Focus on flow: imaging the human microcirculation in perioperative and intensive care medicine
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Chapter 6

Microcirculatory Imaging in Cardiac Anesthesia:
Ketanserin Reduces Blood Pressure
but not Perfused Capillary Density
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Abstract

Objectives: It has become possible to image the human microcirculation at the bedside using sidestream dark field (SDF) imaging. This may help the clinician when correlation between global and microvascular hemodynamics may not be straightforward. Ketanserin, a serotonin and $\alpha$-1 adrenoceptor antagonist, is used in some countries to treat elevated blood pressure after extracorporeal circulation. This might hamper microcirculatory perfusion. Conversely, it is also conceivable that microcirculatory flow is maintained or improved as a result of flow redistribution. In order to introduce SDF imaging in cardiac anesthesia, we set out to directly observe the sublingual microcirculation in this setting.

Design: An observational study.

Setting: A large teaching hospital.

Participants: Mechanically ventilated patients with elevated arterial blood pressure immediately after extracorporeal circulation (ECC).

Intervention: An intravenous bolus of ketanserin, 0.15 mg/kg.

Measurements and main results: Five minutes before and 10 minutes after ketanserin administration, global hemodynamic variables were recorded. In addition, we used SDF imaging to record video clips of the microcirculation. Analysis of these allowed for quantification of microvascular hemodynamics including determination of perfused vessel density (PVD) and microcirculatory flow index (MFI). After ketanserin administration, there was a significant reduction in systolic arterial blood pressure (129 (9) to 100 (15) mm Hg, $p<0.01$). At the level of the microcirculation, the mean MFI did not change significantly for small (diameter <20 µm, 2.79 [interquartile range, 1.38-3] to 2.38 [1.88-2.75], $p=0.62$) or large (diameter >20 µm, 2.83 [1.4-3] to 2.67 [0.35-2.84] $p=1.0$) vessels. There was a significant increase in mean PVD for large vessels (1.23 (0.63) to 1.70 (0.79) mm$^{-1}$, $p=0.02$) but not for small vessels (5.59 (2.60) to 5.87 (1.22) mm$^{-1}$, $p=0.72$) where red blood cell flow was maintained.

Conclusions: SDF imaging clearly showed a discrepancy between global and microvascular hemodynamics after the administration of ketanserin for elevated blood pressure after ECC. Ketanserin effectively lowers arterial blood pressure. However, capillary perfusion is maintained at a steady value. Both effects may be explained by an increase in shunting in the larger vessels of the microcirculation.
Introduction

Clinical research of the microcirculation has long been hampered by a lack of suitable techniques. However, the advent of modern imaging modalities like orthogonal polarized spectral (OPS) imaging [1, 2] and its improved successor, sidestream dark field (SDF) imaging [3], have facilitated observation of the human microcirculation at the bedside in real time. To date, these devices primarily have been used to characterize the microcirculation in sepsis. These studies have consistently shown that there may be large discrepancies between microcirculatory flow and global hemodynamics [4-7].

Interestingly, cardiac anesthesia profoundly relies on monitoring of global hemodynamics, such as arterial and venous pressures. Obviously, these parameters are of great importance. However, if similar discrepancies were to exist in this setting, these routine measurements might not reflect the state of the microcirculation. This would prevent accurate estimation or correction of capillary perfusion and transport of oxygen and nutrients to organs, tissues, and cells. Unfortunately, experience with microcirculatory imaging is limited in this field. To introduce SDF imaging in cardiac anesthesia, we designed an observational pilot study. To this end, we chose to investigate the microcirculation before and after drug treatment for elevated blood pressure after extracorporeal circulation with cardiopulmonary bypass (CPB).

As discussed later, we hypothesized that this clinical setting could show disagreement between microvascular and macrovascular measurements. Thus, it could unveil the maximum potential of the technique.

High blood pressure after CPB may give rise to life-threatening complications such as suture line disruption, aortic dissection, and myocardial ischemia [8]. It may occur in up to 60% of patients undergoing CPB [9] and calls for aggressive treatment to lower blood pressure [8]. At the same time, however, such a reduction in blood pressure must not compromise tissue perfusion to prevent shock and ischemic complications such as stroke.

