higher ( $P < 0.001$ ), but clavulanate adsorption was not dose dependent ( $P = 0.505$ ). Neither ticarcillin adsorption ( $P = 0.645$ ) nor clavulanate adsorption ( $P = 0.721$ ) were significantly affected by the addition of albumin. The time course of adsorption was rapid, with little additional adsorption occurring after 20 min (Fig. 1).

Ticarcillin sieving coefficient was significantly higher with a higher dose of ticarcillin ( $P = 0.021$ ) and without addition of albumin to the blood-crystalloid mixture ( $P = 0.015$ ). Clavulanate sieving coefficient was not significantly affected by dose ( $P = 0.867$ ) or the addition of albumin ( $P = 0.867$ ).

Clavulanate was rapidly eliminated with concentrations falling below the lower limit of quantification within a few 15-min cycles. This precluded any meaningful investigation of the effect of pre- and post-dilution on sieving coefficient. The sieving data presented represent the mean sieving coefficient for each filter used, regardless of pre- or post-dilution.

## 4. Discussion

Substantial adsorption of ticarcillin appears to occur with 46–48% of the dose being adsorbed within 20 min of administration. Proportionally less clavulanate was adsorbed (23–33%). Our data



**Fig. 1.** Median adsorption of ticarcillin and clavulanate against time. Squares represent high dose (180 mg ticarcillin, 6 mg clavulanate), circles represent low dose (60 mg ticarcillin, 2 mg clavulanate). Filled symbols and solid lines indicate added albumin (12 g) and unfilled symbols and dotted lines indicate no added albumin. Error bars show interquartile range.

indicate a sieving coefficient of 0.73–0.99 for ticarcillin and 1.03–2.0 for clavulanate.

Although ticarcillin and, to a lesser extent, clavulanate were adsorbed to the haemofilter and/or circuit, this finding should be interpreted with caution. We have previously shown *in vitro* adsorption of meropenem, piperacillin [14] and vancomycin [11] and very substantial *in vitro* adsorption of aminoglycosides [8,9] but adsorption had only a small effect on peak amikacin concentrations in a porcine model of acute renal failure [15]. Nevertheless, further investigation of the adsorption of ticarcillin and clavulanate is warranted. Where it is available, therapeutic drug monitoring would help to confirm or refute the need for additional dosing to adjust for elimination for any adsorption that may occur *in vivo*.

The data on sieving coefficient are similar to those in the only published clinical study of ticarcillin-clavulanate clearance by haemofiltration [16], which found a ticarcillin sieving coefficient of 0.61–1.06 and clavulanate sieving coefficient of 1.44–1.97 in three children receiving continuous venovenous haemofiltration in predilution mode using a 0.25 m<sup>2</sup> polysulphone filter. Two of the three children were also receiving extracorporeal membrane oxygenation. The mechanism underlying the very high sieving coefficient for clavulanate is unclear. Antibiotics typically have a sieving coefficient that ranges between 0 and 1 with a sieving coefficient that is related to the unbound fraction [6]. However, protein binding clearly cannot explain a sieving coefficient > 1. Clavulanate is an anion and thus its sieving coefficient may be increased by the Gibbs-Donnan effect [6]. This effect occurs as a result of the retention of anionic protein on the blood side of the filter membrane leading to retention of anions and increased elimination of anions. However, the clinical relevance of the Gibbs-Donnan effect is unclear. The negative charge of PAN filters may have enhanced the Gibbs-Donnan effect in our experiment but it should be noted that the high sieving coefficients reported in clinical practice [16] occurred when using polysulphone haemofilters, which have no charge.

