ANESTHESIOLOGY

Heart Rate Control during Experimental Sepsis in Mice

Comparison of Ivabradine and β-Blockers

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ABSTRACT

Background: Tachycardia is a hallmark of sepsis. An elevated heart rate could impair ventricular filling and increase myocardial oxygen demand. β-Blockers and ivabradine (a selective inhibitor of I*f* channels in the sinoatrial node) are both able to control sinus tachycardia, with the latter drug being devoid of negative inotropic effect. This work aimed at assessing the hemodynamic effects of ivabradine as compared with a β-blocker (atenolol) during murine peritonitis.

Methods: Ivabradine (3 μg/g), atenolol (3 μg/g), or placebo was administered intraperitoneally 2 h after induction of peritonitis (cecal ligation and puncture) in male C57BL6 mice. The authors used invasive (left ventricular catheterization) and noninvasive (transthoracic echocardiography) monitoring to assess hemodynamics 20 h after surgery, including heart rate, blood pressure, left ventricular systolic, and diastolic function (n = 10 mice/group). The authors also assessed overall mortality 30 and 60 h after surgery in a distinct subset of animals (n = 20 mice/group). Descriptive data are presented as median (25th to 75th percentile).

Results: As compared with placebo (601 beats/min [547 to 612]), ivabradine (447 beats/min [430 to 496]) and atenolol (482 beats/min [412 to 505]) blunted sepsis-induced tachycardia assessed by transthoracic echocardiography in awake animals ($P < 0.001$ and $P = 0.004$, respectively). Unlike ivabradine, atenolol reduced cardiac output, systolic blood pressure, and left ventricular systolic function (as assessed by ejection fraction, maximal left ventricular pressure rise, and anterior wall strain rate) as compared with septic mice receiving placebo. There was no difference in survival 60 h after sepsis induction with ivabradine (6 of 20, 30%) or atenolol (7 of 20, 35%), as compared with placebo (5 of 20, 25%; $P = 0.224$).

Conclusions: Heart rate control could be similarly achieved by ivabradine or atenolol, with preservation of blood pressure, cardiac output, and left ventricular systolic function with the former drug.

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Tachycardia is known to occur with sepsis and can result in decreased ventricular filling and increased myocardial oxygen demand.
- β-Blockers or ivabradine (a selective inhibitor of I*f* channels in the sinoatrial node) are drugs that can be used to treat tachycardia in the setting of sepsis. However, because of its selective inhibition in the sinoatrial node, ivabradine should not suppress myocardial contractility in the same manner as β-blockers.

What This Article Tells Us That Is New

- This study assesses the effects of ivabradine, atenolol, and placebo in the setting of murine peritonitis. Mice that received atenolol *versus* ivabradine both experienced a similar and significant decline in heart rate. The mice in the atenolol group also experienced a significant decrease in cardiac output, systolic blood pressure, and left ventricular systolic function that was not experienced by the mice who received ivabradine.
- Mice who received atenolol *versus* ivabradine *versus* placebo did not have significantly different survival 60 h after induction of sepsis. Future studies are needed to determine the value of ivabradine *versus* atenolol for heart rate control in human sepsis.

Sepsis remains one of the main causes of death in intensive care units.[1,2] Several treatment modalities aimed at correcting one or more of the underlying derangements of sepsis have led to disappointing results.[3,4] Tachycardia is a hallmark of sepsis, with multiple potential determinants, including fever, hypovolemia, sympathetic tone, and/or exogenous catecholamines. Although the heart rate response to sepsis may be an adaptive reaction to maintain oxygen delivery, tachycardia was also reported as an independent risk factor for mortality during severe sepsis and septic shock, regardless of body temperature.[5–7] An elevated heart rate may impair left ventricular diastolic filling (with a reduction in stroke volume), compromise coronary blood flow, and increase myocardial oxygen demand.

Recent studies showed that β-blockers are effective to control heart rate during septic shock, with beneficial effects on hemodynamics and prognosis.[8–10] However, the

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negative inotropic and hypotensive effects of β-blockers may make their clinical routine use difficult during septic shock, especially in the subgroup of patients with left ventricular systolic dysfunction, a condition that may require the use of β-adrenergic agents.[11]

Unlike β-blockers, ivabradine (a selective inhibitor of I*f* channels in the sinoatrial node) is a pure heart rate–lowering agent devoid of negative effects on inotropic function and arterial load.[12–14] Ivabradine may also have positive effects on endothelial function, microvascular perfusion, and inflammation.[15,16] In addition, ivabradine has antiischemic effects and improves clinical outcomes in patients with chronic left ventricular systolic dysfunction and tachycardia.[17] For these reasons, ivabradine could be interesting in the setting of septic shock to blunt tachycardia, without the negative effects of β-blockade on hemodynamics. This work aimed at assessing the hemodynamic effects of ivabradine as compared with a β-blocker (atenolol) during murine peritonitis. We hypothesized that ivabradine may blunt sepsis-induced tachycardia without detrimental effects on cardiac function.

Materials and Methods

The protocol was reviewed and approved by the local animal care committee (ComEth Anses/National Veterinary School of Alfort/University Paris East Creteil, registration No. 10/02/15-2) to ensure that the procedures were appropriate and humane. All experiments were performed in the same location (small animal experiment lab).

Induction of Sepsis with Cecal Ligation and Puncture

Cecal ligation and puncture is a widely used model in animal research and is considered a gold standard to study experimental sepsis.[18] The animals used were male C57BL6 mice (Janvier Labs, France) aged 10 weeks and weighed approximately 21 to 26 g. A single sex was chosen for homogeneity. After general anesthesia with isoflurane, the cecum was ligated below the ileocecal valve through a 1-cm abdominal midline incision and subjected to a single "through-and-through" perforation (20-gauge needle).[19] Then the abdominal incision was closed in layers. Sham-operated mice underwent the same surgical procedure except for ligation and perforation of the cecum. Food and water were provided *ad libitum*. Pain medication (tramadol, 10 μg/g) was administered after the surgery (sham or cecal ligation and puncture) and every 12 h thereafter. Volume support (0.9% NaCl, 0.05 μg/g) was administered subcutaneously twice daily, immediately after the induction of sepsis.

