Kinetics of galactomannan in surgical patients receiving perioperative piperacillin/tazobactam prophylaxis

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Objectives: The association between piperacillin/tazobactam and the positivity of the galactomannan (GM) detection ELISA test is well described. Little information is available about the kinetics of GM in patients treated with piperacillin/tazobactam. The present study aimed at clarifying the baseline interaction between piperacillin/tazobactam and GM in patients receiving this drug.

Patients and methods: Seven patients undergoing abdominal surgery received perioperative prophylaxis with piperacillin/tazobactam. Each patient received three doses of 4.5 g of the drug, administered at 8 h intervals (one before and two after surgery). Three patients received antibiotic batches with ‘medium’ (GM-index = 1.782) and four patients received antibiotic batches with ‘high’ (GM-index = 6.665) GM content. Serum samples for GM evaluation were collected before drug infusion and at times +1, +3, +6 and +8 h after the first and third infusions.

Results: GM levels increased after infusion, in particular when batches with ‘high’ GM content were used. Moreover, a non-statistically significant increase between the first dose and the third dose was observed. All samples taken >6 h after administration were negative (GM-index < 0.2), both with the ‘medium’ and the ‘high’ GM content batches.

Conclusions: The low content of GM 8 h after piperacillin/tazobactam infusion suggests that in non-neutropenic cancer patients with solid tumours receiving up to three doses of piperacillin/tazobactam, serum sampling for GM detection should be performed immediately before the next piperacillin/tazobactam administration.

Keywords: fungal infections, antigen detection false positives, diagnosis interactions

Introduction

The detection of circulating galactomannan (GM) with the Platelia Aspergillus (PA) ELISA test (Bio-Rad) in the serum of patients at high risk for fungal infections is an important advance in the diagnosis of invasive aspergillosis.1,2 However, recent studies reported the occurrence of false positive results in patients receiving piperacillin/tazobactam as well as the reactivity of piperacillin/tazobactam batches with the PA test.3–7 Moreover, mass spectrometry analysis (MALDITOFF) suggests that GM is likely to be the molecule responsible for the interference between piperacillin/tazobactam and the PA test.8 Physicians might be reluctant to use piperacillin/tazobactam when GM monitoring is indicated, because of the risk of having misleading information. The present study aimed at quantifying the GM content in cancer patients with normal renal and hepatic function, undergoing...
abdominal surgery receiving piperacillin/tazobactam perioperative prophylaxis.

**Patients and methods**

**Patients**

Male or female patients older than 18 years, undergoing abdominal surgery and requiring antibiotic perioperative prophylaxis, were considered eligible for the study provided they had not received any antibiotic therapy with penicillins or cephalosporins during the previous 72 h, had no infection requiring antibiotic treatment, no history of allergy to β-lactam antibiotics, and a normal renal (serum creatinine < 1.2 mg/dL) and hepatic [aspartate aminotransferase (AST) and alanine aminotransferase (ALT) not higher than two times the upper limit of normal] function. The protocol was approved by the Hospital Ethics Committee (no. of approval MI04.001) and patients were required to give written informed consent.

**Piperacillin/tazobactam administration and GM measurement**

Two batches of piperacillin/tazobactam, with a ‘medium’ (batch A) and a ‘high’ (batch B) GM content determined previously,9 were selected for the study. The content of GM was defined by a numerical index (GM-I) between the optical density (OD, measured at 450/620 nm) of the sample tested and the OD of the cut-off positive control (1 ng/mL GM) included in the PA test. For the PA test, each 4.5 g piperacillin/tazobactam vial was resuspended in 100 mL of 0.9% NaCl (as for clinical use) and then assayed with the PA test.

Piperacillin/tazobactam batches A and B had a mean GM-I of 1.782 (range 1.729–1.816) and 6.665 (range 6.478–6.758), respectively. Three patients received batch A (Patients 1–3) and four patients received batch B (Patients 4–7). Each patient received three intravenous (iv) administrations of 4.5 g of piperacillin/tazobactam (every 8 h) during a period of 16 h: one before surgery and two after 8 and 16 h from first dose. Piperacillin/tazobactam was reconstituted and diluted in 100 mL of 0.9% NaCl solution and infused iv in about 30 min.