By augmenting systemic vascular resistance (SVR), both an increase in circulating catecholamines [10] and serotonin (5-HT) [11] are thought to contribute to increased blood pressure in this setting. Thus, a drug blocking adrenergic and 5-HT receptors may be a logical treatment option. Indeed, in Europe, ketanserin, a quinazolinedione derivative with 5-HT-2 receptor and mild 1-α, -β, and -δ adrenoceptor antagonist activity [12], continues to be used successfully in these patients. Intravenous administration reduces systemic blood pressure while moderately increasing heart rate and cardiac output [11].

Ketanserin is generally considered to benefit microcirculatory perfusion based on the observed decrease in SVR. However, this is dependent on the exact site of action. For example, if ketanserin increases precapillary shunting by opening arteriovenous passages, tissue perfusion could actually be hampered. In contrast, if increased capillary flow was the mechanism behind the reduction in SVR, this could possibly increase oxygen and nutrient delivery to tissue. The former could cause a discrepancy between microvascular and global hemodynamic measurements. In addition, it is well known that CPB induces a systemic
inflammatory response [13] which could also contribute to such discrepancy.

For these reasons, we believe that this clinical setting is useful to introduce SDF imaging as a novel technique in cardiac anesthesia. Thus, it was our hypothesis that ketanserin would be able to lower blood pressure but not at the expense of microcirculatory perfusion.

**Methods**

The local institutional review board approved the study. The need for written informed consent was waived in accordance with the national Law on Experiments with Humans because the study was observational and measurements were considered noninvasivse. The study was performed in the intensive care unit (ICU) of our hospital between May and September 2006.

In order to be considered for inclusion, patients had to be over 18 years of age, have undergone cardiac surgery with the use of extracorporeal circulation, and be mechanically ventilated. Patients were considered for eligibility in a nonconsecutive fashion based on the
availability of microcirculatory recording equipment and its operators. Patients were only included definitively if an attending ICU physician decided that ketanserin administration was needed for blood pressure control within 2 hours of arrival in the ICU. Patients with lacerations of the oral mucosa were excluded because this would interfere with microcirculatory imaging. Additional exclusion criteria were a history of any type of diabetes or hypertension because these are known to affect the microcirculation to various extents [14, 15].

All patients underwent routine and continuous monitoring of global hemodynamics through arterial and central venous catheters as well as rectal temperature and ventilation parameters. Two patients had a pulmonary artery catheter. These data were recorded automatically every minute by a patient data-management system.

As per clinical routine, patients were given a 0.15 mg/kg bolus of ketanserin via a peripheral intravenous catheter. In our experience, this dose predictably lowers arterial blood pressure within minutes, whereas no further decline in blood pressure is seen beyond 6 to 8 minutes after drug administration.

Five minutes before and 10 minutes after drug administration, we recorded video clips of the microcirculation using SDF imaging (figure 1). This technique has been described in detail previously [3]. In brief, it consists of a handheld video microscope that emits stroboscopic green light (530 nm) from an outer ring at the end of a probe. This light is absorbed by hemoglobin. Thus, a negative image of moving red blood cells is transmitted back through the isolated optical core of the probe toward a charge-coupled device (CCD) camera. SDF imaging has been shown to provide a higher image quality with more detail and less motion blur than its predecessor OPS imaging [3]. In a recent roundtable conference, international experts reached consensus on how to best evaluate the microcirculation using OPS and SDF imaging [16]. We implemented all recommendations given in this article. Video clips were directly saved as digital AVI-DV files to a hard drive of a personal computer using an analog-to-digital converter (Canopus, Kobe, Japan) and the freeware program WinDV (http://windv.mourek.cz). We used 5x optical magnification, producing images representing approximately 940x750 µm² of tissue surface. Per time point, clips at 3 sublingual sites yielding at least 20 s of stable video per site were recorded.

Special care was taken to avoid pressure artifacts, adhering to the standard operating procedure previously described by Trzeciak et al. [4] and recommended in the roundtable conference [16]. In brief, secretions were removed with gauze, and, after obtaining good image focus, the probe was pulled back gently until contact was lost and then advanced again slowly to the point at which contact was regained. We paid special attention to the larger vessels at the time of recording because alterations in their flow with probe manipulation may indicate pressure artifacts.