Hemodynamic Explorations

The mice were randomly assigned to four groups: sham-operated mice and those undergoing cecal ligation and puncture receiving placebo, ivabradine (3 μg/g per 12 h; Servier, France),[20] or atenolol (3 μg/g per 12 h; AstraZeneca, France), intraperitoneally starting 2 h after cecal ligation and puncture and repeated every 12 h. Intraperitoneal injection was preferred to intravenous infusion, because the latter would have required a continuous sedation with potential hemodynamic effects. The randomization list of cecal ligation and puncture mice involved the replacement of animals who died before hemodynamic assessment, to achieve the target number of 10 mice/group.

Noninvasive (transthoracic echocardiography) and invasive (left ventricular catheterization) hemodynamic explorations were performed in two distinct groups of animals (and different from those used for survival analysis), 20 h after the surgery (*i.e.*, 6 h after the second drug dose).[21] The operators were blinded from knowledge of group assignment when assessing hemodynamic results. Transthoracic echocardiography (without anesthesia) was performed with a 13-MHz linear-array transducer (Vivid 7, GE Medical Systems) for the following measurements: heart rate, left ventricular wall thicknesses, dimensions, fractional shortening, and radial peak systolic strain rate of the anterior wall (obtained from the parasternal short-axis view at the mid-ventricular level). Stroke volume, cardiac output, and ejection fraction were calculated using the estimation of ventricular volumes with the Teicholz formula. Temperature control (servo-controlled homeothermic blanket) and general anesthesia (isoflurane) were used during left ventricular catheterization to preserve cardiovascular homeostasis, especially heart rate. Left ventricular catheterization was performed with a 1.4-Fr conductance microcatheter (Millar Instruments, USA) introduced *via* the right carotid artery into the aorta to measure heart rate and aortic pressure and then into the left ventricle to measure the pic rate of pressure rise and decline.[22] After hemodynamic measurements, the animals were sacrificed by cervical dislocation.

Survival Analysis

In a different group of cecal ligation and puncture mice randomly assigned to receive placebo, ivabradine, or atenolol, survival was assessed 30 and 60 h after the surgery. Distinct animals were therefore used for survival, left ventricular catheterization, and transthoracic echocardiography.

Endpoints and Number of Animals

The primary outcome was the measurement of heart rate assessed by transthoracic echocardiography and left ventricular catheterization. Secondary endpoints included overall mortality 30 and 60 h after the surgery, systolic arterial pressure, stroke volume, cardiac output, and indices of diastolic (developed pressure first maximal negative derivative) and systolic (fractional shortening, ejection fraction, developed pressure first maximal positive derivative, strain rate of anterior wall) cardiac function assessed by transthoracic echocardiography and/or left ventricular catheterization. Outlier values were evaluated, but no action was necessary. We did not assess power calculation for hemodynamic endpoints. The number of animals needed for the survival study was calculated based on a predicted decline in mortality (60 h

after surgery) from 100% in the placebo group[19] to 50% in the ivabradine group. With an overall α risk of 5% (0.0125 for *post hoc* comparisons) and a β risk of 10%, the survival study required at least 15 mice/group. Separate sets of animals were used for survival studies (n = 60), invasive (n = 46) and noninvasive hemodynamics (n = 42; where n refers to the number of animals).

Statistical Analysis

The data were analyzed using the SPSS Base 18.0 statistical software package (SPSS Inc., USA). Normality of continuous data was assessed with the Kolmogorov–Smirnov test. Because not all data sets were normally distributed, we used the median (1st quartile to 3rd quartile) for descriptive statistics. Independent samples were compared using the Kruskal–Wallis test with correction for multiple testing by the Bonferroni test. Categorical variables were compared using the chi-square test or Fisher exact test, as appropriate. Survival data were also analyzed with standard Kaplan–Meier actuarial techniques for estimation of survival probabilities. The animals who survived after 60 h were right-censored. Mortality was examined with an animal as the unit of analysis. Two-tailed *P* values smaller than 0.05 were considered significant.

Results

Heart Rate Control

Eight animals did not survive the surgery: six in the left ventricular catheterization group and two in the transthoracic echocardiography group. They were excluded from the analysis (fig. 1). Hemodynamic parameters assessed by transthoracic echocardiography (without anesthesia) and left ventricular catheterization (under general anesthesia) are reported in table 1. Septic mice exhibited the highest values of heart rate (echocardiography: 601 beats/min [547 to 612]; left ventricular catheterization: 468 beats/min [459 to 490]). Both ivabradine (echocardiography: 447 beats/min [430 to 496]; left ventricular catheterization: 376 beats/min [356 to 403]) and atenolol (echocardiography: 482 beats/min [412 to 505]; left ventricular catheterization: 372 beats/min [322 to 399]) similarly blunted sepsis-induced

