Effects of quinupristin/dalfopristin on vasomotor tone in the intact peripheral microcirculation

Takaya Tsueshita1,2, Salil Gandhi3 and Israel Rubinstein1,2,3*

Departments of 1Medicine and 3Pharmaceutics and Pharmacodynamics, Colleges of Medicine and Pharmacy, University of Illinois at Chicago; 2Chicago VA Health Care System, West Side Division, Chicago, IL 60612, USA

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The purpose of this study was to determine whether quinupristin/dalfopristin modulates vasomotor tone in the intact peripheral microcirculation. Using intravital microscopy, we found that superfusion of quinupristin/dalfopristin (120 mg/L) on the intact hamster cheek pouch microcirculation had no significant effects on arteriolar diameter. In addition, superfusion of quinupristin/dalfopristin (120 mg/L) had no significant effects on vasodilation evoked by bradykinin and acetylcholine, two endothelium-dependent agonists, or by vasoactive intestinal peptide, an endothelium-independent agonist, on to the cheek pouch. Collectively, these data indicate that quinupristin/dalfopristin has no significant effects on vasomotor tone in the intact peripheral microcirculation.

Introduction

Quinupristin/dalfopristin (Synercid), a novel streptogramin antibiotic, is used to treat serious infections caused by vancomycin-resistant Enterococcus faecium and complicated skin infections caused by Streptococcus pyogenes or methicillin-susceptible Staphylococcus aureus.1,2 The drug has been shown in vitro to suppress elaboration of certain pro-inflammatory mediators that modulate vasoreactivity, such as interleukin (IL)-1α, IL-1β, IL-6 and tumor necrosis factor (TNF)-α.3,4 This observation indicates that quinupristin/dalfopristin expresses immunomodulatory properties distinct from its anti-infective activity. However, the relevance of these attributes to the clinical efficacy of the drug is uncertain.

A prominent feature of the host inflammatory response to infection is pronounced vasodilation in the peripheral microcirculation thought to be mediated by potent pro-inflammatory mediators, such as bradykinin and cytokines.5 Whether quinupristin/dalfopristin modulates vasomotor tone in the peripheral microcirculation during the host inflammatory response to infection is uncertain.

Hence, the purpose of this study was to begin to address this issue by determining whether quinupristin/dalfopristin modulates vasodilation evoked by bradykinin and acetylcholine, two endothelium-dependent agonists,6,7 and vasoactive intestinal peptide (VIP), an endothelium-independent vasodilator,8,9 in the intact peripheral microcirculation.

Materials and methods

Preparation of animals

This research adhered to the ‘Principles of Laboratory Animal Care’ (NIH publication 85-23 revised 1985) and was approved by the University of Illinois at Chicago Animal Care Committee. To determine the effects of quinupristin/dalfopristin on vasomotor tone, we used the intact hamster cheek pouch model previously used by us and other investigators.6–8,10 Adult, male golden Syrian hamsters weighing 129–135 g (Sasco, Omaha, NE, USA) were anaesthetized with pentobarbital sodium (6 mg/100 g body weight, ip). A tracheostomy was carried out to facilitate spontaneous breathing. A femoral vein was cannulated to inject supplemental anaesthesia during the experiment (2–4 mg/100 g body weight/h). A femoral artery was cannulated to record systemic arterial pressure and heart rate. Body temperature was monitored and kept constant (37–38°C) via a feedback controller and heating pad throughout the duration of the experiment.

*Correspondence address. Department of Medicine (M/C 719), University of Illinois at Chicago, 840 S. Wood Street, Chicago, IL 60612-7323, USA. Tel: +1-312-996-8039; Fax: +1-312-996-4665; E-mail: IRubinst@uic.edu
To visualize the microcirculation of the cheek pouch, the left cheek pouch was spread over a plastic base plate and an incision was made in the overlying skin to expose the cheek pouch membrane. The avascular connective tissue layer of the membrane was carefully removed and an upper plastic chamber was positioned over the base plate. This arrangement forms a triple-layered complex: the base plate, the upper chamber and the cheek pouch membrane exposed between the two plates. The chamber was connected via a three-way valve to a reservoir that allowed continuous superfusion of the cheek pouch chamber with warm (37–38°C) bicarbonated buffer (pH 7.4) bubbled continuously with 95% N₂/5% CO₂. The chamber was connected via a three-way valve to an infusion pump (Sage Instruments, Boston, MA, USA) for controlled administration of drugs into the superfusate.6–8

**Determination of arteriolar diameter**

The cheek pouch microcirculation was visualized with a microscope (Nikon, Tokyo, Japan) coupled to a 100 W mercury light source at a magnification of x40. The microscope image was projected through a low-light TV camera (Panasonic TR-124 MA; Matsushita Communication Industrial Co., Ltd, Yokohama, Japan) on to a video screen (Panasonic). The inner diameter of second order arterioles (42–57 µm), which regulate vascular resistance in the cheek pouch,10 was determined during the experiment from the video display of the microscope image using a videomicroscope (VIA 100; Boeckler Instruments, Tucson, AZ, USA) as previously described in our laboratory.6–8 In each animal, the same arteriolar segment was used to measure changes in diameter during the experiment.