Blood samples for GM measurement were collected before infusion and at times +1, +3, +6, +8 h after the first and third infusions of a 4.5 g piperacillin/tazobactam dose. Two further samples were collected at 48 and 72 h after the first dose of antibiotic. Serum samples were immediately separated, aliquotted and frozen at −20°C until the analysis. Piperacillin/tazobactam was infused by an iv line, while blood samples for GM determination were drawn from a peripheral vein or from an iv line, depending on the specific clinical status of the patients. When the same iv line was used both for piperacillin/tazobactam infusions and for blood specimen collection, it was accurately washed with NaCl 0.9% before blood drawing. The PA test was performed in duplicate, following the manufacturer’s instructions. A positivity cut-off of GM-I ≥0.7 was used as from published indications.10 Means and standard deviations of GM-I from all patients had gastrointestinal cancer and needed to undergo surgery with antibiotic chemotherapy. Results obtained as GM-I from serum of patients treated with ‘medium’ and ‘high’ GM content piperacillin/tazobactam batches were plotted against time in order to obtain a kinetic curve of GM levels.

As shown in Figure 1, the administration of piperacillin/tazobactam resulted in an increase in the GM-I immediately after the first and third doses. The increase was particularly evident with the ‘high’ GM content batch. Patient 4 and patient 5 indeed had the highest signal increase after the third administration. However, only in Patient 4 did the GM-I go above the threshold for positivity (two specimens collected 1 h after the first and third doses, with a GM-I of 0.87 and 0.95, respectively).

Lower increases were observed in the group receiving ‘medium’ GM content batches (Figure 2). The mean of the GM-I of the two groups of patients (Figure 3) showed a remarkable although non-statistically significant GM-I increase from 0.45 to 0.51 between the first and third dose (Table 2). All samples taken at times +8 h after piperacillin/tazobactam administration were <0.3, either with the ‘medium’ or the ‘high’ GM content batches.

**Discussion**

In 2003, some authors (including ourselves) found that a relevant proportion of patients treated with piperacillin/tazobactam (36–38%) were falsely positive by the PA test and that the antibiotic itself contained a variable amount of GM in 75–100% of the tested vials.3,5–7,11 On the contrary, other authors did not confirm these findings.12 We found that this apparent discrepancy might well be explained by the fact that the content of GM in piperacillin/tazobactam batches and vials is variable, resulting in a wide range of results from the PA test (GM-I 0.19–6.98), and that 24% of the piperacillin/tazobactam batches test negative by the PA test. Moreover, GM-positive piperacillin/tazobactam vials show a bimodal distribution in GM content, allowing the distinction of ‘medium’ (GM-I 0.77–2.87) and ‘high’ (GM-I 3.86–6.98) GM-positive piperacillin/tazobactam batches.9 This new cause for false-positive GM tests appears less manageable than those previously described, such as other microorganisms, cyclophosphamide, food, cotton swabs, chronic graft-versus-host disease (GVHD).13 The only thing to do would be to check for GM in every piperacillin/tazobactam batch, but this would be an unacceptable and too time-consuming practice. In the present study we tried to evaluate the kinetics of GM in non-neutropenic patients receiving piperacillin/tazobactam from two different GM content batches (‘medium’ and ‘high’), and we found an increase in GM level, particularly within the first 3 h.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>GOT/GPT (U/L)</th>
<th>Creatinine (mg/dL)</th>
<th>Kind of surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>58</td>
<td>46/52</td>
<td>0.75</td>
<td>resection of rectosigmoid</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>75</td>
<td>17/20</td>
<td>0.83</td>
<td>resection of rectum</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>72</td>
<td>13/7</td>
<td>0.56</td>
<td>resection of colon</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>73</td>
<td>29/24</td>
<td>0.94</td>
<td>resection of colon</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>70</td>
<td>8/4</td>
<td>0.47</td>
<td>resection of colon</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>76</td>
<td>19/12</td>
<td>0.57</td>
<td>gastrectomy</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>79</td>
<td>11/7</td>
<td>0.91</td>
<td>resection of colon</td>
</tr>
</tbody>
</table>

GOT, gultamyl oxaloacetic transaminase; GTP, gultamyl pyruvic transaminase.
after drug infusion. GM returned to very low levels within 8 h after piperacillin/tazobactam administration. There was an evident, although not statistically significant, trend towards GM accumulation during prophylaxis. As expected, both the increase and the trend towards accumulation was more noticeable using the 'high' GM content piperacillin/tazobactam batch.