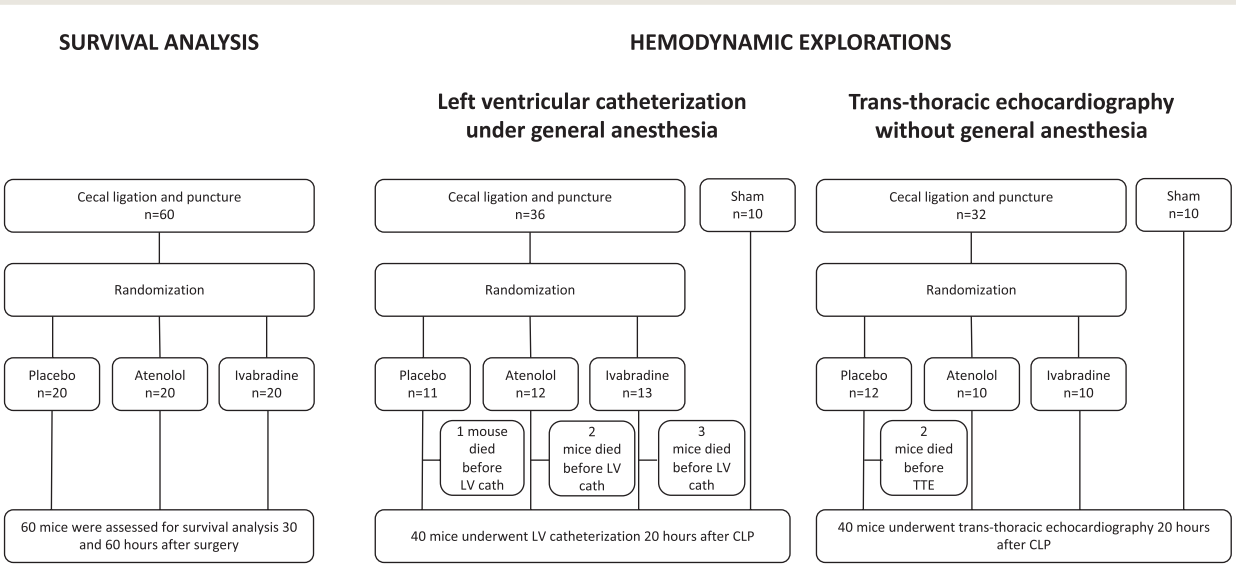

Fig. 1. Study work flow. A total of 148 mice were allocated to survival analysis (n = 60), invasive hemodynamic monitoring by left ventricular catheterization under general anesthesia (n = 46), or noninvasive hemodynamic monitoring by transthoracic echocardiography (TTE) without general anesthesia (n = 42). Distinct animals were therefore used for survival, left ventricular catheterization, and TTE. Survival analysis was assessed 30 and 60 h after cecal ligation and puncture in all 60 mice dedicated to survival analysis and randomly assigned to receive placebo (n = 20), atenolol (n = 20), or ivabradine (n = 20). Left ventricular catheterization and TTE were assessed 20 h after sham operation or cecal ligation and puncture, with randomization to receive placebo, atenolol, or ivabradine in case of cecal ligation and puncture. The randomization list of cecal ligation and puncture mice involved the replacement of animals who died before hemodynamic assessment, to achieve the target number of 10 mice/group). Among 46 mice dedicated to invasive hemodynamic monitoring, 6 died before left ventricular catheterization (including 1 with cecal ligation and puncture receiving placebo, 2 with cecal ligation and puncture receiving atenolol, and 3 with cecal ligation and puncture receiving ivabradine) and could not be explored, whereas 40 mice underwent left ventricular catheterization, including10 sham-operated mice and 30 cecal ligation and puncture mice receiving placebo (n = 10), atenolol (n = 10), or ivabradine (n = 10). Among 42 mice dedicated to noninvasive hemodynamic monitoring, 2 with cecal ligation and puncture receiving placebo died before TTE and could not be explored, whereas 40 mice underwent TTE, including 10 sham-operated mice and 30 cecal ligation and puncture mice receiving placebo (n = 10), atenolol (n = 10), or ivabradine (n = 10). cath, catheterization; CLP, cecal ligation and puncture; LV, left ventricular.

Table 1. Hemodynamic Parameters Assessed by Transthoracic Echocardiography and LV Catheterization

	Cecal Ligation and Puncture				
	Sham-operated (n = 10)	Placebo (n = 10)	Atenolol (n = 10)	Ivabradine (n = 10)	*P* Value*
Transthoracic echocardiography					
Heart rate, beats/min	527 (502–557)	601 (547–612)	482 (412–505)†	447 (430–496)†	< 0.001
Septal wall thickness, mm	0.79 (0.78–0.80)	0.81 (0.79–0.82)	0.80 (0.78–0.82)	0.80 (0.78–0.82)	0.532
Posterior wall thickness, mm	0.74 (0.71–0.77)	0.72 (0.71–0.78)	0.76 (0.67–0.80)	0.75 (0.72–0.78)	0.995
LV end-diastolic diameter, mm	2.94 (2.81–3.01)	3.08 (2.97–3.27)	3.13 (3.01–3.48)	3.04 (2.74–3.23)	0.138
LV end-systolic diameter, mm	1.75 (1.67–1.98)	1.80 (1.66–1.97)	2.21 (2.02–2.43)†‡	1.75 (1.66–2.00)§	0.001
LV diastolic volume, µl	77 (68–86)	89 (79–105)	93 (83–126)	85 (63–102)	0.138
LV stroke volume, µl	57 (52–65)	70 (64–84)	61 (53–69)	68 (47–78)	0.211
Cardiac output, ml/min	30 (27–35)	41 (36–52)	28 (22–34)†	31 (24–34)	0.031
LV fractional shortening, %	38 (34–43)	42 (40–44)	32 (24–33)†‡	39 (38–42)§	< 0.001
LV ejection fraction, %	75 (70–80)	79 (78–81)	67 (54–68)†‡	76 (74–79)§	< 0.001
Anterior wall strain rate, s^{-1}	24 (23–25)	22 (19–24)	13 (7–18)†‡	22 (20–25)§	< 0.001
LV catheterization					
Heart rate, beats/min	438 (420–466)	468 (459–490)	372 (322–399)†‡	376 (356–403)†‡	< 0.001
Systolic blood pressure, mmHg	105 (98–110)	101 (95–103)	93 (91–98)‡	102 (100–108)§	0.021
LV dP/dT_{max}, mmHg/s	6,973 (6,234–7,276)	8,429 (8,230–8,689)‡	5,070 (4,877–5,677)†	6,813 (6,238–7,990)§	< 0.001
LV dP/dT_{min}, mmHg/s	5,521 (4,980–6,200)	7,666 (7,337–7,805)‡	4,276 (3,712–5,389)†	5,941 (5,443–6,293)†	< 0.001

The data are presented as medians (1st quartile to 3rd quartile).