One video file was recorded for each site at each time point. These were stored under a random number. At a later time, these were analyzed by one of the authors (PWGE) using the AVA 3.0 Software program (Microvision Medical, Amsterdam, The Netherlands). According to the recommendations, microvascular flow index (MFI), perfused vessel density
(PVD), proportion of perfused vessels (PPV), and indices of heterogeneity were determined for every patient at both time points. All have been validated previously [4, 17, 18]. As published recently [19], each score was determined for both large and small microvessels with a cut-off diameter of 20 µm. In addition, we defined large-type vessels that split into more large vessels as arterioles. Other large vessels were defined as venules. For PPV and PVD, vessel density was calculated as the number of vessels crossing 3 horizontal and 3 vertical equidistant lines spanning the screen divided by the total length of the lines. Perfusion at each crossing was then scored semi-quantitatively by the eye as follows: 0 - no flow (no flow present for the entire duration of the clip), 1 - intermittent flow (flow present >50% of the duration of the clip), 2 - sluggish flow (flow present >50% but <100% of the duration of the clip or very slow flow for the entire duration of the clip), and 3 - continuous flow (flow present for the entire duration of the clip). PVD was then calculated as the number of crossings with flow scores greater than 1. PPV was calculated as the proportion of crossings with flow scores greater than 1 divided by the total number of crossings. For each time point and each patient, the scores for PPV and PVD were averaged. PPV is expressed as n/mm, whereas PPV is expressed as a percentage. Intraobserver variability ranges between 2.5% and 4.7% for PVD and between 0.9% and 4.5% for PPV. The interobserver variability is slightly higher: between 3.0% and 6.2% and between 4.1% and 10%, respectively [18].

MFI was based on the determination of the predominant type of flow in 4 quadrants.
adhering to the same scoring system. MFI is the sum of these flow scores divided by the number of quadrants in which the vessel type is visible. The intraobserver agreement of MFI is about 85% (kappa score 0.78) and interobserver agreement about 90% (kappa score 0.85) [17]. For each time point and each patient, the scores for MFI were averaged.

Heterogeneity was assessed in 2 different ways. For PVD, the coefficient of variation was determined. For MFI, we assessed heterogeneity in each patient by subtracting the lowest from the highest quadrant MFI and dividing the result by the mean MFI [4, 18]. We used nonparametric tests for MFI and paired t tests for other data. Results are reported as median and interquartile ranges (IQR) for MFI and as the mean (standard deviation (SD)) for other parameters, unless indicated otherwise. The study was powered to detect a 20% difference in small vessel PVD after ketanserin administration based on the PVD and its SD in patients undergoing thoracic surgery as found by De Backer et al [20]. This showed the need for the inclusion of 6 patients.

Results

Six patients were included. Patient characteristics are shown in table 1, including their analgesic, vasoactive, and sedative drugs. One patient had an intra-arterial balloon pump in place, and one patient had a history of systemic lupus erythematosus and a kidney transplant for which she received immunosuppressants. One patient had a pacemaker functioning in AAI mode. This patient was excluded from heart rate analysis. Intravenous administration of ketanserin, 0.15 mg/kg, had a similar impact on global hemodynamic parameters in all patients. These are shown in table 2. There was no change in temperature or oxygen saturation throughout the experiment.

We were successful in obtaining high-quality images in each patient. Figure 2 shows a typi-
The standard operating procedure for acquisition and analysis of the microcirculation was strictly adhered to in order to avoid artifacts. A total of 36 SDF video clips were recorded. In only 2 of these, an arteriole could be identified. Typically, microvascular architecture consisted of tortuous capillaries and venules. The results for PVD, PPV, MFI, and indices of heterogeneity are shown in Table 3 and Figure 3.

The administration of ketanserin lowered arterial blood pressure. At the same time, we observed a significant increase in mean perfused vessel density for large vessels. However, capillary perfusion was unaltered by the administration of ketanserin. Other microcirculatory parameters including microvascular heterogeneity remained statistically unaltered.

Figure 4 depicts the relationship between changes in global hemodynamics versus changes in microvascular parameters in individual patients. Individually, there does not seem to be a clear association between the degree of reduction in systemic blood pressure and the response of the microcirculation. Again, on average, PVD in microvessels with a diameter smaller than 20 µm remained fairly stable, whereas that of microvessels with a diameter larger than 20 µm increased.