By using the sieving coefficient and the effluent rate it is possible to calculate the clearance by haemofiltration and hence make

an appropriate dose adjustment in patients receiving haemofiltration [6]. As the sieving coefficient for clavulanate is substantially higher than for ticarcillin, at any given effluent rate, clearance of clavulanate will be substantially higher than that of ticarcillin. This raises the question of whether additional doses of clavulanate (without ticarcillin) need to be given. Our data cannot answer that question as the pharmacokinetic-pharmacodynamic relationship for clavulanate is poorly understood. However, our data do indicate a need for further investigation of this issue. Similar considerations are likely to apply to dosing of amoxicillin-clavulanate but there are no data on the pharmacokinetics of this agent in patients receiving continuous renal replacement therapy.

Our study has several limitations. Firstly, and most importantly, this study utilized a single compartment bench model. As noted above this may overestimate the extent of adsorption *in vivo*. Limited data on other antimicrobials indicate that *in vitro* sieving coefficients are similar to *in vivo* coefficients in most [12,14,17,18] but not all [19] cases. The sieving coefficients for ticarcillin and clavulanate obtained in this study are similar to those seen in the three patients that have been studied, albeit using haemofilters made from a different material. However, although previous studies using the same experimental model confirmed that the circulating fluid is hypoalbuminaemic [8], as are most critically ill patients, the model does not take into account possible binding to acute phase proteins, which are elevated in critical illness. Secondly, we were unable to assess the effect of dilution mode. Our previous experiment with levofloxacin showed only a minor decrease in sieving coefficient when post-dilution was used compared with predilution [12], although a large effect was shown *in vivo* for vancomycin [20]. Thirdly, we only studied PAN haemofilters. Sieving coefficients were similar when polysulphone filters were used *in vivo* but different results might be obtained with other commonly used filter membranes, such as polyethersulphone or polyamide [10,11,12]. Finally, we are unable to categorically exclude the possibility of sampling or measurement error as a cause for a clavulanate sieving coefficient > 1, e.g., greater instability of clavulanate

in stored plasma compared with ultrafiltrate specimens. However, our data are similar to the only other data on clavulanate sieving coefficient and clavulanate rapidly became undetectable in the circulating blood-crystalloid mixture, indicating a high clearance. As clearance is mainly dependent on sieving coefficient and effluent rate, we believe the high sieving coefficient was not due to error.

In conclusion, our data confirm previous, extremely limited, clinical data regarding the sieving coefficient for ticarcillin (0.73–0.99) and the extremely high sieving coefficient for clavulanate (1.03–2.0). The clinical significance of the high clavulanate sieving coefficient is unclear but it may indicate the need to evaluate whether additional doses of clavulanate alone are warranted in the clinical setting. Similar considerations may also apply to amoxicillin-clavulanate. The limitations of our experimental model mean that our *in vitro* sieving coefficients should only be used to aid dosing until further research establishes *in vivo* coefficients, and only when using PAN haemofilters. Clinical dose adjustment based on *in vitro* adsorption data is not recommended but these data indicate that further investigation of ticarcillin and clavulanate adsorption are warranted.

## Declarations

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## Competing Interests

Professor Lipman is a member of the Bayer ESICM Advisory Board, MSD Antibacterials Advisory Board, has received honoraria for lectures from Pfizer South Africa and MSD South Africa and is a committee member of Pfizer International: 2018 Anti-infectives. Other authors have no interests to declare.

## Ethical Approval

Not required.

## References

[1] Watson ID, Boulton-Jones M, Stewart MJ, Henderson I, Payton CD. Pharmacokinetics of clavulanic acid-potentiated ticarcillin in renal failure. *Ther Drug Monit* 1987;9:139–47.