*Kruskal–Wallis test. †Bonferroni corrected *P* value < 0.05 *versus* placebo. ‡Bonferroni corrected *P* value < 0.05 *versus* sham. §Bonferroni corrected *P* value < 0.05 *versus* atenolol. dP/dT_{max}, developed pressure first maximal positive derivative; dP/dT_{min}, developed pressure first maximal negative derivative; LV, left ventricle.

tachycardia, whatever the hemodynamic tool used to record heart rate (table 1, fig. 2).

Cardiac Output, Left Ventricular Function, and Systolic Blood Pressure

All hemodynamic measurements were fulfilled on the next day after the induction of sepsis (20 h after cecal ligation and puncture procedure). Systolic blood pressure was similar between sham-operated and septic mice receiving placebo (sham: 105 mmHg [98 to 110] *vs.* cecal ligation and puncture plus placebo: 101 mmHg [95 to 103]; $P > 0.999$). Cardiac output and left ventricular systolic parameters (ejection fraction, anterior wall strain rate) were also comparable between these groups, except for a significantly increased developed pressure first maximal positive derivative in septic mice receiving placebo (sham: 6,973 mmHg/s [6,234 to 7,276] *vs.* cecal ligation and puncture plus placebo: 8,429 mmHg/s [8,230 to 8,689]; $P = 0.040$).

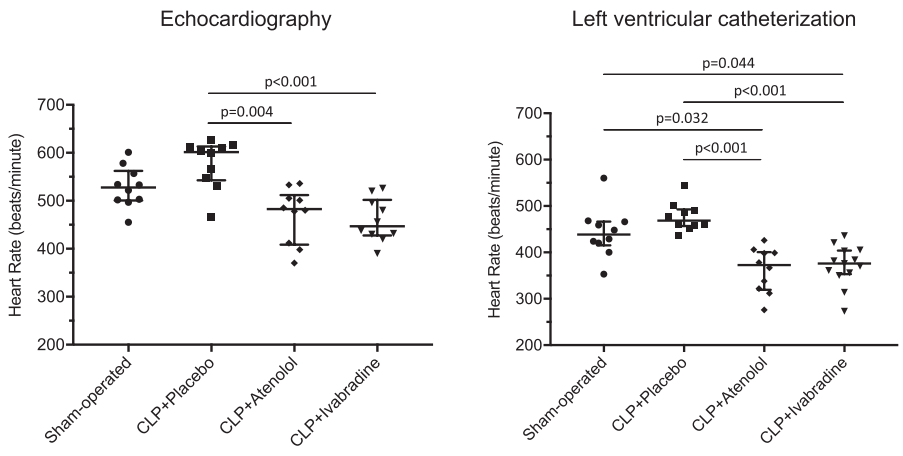

Fig. 2. Heart rate assessed by transthoracic echocardiography and left ventricular catheterization. *p* = *P* value for Kruskal–Wallis test with correction for multiple testing by the Bonferroni test. CLP, cecal ligation and puncture.

In septic mice, atenolol reduced cardiac output (cecal ligation and puncture plus atenolol: 28 ml/min [22 to 34] *vs.* cecal ligation and puncture plus placebo: 41 ml/min [36 to 52]; $P = 0.040$), left ventricular ejection fraction (cecal ligation and puncture plus atenolol: 67% [54 to 68] *vs.* cecal ligation and puncture plus placebo: 79% [78 to 81]; $P < 0.001$), developed pressure first maximal positive derivative (cecal ligation and puncture plus atenolol: 5,070 mmHg/s [4,877 to 5,677] *vs.* cecal ligation and puncture plus placebo: 8,429 [8,230 to 8,689]; $P < 0.001$), developed pressure first maximal negative derivative (cecal ligation and puncture plus atenolol: 4,276 mmHg/s [3,712 to 5,389] *vs.* cecal ligation and puncture plus placebo: 7,666 mmHg/s [7,337 to 7,805]; $P < 0.001$), and anterior wall strain rate (cecal ligation and puncture plus atenolol: 13 s^{-1} [7 to 18] *vs.* cecal ligation and puncture plus placebo: 22 s^{-1} [19 to 24]; $P = 0.015$), as compared with placebo (table 1, fig. 3). Ivabradine was not associated with any modification of these parameters, as compared with placebo.

As compared with atenolol, ivabradine improved systolic blood pressure (cecal ligation and puncture plus ivabradine: 102 mmHg [100 to 108] *vs.* cecal ligation and puncture plus atenolol: 93 mmHg [91 to 98]; $P = 0.034$) and left ventricular ejection fraction (cecal ligation and puncture plus ivabradine: 76% [74 to 79] *vs.* cecal ligation and puncture plus plus atenolol: 67% [54 to 68]; $P = 0.004$) and developed pressure first maximal positive derivative (cecal ligation and puncture plus ivabradine: 6,813 mmHg/s [6,238 to 7,990] *vs.* cecal ligation and puncture plus atenolol: 5,070 mmHg/s [4,877 to 5,677]; $P = 0.026$) and anterior wall strain rate