Discussion

We successfully used SDF imaging in cardiac anesthesia to study the microcirculatory effects of ketanserin when administered to correct elevated blood pressure after extracorporeal circulation. Using this novel technique, it was shown that changes in global hemody-
Ketanserin Reduces Blood Pressure but not Perfused Capillary Density

...namics were not reflected in the microcirculation. Such discrepancies previously have been described for other clinical settings, most notably in sepsis.

In the present patients, an intravenous bolus of ketanserin significantly increased mean perfused vessel density in vessels larger than 20 µm. Perhaps more importantly, ketanserin did not alter PVD, PPV, or MFI for small vessels. This implies that capillary density remained intact, suggesting that microcirculatory perfusion was not compromised even in the face of...

Figure 3. The effect of ketanserin, 0.15 mg/kg, on perfused vessel density for vessels with a diameter (top) smaller and (bottom) larger than 20 µm. Data points represent individual patients. Perfused large vessel density increased significantly (*, table 3), whereas perfused small vessel density remained statistically unaltered.
markedly different global hemodynamics. The latter followed the same pattern as described earlier by Van der Stroom et al. [8] with a slightly elevated heart rate and a reduction in arterial blood pressure.

This is the first study that reports on the state of the microcirculation in normovolemic patients after cardiac surgery using all international consensus recommendations. Bauer et al. [21] reported on functional capillary density after cardiac surgery using OPS imaging. However, they used capillary length per area as a parameter that precludes direct comparison with the present data. Using the same technique, De Backer et al. [20] reported on PVD in patients before cardiac surgery. They found a median of 1.9 (IQR 1.4-2.3) mm$^{-1}$ for large vessels and 3.8 (IQR 3.2-4.5) mm$^{-1}$ for small vessels. This is quite different from the present findings: 5.8 (IQR 4.0-7.7) for small vessels and 1.1 (IQR 0.7-1.7) for large vessels.

Focusing on small vessels, the study by De Backer et al. [6] showed median PVD values of 3.1 (IQR 2.8-3.2), 3.1 (IQR 2.9-3.3), and 1.1 (IQR 0.7-1.5) for healthy controls, nonseptic acutely ill ICU patients, and septic patients, respectively. Interestingly, Trzeciak et al. [4] also reported on PVD in septic patients and healthy volunteers but found very different numbers, 8.20 (1.9) and 10.75 (1.2) mm$^{-1}$.

Only 3 studies have reported on MFI in humans. Boerma et al. [22] used this index in their
study comparing stoma and sublingual microcirculation. In septic patients, they reported a median MFI value for small vessels of 2.08 (IQR 1.25-2.42).

Tzreciak et al. [4] also used MFI, although it is not clear for which vessel size. Their reported median MFI was 1.48 (SD 0.4) for septic patients and 2.82 (SD 0.1) for healthy controls. Den Uil et al. [23] reported the maximum value of 3 as median MFI for all vessel types after cardiac surgery. The present MFI values for small vessels are slightly lower but seem to be in the range of healthy volunteers.

Some of the observed differences in vessel density may be explained by the higher quality of SDF imaging as compared with OPS imaging. It is important to note that none of the previous studies quoted fully adhered to the recommendations of the round table conference. Therefore, differences in methodology between the present study and those by De Backer et al. [6] and Trzeciak et al. [4] may explain part of the large differences in capillary density. For example, Trzeciak et al.’s capillary density was simply a count of vessels seen, whereas De Backer et al., like us, only counted vessels that showed flowing hemoglobin. The importance of standardization in microvascular research cannot be emphasized enough. As discussed in more detail later, the remainder of the difference may be explained by the fact that we studied patients with elevated blood pressure.

Before the present study, only 1 article focused on the human microcirculatory effects of ketanserin. Based on peripheral plethysmography, Konishi et al. [24] concluded that ketanserin beneficially affects microhemodynamics and rheology. As stated earlier, the present study confirmed an increase in large vessel PPV, but capillary density remained unchanged. Animal experiments have shown that 5-HT-2 receptor–mediated constriction of arteries larger than 50 µm is blocked by ketanserin [12]. This is consistent with the observation of an increase in PVD for large vessels, almost exclusively venules.