Other studies regarding the kinetics and interaction between piperacillin/tazobactam and the PA test have recently been reported.
Kinetics of galactomannan in patients receiving piperacillin/tazobactam

Table 2. Mean GM-index at different times from piperacillin/tazobactam administration in the two groups of patients, receiving piperacillin/tazobactam with ‘medium’ and ‘high’ GM content

<table>
<thead>
<tr>
<th>Time of blood sampling with respect to piperacillin/tazobactam infusion</th>
<th>Mean GM-index ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately before the first piperacillin/tazobactam infusion</td>
<td>0.106 ± 0.09 (0.044–0.210)</td>
</tr>
<tr>
<td>1 h after the end of the first piperacillin/tazobactam infusion</td>
<td>0.121 ± 0.045 (0.081–0.170)</td>
</tr>
<tr>
<td>3 h after the end of the first piperacillin/tazobactam infusion</td>
<td>0.121 ± 0.045 (0.081–0.170)</td>
</tr>
<tr>
<td>6 h after the end of the first piperacillin/tazobactam infusion</td>
<td>0.120 ± 0.098 (0.052–0.233)</td>
</tr>
<tr>
<td>Immediately before the second piperacillin/tazobactam infusion</td>
<td>0.138 ± 0.039 (0.102–0.180)</td>
</tr>
<tr>
<td>1 h after the end of the second piperacillin/tazobactam infusion</td>
<td>0.120 ± 0.098 (0.052–0.233)</td>
</tr>
<tr>
<td>3 h after the end of the second piperacillin/tazobactam infusion</td>
<td>0.102 ± 0.056 (0.049–0.160)</td>
</tr>
<tr>
<td>6 h after the end of the second piperacillin/tazobactam infusion</td>
<td>0.138 ± 0.039 (0.102–0.180)</td>
</tr>
<tr>
<td>8 h after the end of the second piperacillin/tazobactam infusion</td>
<td>0.102 ± 0.056 (0.049–0.160)</td>
</tr>
<tr>
<td>48 h after the first piperacillin/tazobactam infusion</td>
<td>0.049 ± 0.110–0.204</td>
</tr>
<tr>
<td>72 h after the first piperacillin/tazobactam infusion</td>
<td>0.030 ± 0.119–0.177</td>
</tr>
</tbody>
</table>

Walsch et al. studied the kinetics of GM in normal rabbits treated with piperacillin/tazobactam and they found that piperacillin/tazobactam administration resulted in a marked increase in GM from pre-infusion (GM-I 0.27) to 30 min post-infusion (GM-I 0.83) and in a decline to 0.44 within 24 h from the first infusion. The rabbits were treated over a period of 7 days. At this time point there was evidence of GM accumulation. In another study, PA test negativity was obtained within 2 h after a single dose in healthy volunteers, while in patients receiving repeated doses of antibiotics, the GM test became negative within 12–96 h upon discontinuation, again suggesting a trend to accumulation. Furthermore, the kinetics of GM varied according to the duration of treatment, and the amount of GM in the antibiotic batches failed to predict the GM levels in vivo.

In contrast with the GM accumulation trend reported in these two studies, Singh et al. suggested that low concentrations of piperacillin/tazobactam in serum (<1.4 µg/mL 6 h after the dose or at a trough) should correlate with negative GM test (GM-I < 0.2), thus suggesting the absence of any trend towards accumulation. An explanation for these discrepancies could probably be found in the different patient populations used. Indeed, any alteration in hepatic and/or renal functionality might impact on the kinetics of GM in patients. In addition, since GM undergoes antigenic uptake by macrophages, its elimination pattern may differ substantially from the one expressed by drugs such as piperacillin/tazobactam.

In conclusion, our study shows that in non-neutropenic oncological patients receiving up to three doses of piperacillin/tazobactam, the interaction between the drug and the GM test is present but minimal. No information is available in neutropenic patients receiving prolonged therapy, and since haematological patients are the main target of GM detection, it appears necessary to perform further studies on this kind of patient.

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Transparency declarations

None to declare.

Author contributions

M.M. conceived the study, conducted galactomannan tests, statistical analysis, results interpretation and drafted the manuscript. M.J.M. collected and managed clinical data and monitored collection of clinical samples. E.F. helped to perform galactomannan test, conducted results interpretation and drafted the manuscript. N.S. monitored surgery procedure and clinical management of patients. C.V. conceived the study, evaluated clinical and laboratory data, and drafted the manuscript. All authors read and approved the final manuscript.

References