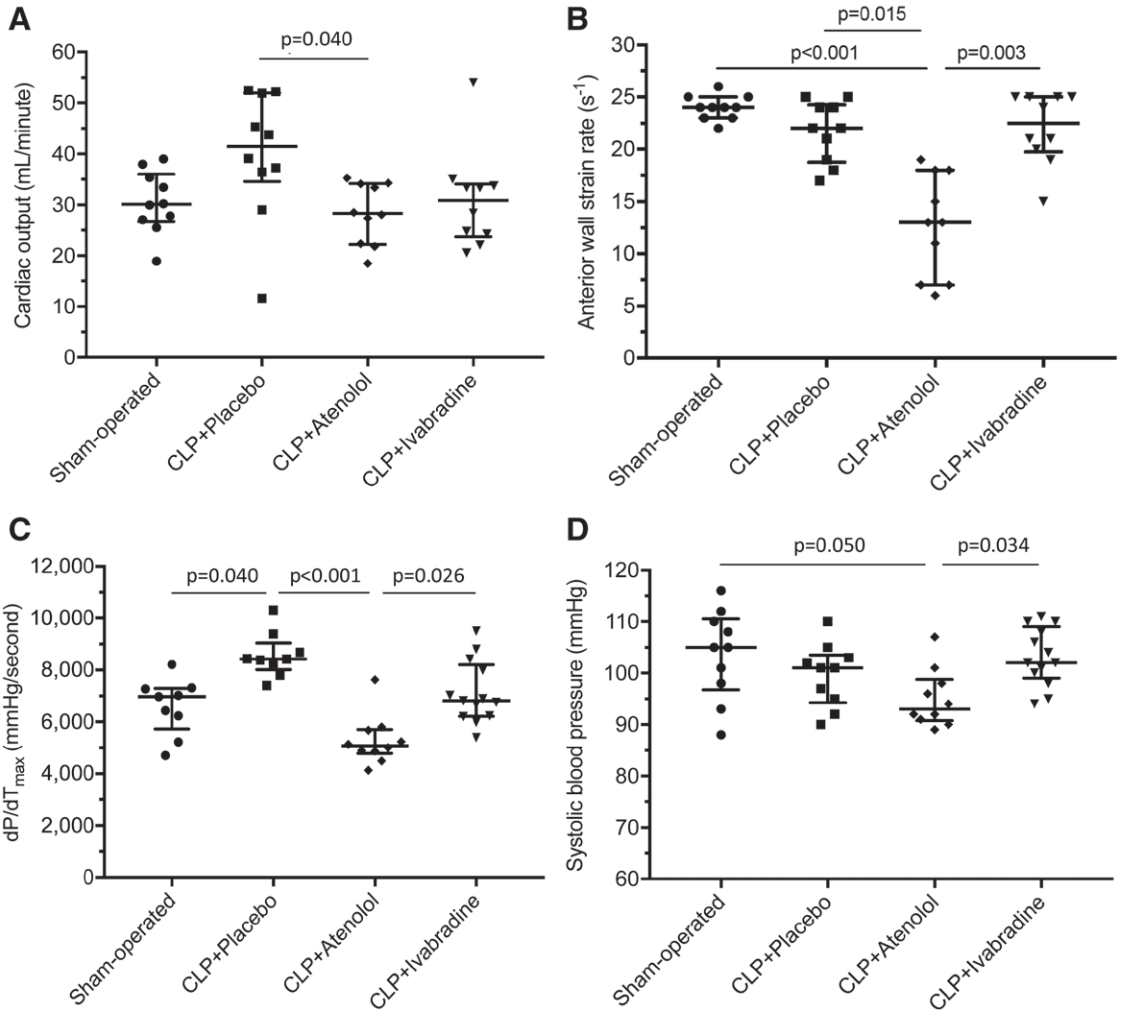

Fig. 3. Hemodynamic parameters. Cardiac output was assessed by transthoracic echocardiography (*A*); anterior wall strain rate assessed by transthoracic echocardiography (*B*); maximum first derivative of left ventricular pressure (dP/dT_{max}) assessed by left ventricular catheterization (*C*); and systolic blood pressure assessed by left ventricular catheterization (*D*). *p* = *P* value for Kruskal–Wallis test with correction for multiple testing by the Bonferroni test. CLP, cecal ligation and puncture.

(cecal ligation and puncture plus ivabradine: 22 s^{-1} [19 to 24] *vs.* cecal ligation and puncture plus atenolol: 13 s^{-1} [7 to 18]; $P = 0.003$).

Outcomes

In septic mice, mortality 30 h after surgery was reduced with the use of atenolol or ivabradine as compared with placebo (3 of 20, 15% *vs.* 5 of 20, 25% *vs.* 10 of 20, 50%; $P = 0.048$), but this result did not persist long-term (13 of 20, 65% *vs.* 14 of 20, 70% *vs.* 5 of 20, 75% at 60 h; $P = 0.224$; fig. 4).

Discussion

We herein report the comparable efficacy of ivabradine and atenolol to blunt tachycardia in a murine model of sepsis. Blood pressure and most indices of cardiac function were preserved with ivabradine but not with atenolol in septic mice. Neither drug could significantly alter overall mortality in this model.

Tachycardia and Its Control

During human sepsis, tachycardia was reported as an independent predictor of mortality.[6,7] Several studies questioned the hypothesis that some septic patients had inappropriate activation of the sympathetic tone leading to excessive tachycardia.[23] This phenomenon could alter the left ventricular diastolic function and the tolerance of vasopressor support of these patients. Recent experimental studies have suggested a beneficial effect of β-blockade in septic shock, with a control of heart rate, an increase in stroke volume, and an enhancement of cardiac efficiency.[10] We herein confirm the efficacy of βblockers in blunting sepsis-induced tachycardia, with ivabradine displaying a comparable effect. Heart rates were lower when measured with left ventricular catheterization as compared with transthoracic echocardiography, probably because general anesthesia with isoflurane was used for the former technique. In contrast to previous studies,[10,24] we witnessed significant alterations of blood pressure and cardiac function in septic animals receiving atenolol as compared with those treated with placebo or ivabradine. The model used and the type of β-blocker may explain these discrepancies. Indeed, our model of sepsis did not seem alter left ventricular systolic function or cardiac output *per se*, unlike former studies testing β-blockers during lipopolysaccharide infusion or intraperitoneal injection of fecal slurry.[10,24] Another study even found an improvement in cardiac output with esmolol in septic rats, but hemodynamic explorations were performed *ex vivo*, and septic animals had a decreased cardiac output compared with controls,[25] which was not the case in our study. Data interpretation should be cautious considering these discrepancies between murine models of sepsis. Loading conditions, which are poorly controlled in animal models, may heavily influence systolic function during sepsis.[26] Further *in vivo* research should be conducted to better scrutinize the effect of cardiotropic drugs both on cardiac and peripheral vascular function during sepsis.