Regrettably, the number of arterioles in the present recordings was too small to yield meaningful results. In contrast, the α1-blocking effect of ketanserin is known to dilate venous capacitance vessels [25]. Possibly, the increase in PVD for large vessels is the microcirculatory substrate for this observation. However, this is not reflected in the central venous pressure of the present patients, which changed very little after ketanserin administration.

Again, from animal experiments, it has been suggested that ketanserin may open arteriovenular shunts [26]. This is consistent with our finding that small-vessel microcirculatory parameters remained unchanged in the face of lower systemic blood pressure and an increased density of larger microcirculatory vessels. If ketanserin were truly able to open arteriovenous shunts, this means that, in the current patient group, these shunts were closed. This may have been the cause for their elevated blood pressure.

Microcirculatory imaging has been used extensively in sepsis. This led Ince [27] to introduce the concept of microvascular weak units, meaning that in sepsis these weak units feature impaired perfusion even when macrohemodynamics are normalized. The units can exist next to areas in which the microcirculation is normal. In other words, the hallmark of these microcirculatory derangements is heterogeneity. In fact, the distributive shock
seen in sepsis and systemic inflammation may be classified according to microcirculatory observations [28].

This may warrant treating the microcirculation as an organ that may be in need of resuscitation in certain clinical settings [5]. It is feasible that the concept of microcirculatory weak units is also applicable after CPB because this is known to induce a systemic inflammatory response state associated with hypermetabolism and an increase in oxygen consumption. Oudemans-Van Straaten et al. [29] showed that ketanserin decreases endotoxin levels and attenuates the increased VO$_2$ in this setting. However, this could be a reflection of ketanserin compromising the microcirculation by shunting blood away from it, thus preventing cells from using the oxygen. A decreased PPV and PVD and/or an increased heterogeneity of the microcirculation would be consistent with this idea. However, the present study was not able to identify changes in these parameters by ketanserin administration.

The most important finding is that changes in global hemodynamic parameters do not necessarily reflect alterations in the microcirculation and vice versa. This is also a consistent finding in microcirculatory sepsis and systemic inflammatory response state research. It may therefore be prudent to directly visualize this vital organ when trying to optimize perfusion in clinical medicine. Figure 4 is supportive of this statement in the clinical setting we studied.

A limitation of the present study may be that the sample size is small. However, because of its design in which patients function as their own controls, power analysis showed that the sample was adequate to address the specific aims of this study. Theoretically, the problem of sample size would be more severe for the analysis of larger vessels. Since large vessels are less abundant than smaller ones, some may be missed because of the small sampling area of the SDF device. That is why the consensus on microcirculatory measurements recommends a minimum of 3 sites to be evaluated. Still, the low number of subjects may hinder comparison with microcirculatory data from other studies. On the other hand, we found a statistically significant and clinically relevant result for PVD for larger microvessels.

The sublingual area was chosen for microcirculatory assessment for 3 reasons. First, it is not affected by changes in peripheral temperature. Second, it is close to the brain, which is of primary concern from a perfusion standpoint. Third, it is easily accessible. It should be noted that sublingual microvascular perfusion does not always correlate with other microcirculatory beds. Boerma et al. [22] showed this difference comparing sublingual and stoma microcirculation beds.

Further study is needed to assess the microcirculatory effects of other commonly used agents to treat elevated blood pressure such as nitroglycerin, nitroprusside, and nicardipine. Differences in microvascular response may have implication for future drug selection. In addition, our technique now allows for investigation of microvascular effects of other vasoactive agents commonly used in cardiac anesthesia.
Conclusion

We successfully have used SDF imaging in the clinical setting of cardiac anesthesia to assess the effects of ketanserin on the microcirculation. This study adds to the growing body of evidence that changes in global circulatory parameters are poor predictors of microcirculatory changes.

Ketanserin, when given for elevated blood pressure after extracorporeal circulation, reduces systemic blood pressure while increasing microvascular perfused vessel density for vessels with a diameter larger than 20 µm. All other indices of perfusion including those for smaller microvessels remained unaltered.

This suggests that increased arteriovenous shunting is the mechanism of the known reduction in systemic vascular resistance by ketanserin. The fact that indices of capillary perfusion did not change significantly, despite the low range of blood pressures after ketanserin administration, may indicate that ketanserin does not impair oxygen and nutrient delivery in this clinical setting while effectively reducing blood pressure.

References

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