**Effects of quinupristin/dalfopristin on agonist-induced vasodilation**

The purpose of these studies was to determine whether quinupristin/dalfopristin modulates vasodilation elicited by bradykinin, VIP and acetylcholine in the cheek pouch. In preliminary experiments, we determined that superfusion of the cheek pouch with increasing concentrations of quinupristin/dalfopristin (10, 40, 80 and 120 mg/L) for 1 h each was associated with no significant changes in arteriolar diameter from baseline (n = 8; P > 0.5). Hence, the highest concentration of quinupristin/dalfopristin (120 mg/L) was used in subsequent experiments. This concentration is 12-fold higher than that achieved in human serum with conventional dosing.2 The 1 h superfusion of quinupristin/dalfopristin used in these studies is consistent with the duration of intravenous infusion of the drug in humans.1,2

In the first series of experiments, after the equilibration period (30 min), bradykinin (10⁻⁷ M) was superfused for 7 min. Arteriolar diameter was determined before, every minute during and after superfusion of bradykinin was stopped for 30 min. The increase in arteriolar diameter was observed within 2–3 min after the start of the superfusion, was maximal within 5 min and returned to baseline c. 20 min thereafter. Once arteriolar diameter returned to baseline, quinupristin/dalfopristin (120 mg/L) was superfused for 1 h before and during repeated superfusion of bradykinin (10⁻⁷ M). Changes in arteriolar diameter were determined as outlined above. In another series of experiments, VIP (0.1 nmol) or acetylcholine (10⁻⁸ M) was superfused for 7 min before and after 1 h superfusion of quinupristin/dalfopristin (120 mg/L). Changes in arteriolar diameter were determined as outlined above. In preliminary studies, we determined that repeated superfusions of bradykinin (10⁻⁷ M), VIP (0.1 nmol) and acetylcholine (10⁻⁸ M) were associated with reproducible results (n = 6; data not shown). The vasorelaxant effects of bradykinin, VIP and acetylcholine in the intact hamster cheek pouch microcirculation are mediated by agonist-specific receptors.6–8,10 The concentrations of bradykinin, VIP and acetylcholine used in these experiments are based on previous studies in our laboratory.6–8

**Drugs**

Bradykinin and acetylcholine were obtained from Sigma Chemical Co. (St Louis, MO, USA). Human vasoactive intestinal peptide was obtained from American Peptide Company (Sunnyvale, CA, USA). Quinupristin/dalfopristin was obtained from Aventis Pharmaceuticals (Parsippany, NJ, USA). Quinupristin/dalfopristin was dissolved in dimethylsulphoxide (DMSO) as a 1 mM stock solution and diluted in saline to the desired concentration. The final concentration of DMSO in cheek pouch superfusate was 0.05%. All other drugs were dissolved and diluted in saline to the desired concentrations on the day of the experiment.

**Data and statistical analyses**

When a drug was superfused on the cheek pouch, we determined the maximal change in arteriolar diameter and used it as the response to that drug in each animal. Arteriolar diameter was expressed as the ratio of experimental to control diameter, with control diameter normalized to 100% to account for intra- and inter-animal variability. Data are expressed as means ± S.E.M. Statistical analysis was carried out using repeated-measures analysis of variance with Neuman–Keuls multiple-range post hoc test to detect values that were different from control values (StatView; SAS Institute Inc., Cary, NC, USA). P < 0.05 was considered statistically significant; n is given as the number of experiments, with each experiment representing a separate animal.
Quinupristin/dalfopristin and vasodilation

Table 1. Effects of quinupristin/dalfopristin on agonist-induced vasodilation in the hamster cheek pouch