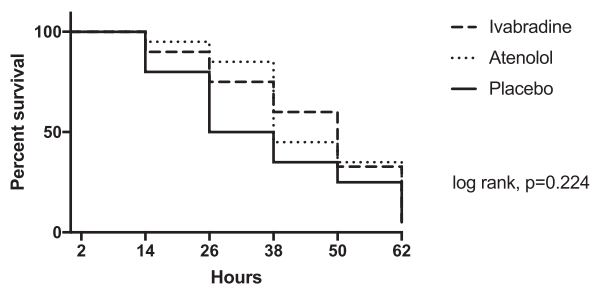

Fig. 4. Kaplan–Meier survival curve of 60 mice, 60 h after sepsis induction by cecal ligation and puncture and treatment with placebo (n = 20), atenolol (n = 20), or ivabradine (n = 20).

Cardiac Function

Ivabradine is a selective bradycardic agent devoid of negative effects on cardiac function even in patients with markedly depressed left ventricular function.[27] Several studies have demonstrated that ivabradine effectively reduces heart rate while preserving myocardial contractility, cardiac output, and blood pressure.[21] In addition, ivabradine could help prevent the unwanted chronotropic effects of inotropic therapy.[28] Our study demonstrates that ivabradine has no significant deleterious consequences on blood pressure, cardiac output, or cardiac function in the specific setting of murine sepsis. A recent experimental study using a similar model of cecal ligation and puncture applied to rats receiving ivabradine or placebo (without β-blockade comparison) found similar results on cardiac function.[29] In this work by Wei *et al.*,[29] ivabradine reduced heart rate during sepsis (cecal ligation and puncture: 425 beats/min [402 to 467] *vs.* cecal ligation and puncture plus ivabradine: 343 beats/min [269 to 368]; $P = 0.0001$), a magnitude similar to our work (approximately 20%). Ivabradine had no effect on mean arterial pressure or cardiac output, assessed by echocardiography. In addition, the authors also assessed vascular function by studying vasoreactivity to phenylephrine and acetylcholine of thoracic aorta and small mesenteric artery rings. Vascular responsiveness was not different with ivabradine, as compared with placebo. The inflammatory response to sepsis (plasma levels of some cytokines, such as tumor necrosis factor-α and interleukin-6) was not modified with ivabradine administration. This experimental study and ours jointly demonstrate the ability of ivabradine to reduce sepsis-induced tachycardia without adverse effects on cardiac and vascular function. They may also suggest its usefulness during sepsis with major hemodynamic instability or in case of septic myocardial dysfunction.[26] Further investigations are needed to scrutinize the hemodynamic effects of β-blockers and ivabradine in the subgroup of patients with

septic systolic dysfunction, with an emphasis on patients requiring inotropic therapy. Because diastolic dysfunction is frequent during sepsis and associated with higher mortality,[30] a full assessment of diastolic function is also needed to analyze the consequences of reducing heart rate on left ventricular relaxation and filling.

Mortality

A recent clinical trial suggested that β-blocker–driven heart rate control could improve mortality and organ failures in human sepsis.[8] However, it remains unclear how β-blockade may improve outcome, because this class of medication had pleiotropic hemodynamic and metabolic effects.[31,32] β-Blockers may mitigate noncardiac effects of excessive adrenergic stress, including insulin resistance, thrombogenicity, and immunosuppression. In our model of murine sepsis with cecal ligation and puncture, we were unable to demonstrate a significant impact of heart rate control on outcome, whatever the drug used. The limited sample size and the marked severity of our model (with a mortality rate of 95% in septic mice receiving placebo) may have overwhelmed any effect of the drugs on mortality.

Strengths and Limitations

Our study compared the hemodynamic effects of β-blockers and ivabradine during experimental sepsis. We used invasive (left ventricular catheterization) and noninvasive (transthoracic echocardiography) hemodynamic tools and found consistent results with both methods. Transthoracic echocardiography included advanced imaging techniques (strain rate imaging) and was performed without anesthesia to avoid the potential hemodynamic effects of isoflurane.[33] Our results add some knowledge to the field and may contribute to replacement, refinement, or reduction of animal subjects in future research. Our study has several limitations. First, we did not assess the dose response of tested drugs and used only male mice. Our findings will need to be validated in female mice. Second, although heart rate control may have major effects on diastolic filling time, the assessment of diastolic function was not comprehensive. Third, this study only focused on the cardiac effects of heart rate control. Further studies should be conducted to compare the pleiotropic effects of the tested drugs on microvascular perfusion, endothelial function, and markers of inflammation. Last, although cecal ligation and puncture is a clinically closed model of sepsis, the extrapolation of our results to the clinical scenario is questionable given the complexity of human sepsis.

Conclusions

Ivabradine and atenolol were similarly effective in blunting tachycardia in a murine model of sepsis. Atenolol (but not ivabradine) altered blood pressure, cardiac output, and left ventricular systolic function in septic mice. These findings may suggest a role for ivabradine for heart rate control during sepsis. Further studies are needed to assess whether ivabradine could be useful in controlling heart rate during human sepsis, especially in case of severe hemodynamic instability or septic cardiomyopathy.

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Competing Interests

The authors declare no competing interests.