- [2] GlaxoSmithKline. Timentin prescribing information. 2008; [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2010/050658s023,050590s058,050590s059lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/050658s023,050590s058,050590s059lbl.pdf). Last accessed 22nd February 2019.
- [3] Dalet F, Amado E, Cabrera E, Donate T, del Rio G. Pharmacokinetics of the combination of ticarcillin with clavulanic acid in renal insufficiency. *J Antimicrob Chemother* 1986;17:57–64.
- [4] Parry MF, Neu HC. Pharmacokinetics of ticarcillin in patients with abnormal renal function. *J Infect Dis* 1976;133:46–9.
- [5] Hoste EA, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med* 2015;41:1411–23.
- [6] Choi G, Gomersall CD, Tian Q, Joynt GM, Li AM, Lipman J. Principles of antibacterial dosing in continuous renal replacement therapy. *Blood Purif* 2010;30:195–212.
- [7] Wong WT, Choi G, Gomersall CD, Lipman J. To increase or decrease dosage of antimicrobials in septic patients during continuous renal replacement therapy: the eternal doubt. *Curr Opin Pharmacol* 2015;24:68–78.
- [8] Tian Q, Gomersall CD, Ip M, Tan PE, Joynt GM, Choi GY. Adsorption of amikacin, a significant mechanism of elimination by hemofiltration. *Antimicrob Agents Chemother* 2008;52:1009–13.
- [9] Lam KN, Tian Q, Ip M, Gomersall CD. *In vitro* adsorption of gentamicin and netilmicin by polyacrylonitrile and polyamide hemofiltration filters. *Antimicrob Agents Chemother* 2010;54:963–5.
- [10] Tian Q, Gomersall CD, Wong A, Leung P, Choi G, Joynt GM, et al. Effect of drug concentration on adsorption of levofloxacin by polyacrylonitrile haemofilters. *Int J Antimicrob Agents* 2006;28:147–50.
- [11] Tian Q, Gomersall CD, Leung PP, Choi GY, Joynt GM, Tan PE, et al. The adsorption of vancomycin by polyacrylonitrile, polyamide and polysulfone hemofilters. *Artificial Organs* 2008;32:81–4.
- [12] Choi G, Gomersall CD, Lipman J, Wong A, Joynt GM, Leung P, et al. The effect of adsorption, filter material and point of dilution on antibiotic elimination by haemofiltration. An *in vitro* study of levofloxacin. *Int J Antimicrob Agents* 2004;24:468–72.
- [13] Murillo J, Standiford HC, Schimpff SC. Gentamicin and ticarcillin serum levels. *JAMA* 1979;241:2401–3.
- [14] Jamal JA, Udy AA, Wallis SC, Ranganathan D, McWhinney BC, Ungerer JP, et al. Can we use an *ex vivo* continuous hemofiltration model to describe the adsorption and elimination of meropenem and piperacillin? *Int J Artif Organs* 2015;38:419–24.
- [15] Gomersall CD, Tian Q, Reynolds D, Ip M, Choi G, Joynt G. Adsorption of amikacin during continuous venovenous haemofiltration in a swine model of acute renal failure. *Crit Care* 2015;19:117.
- [16] Lindsay CA, Bawdon R, Quigley R. Clearance of ticarcillin-clavulanic acid by continuous venovenous hemofiltration in three critically ill children, two with and one without concomitant extracorporeal membrane oxygenation. *Pharmacotherapy* 1996;16:458–62.
- [17] Gruber PC, Tian Q, Gomersall CD, Joynt GM, Choi GY. An *in vitro* study of the elimination of oseltamivir carboxylate by haemofiltration. *Int J Antimicrob Agents* 2007;30:95–7.
- [18] Taylor WR, Thinh BN, Anh GT, Horby P, Werthelme H, Lindegardh N, et al. Oseltamivir is adequately absorbed following nasogastric administration to adult patients with severe H5N1 influenza. *PLoS ONE* 2008;3:e3410.
- [19] Karsch K, Chen X, Miera O, Peters B, Obermeier P, Francis RC, et al. Pharmacokinetics of oral and intravenous oseltamivir treatment of severe influenza b virus infection requiring organ replacement therapy. *Eur J Drug Metab Pharmacokinet* 2017;42:155–64.
- [20] Uchino S, Cole L, Morimatsu H, Goldsmith D, Bellomo R. Clearance of vancomycin during high-volume hemofiltration: impact of pre-dilution. *Intensive Care Med* 2002;28:1664–7.