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Change from baseline arteriolar diameter (%)&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Quinupristin/dalfopristin (120 mg/L)</td>
<td>nil</td>
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<tr>
<td>Bradykinin (10&lt;sup&gt;−7&lt;/sup&gt; M)</td>
<td>13.0±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Quinupristin/dalfopristin (120 mg/L), bradykinin (10&lt;sup&gt;−7&lt;/sup&gt; M)</td>
<td>13.6±1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VIP (0.1 nmol)</td>
<td>21.4±1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quinupristin/dalfopristin (120 mg/L), VIP (0.1 nmol)</td>
<td>17.5±1.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetylcholine (10&lt;sup&gt;−8&lt;/sup&gt; M)</td>
<td>19.3±1.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quinupristin/dalfopristin (120 mg/L), acetylcholine (10&lt;sup&gt;−8&lt;/sup&gt; M)</td>
<td>17.8±0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DMSO (0.05%)</td>
<td>nil</td>
</tr>
<tr>
<td>Saline</td>
<td>nil</td>
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<sup>a</sup>Data are means ± S.E.M.; each group, n = 4 animals.  
<sup>b</sup><i>P</i> < 0.05 in comparison with baseline.

Results

Effects of quinupristin/dalfopristin on agonist-induced vasodilation

Systemic arterial pressure and heart rate did not change significantly throughout the duration of the experiments. Superfusion of quinupristin/dalfopristin (120 mg/L) alone for 67 min had no significant effects on arteriolar diameter (Table 1; n = 4 animals; <i>P</i> > 0.5). Superfusion of quinupristin/dalfopristin (120 mg/L) for 1 h before and during a 7 min superfusion of bradykinin (10<sup>−7</sup> M) had no significant effects on bradykinin-induced vasodilation (13.0 ± 0.4% versus 13.6 ± 1.2% increase in arteriolar diameter from baseline before and after superfusion of quinupristin/dalfopristin, respectively; Table 1; each group, n = 4 animals; <i>P</i> > 0.5). Likewise, superfusion of quinupristin/dalfopristin (120 mg/L) for 1 h before and during a 7 min superfusion of VIP (0.1 nmol) or acetylcholine (10<sup>−8</sup> M) had no significant effects on agonist-induced vasodilation (VIP, 21.4 ± 1.2% versus 17.5 ± 1.4%; acetylcholine, 19.3 ± 1.8% versus 17.8 ± 0.8% increase in arteriolar diameter from baseline before and after superfusion of quinupristin/dalfopristin, respectively; Table 1; each group, n = 4 animals; <i>P</i> > 0.5). Superfusion of DMSO (0.05%), the vehicle of quinupristin/dalfopristin, for 67 min and superfusion of saline for the entire duration of the experiment had no significant effects on arteriolar diameter (Table 1; each group, n = 4 animals; <i>P</i> > 0.5).

Discussion

There are two new findings from this study. First, we found that a 1 h superfusion of quinupristin/dalfopristin, at a concentration 12-fold higher than that achieved in human serum with conventional dosing,<sup>2</sup> on to the intact hamster cheek pouch microcirculation had no significant effects on vasomotor tone. Secondly, superfusion of quinupristin/dalfopristin had no significant effects on vasodilation evoked by bradykinin and acetylcholine, two structurally unrelated compounds that evoke endothelium-dependent, nitric oxide-mediated vasodilation, and by VIP, a neuropeptide that elicits nitric oxide-independent vasorelaxation.<sup>6–9</sup> Taken together, these data indicate that administration of a relatively high dose of quinupristin/dalfopristin does not impair the contractile apparatus of resistance arterioles in the intact peripheral circulation.

The hamster cheek pouch is an established model to elucidate mechanisms regulating vasomotor tone in the intact peripheral microcirculation.<sup>6–9</sup> To this end, successive superfusions of bradykinin, VIP and acetylcholine on the cheek pouch at appropriate time intervals are associated with reproducible vasodilation in the absence of tachyphylaxis.<sup>6–9</sup> The cheek pouch preparation is stable for at least 6 h so that the effects of these drugs on vasomotor tone can be tested repeatedly in the same animal. Hence, each animal serves as its own control, which, in turn, reduces the number of animals required per experiment and facilitates data analysis.

Quinupristin/dalfopristin had no significant effects on endothelium-dependent, nitric oxide-mediated and nitric oxide-independent vasodilation in the intact hamster cheek pouch. These findings imply that quinupristin/dalfopristin does not impair the ability of the host to combat Gram-positive infection by mounting effective vasodilation in injured tissues. Vasodilation, in turn, increases local blood flow and promotes clearance of bacteria, bacterial products and pro-inflammatory mediators. Quinupristin/dalfopristin may exert similar effects when elaboration of pro-inflammatory cytokine is up-regulated in the peripheral microcirculation during the host inflammatory response to Gram-positive bacterial infections.

In summary, we found that 1 h superfusion of quinupristin/dalfopristin at a concentration 12-fold higher than that achieved in human serum with conventional dosing does not impair the contractile apparatus of resistance arterioles in the intact peripheral circulation.
Acknowledgements

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References