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References

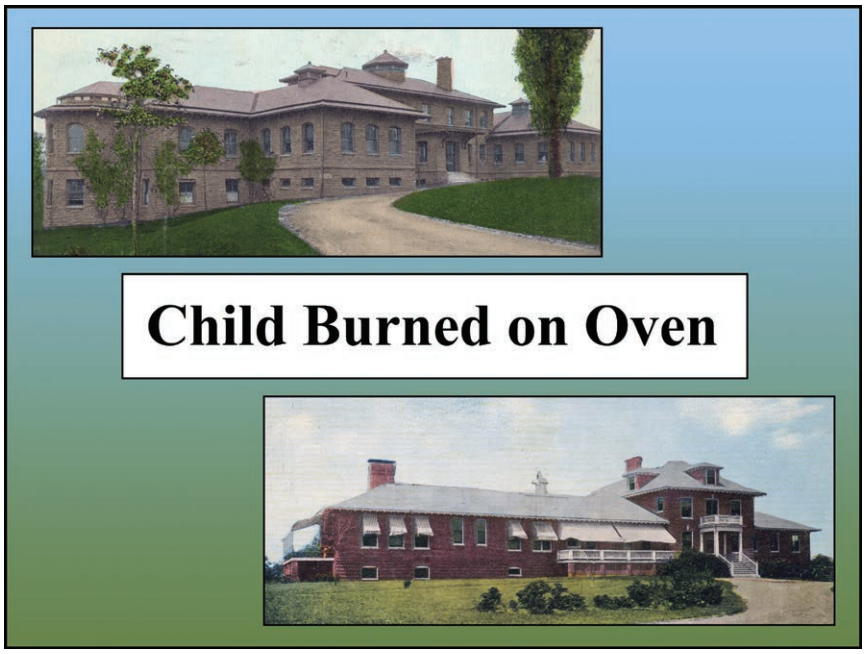
1. Martin GS, Mannino DM, Eaton S, Moss M: The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003; 348:1546–54
2. Annane D, Aegerter P, Jars-Guincestre MC, Guidet B; CUB-Réa Network: Current epidemiology of septic shock: The CUB–Réa Network. Am J Respir Crit Care Med 2003; 168:165–72
3. Werdan K, Pilz G, Bujdoso O, Fraunberger P, Neeser G, Schmieder RE, Viell B, Marget W, Seewald M, Walger P, Stuttmann R, Speichermann N, Peckelsen C, Kurowski V, Osterhues HH, Verner L, Neumann R, Müller-Werdan U; Score-Based Immunoglobulin Therapy of Sepsis (SBITS) Study Group: Score-based immunoglobulin G therapy of patients with sepsis: The SBITS study. Crit Care Med 2007; 35:2693–701
4. Ranieri VM, Thompson BT, Barie PS, Dhainaut JF, Douglas IS, Finfer S, Gårdlund B, Marshall JC, Rhodes A, Artigas A, Payen D, Tenhunen J, Al-Khalidi HR, Thompson V, Janes J, Macias WL, Vangerow B, Williams MD; PROWESS-SHOCK Study Group: Drotrecogin alfa (activated) in adults with septic shock. N Engl J Med 2012; 366:2055–64
5. Parker MM, Shelhamer JH, Natanson C, Alling DW, Parrillo JE: Serial cardiovascular variables in survivors

and nonsurvivors of human septic shock: Heart rate as an early predictor of prognosis. Crit Care Med 1987; 15:923–9
6. Leibovici L, Gafter-Gvili A, Paul M, Almanasreh N, Tacconelli E, Andreassen S, Nielsen AD, Frank U, Cauda R; TREAT Study Group: Relative tachycardia in patients with sepsis: An independent risk factor for mortality. QJM 2007; 100:629–34
7. Hoke RS, Müller-Werdan U, Lautenschläger C, Werdan K, Ebelt H: Heart rate as an independent risk factor in patients with multiple organ dysfunction: A prospective, observational study. Clin Res Cardiol 2012; 101:139–47
8. Morelli A, Ertmer C, Westphal M, Rehberg S, Kampmeier T, Ligges S, Orecchioni A, D'Egidio A, D'Ippoliti F, Raffone C, Venditti M, Guarracino F, Girardis M, Tritapepe L, Pietropaoli P, Mebazaa A, Singer M: Effect of heart rate control with esmolol on hemodynamic and clinical outcomes in patients with septic shock: A randomized clinical trial. JAMA 2013; 310:1683–91
9. Morelli A, Singer M, Ranieri VM, D'Egidio A, Mascia L, Orecchioni A, Piscioneri F, Guarracino F, Greco E, Peruzzi M, Biondi-Zoccai G, Frati G, Romano SM: Heart rate reduction with esmolol is associated with improved arterial elastance in patients with septic shock: A prospective observational study. Intensive Care Med 2016; 42:1528–34
10. Aboab J, Sebille V, Jourdain M, Mangalaboyi J, Gharbi M, Mansart A, Annane D: Effects of esmolol on systemic and pulmonary hemodynamics and on oxygenation in pigs with hypodynamic endotoxin shock. Intensive Care Med 2011; 37:1344–51
11. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP: Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med 2017; 43:304–77
12. Manz M, Reuter M, Lauck G, Omran H, Jung W: A single intravenous dose of ivabradine, a novel I(f) inhibitor, lowers heart rate but does not depress left ventricular function in patients with left ventricular dysfunction. Cardiology 2003; 100:149–55
13. Lopatin YM, Vitale C: Effect of ivabradine on central aortic blood pressure in patients with stable coronary artery disease: What do we know? Int J Cardiol 2016; 224:145–8
14. Fasullo S, Cannizzaro S, Maringhini G, Ganci F, Giambanco F, Vitale G, Pinto V, Migliore G, Torres D, Sarullo FM, Paterna S, Di Pasquale P: Comparison of ivabradine *versus* metoprolol in early phases of reperfused anterior myocardial infarction with impaired left ventricular function: Preliminary findings. J Card Fail 2009; 15:856–63
15. Nerla R, Di Franco A, Milo M, Pitocco D, Zaccardi F, Tarzia P, Sarullo FM, Villano A, Russo G, Stazi A, Ghirlanda G, Lanza GA, Crea F: Differential effects of heart rate reduction by atenolol or ivabradine on peripheral endothelial function in type 2 diabetic patients. Heart 2012; 98:1812–6
16. Miranda ML, Balarini MM, Balthazar DS, Paes LS, Santos MS, Bouskela E: Ivabradine attenuates the microcirculatory derangements evoked by experimental sepsis. Anesthesiology 2017; 126:140–9
17. Fox K, Ford I, Steg PG, Tendera M, Ferrari R; BEAUTIFUL Investigators: Ivabradine for patients with stable coronary artery disease and left-ventricular systolic dysfunction (BEAUTIFUL): A randomised, double-blind, placebo-controlled trial. Lancet 2008; 372:807–16
18. Toscano MG, Ganea D, Gamero AM: Cecal ligation puncture procedure. J Vis Exp 2011; 2860
19. Merx MW, Liehn EA, Janssens U, Lütticken R, Schrader J, Hanrath P, Weber C: HMG-CoA reductase inhibitor simvastatin profoundly improves survival in a murine model of sepsis. Circulation 2004; 109:2560–5
20. Mesirca, P, Marger, L, Torrente, A, Bouly, M, Cohen-Solal, A, Leoni, A.L., Nargeot, J, Mangoni, M.E.: Direct relationship between *in vitro* I*f* current inhibition by ivabradine and heart rate in the mouse. Eur Heart J 31:697–8
21. Vilaine JP: The discovery of the selective I(f) current inhibitor ivabradine: A new therapeutic approach to ischemic heart disease. Pharmacol Res 2006; 53:424–34
22. Pacher P, Nagayama T, Mukhopadhyay P, Bátkai S, Kass DA: Measurement of cardiac function using pressure–volume conductance catheter technique in mice and rats. Nat Protoc 2008; 3:1422–34
23. Annane D, Trabold F, Sharshar T, Jarrin I, Blanc AS, Raphael JC, Gajdos P: Inappropriate sympathetic activation at onset of septic shock: A spectral analysis approach. Am J Respir Crit Care Med 1999; 160:458–65
24. Ackland GL, Yao ST, Rudiger A, Dyson A, Stidwill R, Poputnikov D, Singer M, Gourine AV: Cardioprotection, attenuated systemic inflammation, and survival benefit of β1-adrenoceptor blockade in severe sepsis in rats. Crit Care Med 2010; 38:388–94

25. Suzuki T, Morisaki H, Serita R, Yamamoto M, Kotake Y, Ishizaka A, Takeda J: Infusion of the β-adrenergic blocker esmolol attenuates myocardial dysfunction in septic rats. Crit Care Med 2005; 33:2294–301
26. Boissier F, Razazi K, Seemann A, Bedet A, Thille AW, de Prost N, Lim P, Brun-Buisson C, Mekontso Dessap A: Left ventricular systolic dysfunction during septic shock: The role of loading conditions. Intensive Care Med 2017; 43:633–42
27. Swedberg K, Komajda M, Böhm M, Borer JS, Ford I, Dubost-Brama A, Lerebours G, Tavazzi L; SHIFT Investigators: Ivabradine and outcomes in chronic heart failure (SHIFT): A randomised placebo-controlled study. Lancet 2010; 376:875–85
28. Roubille F, Tardif JC: New therapeutic targets in cardiology: Heart failure and arrhythmia: HCN channels. Circulation 2013; 127:1986–96
29. Wei C, Al Kattani N, Louis H, Albuisson E, Levy B, Kimmoun A: I*f* channel inhibition with ivabradine does not improve cardiac and vascular function in experimental septic shock. Shock 2016; 46:297–303
30. Sanfilippo F, Corredor C, Fletcher N, Landesberg G, Benedetto U, Foex P, Cecconi M: Diastolic dysfunction and mortality in septic patients: A systematic review and meta-analysis. Intensive Care Med 2015; 41:1004–13
31. Rudiger A, Singer M: Mechanisms of sepsis-induced cardiac dysfunction. Crit Care Med 2007; 35:1599–608
32. Montmollin E de, Aboab V, Mansart A, Annane D: Bench-to-bedside review: β-Adrenergic modulation in sepsis. Crit Care Lond Engl 2009; 13:230
33. Gentry-Smetana S, Redford D, Moore D, Larson DF: Direct effects of volatile anesthetics on cardiac function. Perfusion 2008; 23:43–7

ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Byline Backstory No. 1: A Twice-Burned Toddler Finds Anesthetic Relief



Southwest of Philadelphia, my father, Hank Bause, attended Crozer Seminary and heard a Crozer alumnus, Reverend Martin Luther King, Jr., practice his sermons. One of Dad's vivid memories as a seminarian was driving me, his 9-month-old son, to Crozer Hospital (*upper left*) after I had toddled into the kitchen and planted both hands down upon the open door of our heated oven. The *Chester Times* headlined this incident as "Child Burned on Oven." After my father graduated from Crozer, he drove my mother and me to Stratham, New Hampshire, so he could serve as a fledgling pastor there. Less than a year after my first-degree burns at Crozer, I suffered second-degree ones in Stratham. From there, my mother Suzanne would rush her hyperactive toddler to nearby Exeter Hospital (*lower right*) for burns from scalding tea water that I had pulled down over my right arm. Perhaps the anguish of my subsequent scarring and childhood nightmares of "roasting, like a turkey in an oven" motivated my future pursuit of the relief of pain…as an anesthesiologist. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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