Sivelestat Reduces Inflammatory Mediators and Preserves Neutrophil Deformability During Simulated Extracorporeal Circulation

Kanji Matsuzaki, MD, Yuji Hiramatsu, MD, PhD, Satoshi Homma, MD, PhD, Shoko Sato, BMT, Osamu Shigeta, MD, PhD, and Yuzuru Sakakibara, MD, PhD

Departments of Cardiovascular Surgery and Cardiology, Institute of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan

Background. **Neutrophil is a major focus in efforts to ameliorate the systemic inflammatory response associated with cardiopulmonary bypass. Neutrophil elastase is a powerful proteolytic enzyme, and plays a pivotal role in the development of the inflammatory response. This study assesses the inhibitory effects of sivelestat, a highly specific neutrophil elastase inhibitor, on elastase levels, cytokine production, and the functional changes of neutrophils in a simulated extracorporeal circulation model.**

Methods. **Simulated recirculation was established by recirculating heparinized (3.75 U/mL) human blood for 120 minutes in an oxygenator and a roller pump circuit with and without 100 μmol/L of sivelestat (n = 7 for each group). Neutrophil elastase and interleukin-8 were measured with an enzyme immunoassay. Neutrophil deformability was evaluated by simulated microcapillaries. The neutrophil F-actin and the expression of CD11b and L-selectin were measured by flow cytometry.**

Results. **Sivelestat reduced both neutrophil elastase levels ($p = 0.0006$) and interleukin-8 production ($p < 0.0001$) at 120 minutes of recirculation. Sivelestat also significantly preserved neutrophil deformability ($p = 0.017$) and reduced F-actin expression ($p = 0.0037$). The drug did not modulate the changes of CD11b or L-selectin.**

Conclusions. **This study suggests that specific elastase inhibition with sivelestat could be a feasible therapeutic strategy for patients undergoing cardiopulmonary bypass to attenuate neutrophil-derived inflammatory response and organ injuries.**

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Cardiopulmonary bypass (CPB) induces a systemic inflammatory response that contributes to the morbidity of open heart surgery [1, 2]. One of the most important initiating events of this phenomenon is neutrophil activation and sequestration. Neutrophil sequestration in microvessels is due to a loss of deformability and the changes of adhesive qualities between neutrophils and endothelial cells [3]. Neutrophils also secrete toxic oxygen species and proteolytic enzymes, including neutrophil elastase. Neutrophil elastase is an extremely cytotoxic enzyme in plasma and interstitial fluid. It degrades connective tissue components such as elastin, proteoglycan, fibronectin, and collagen, and potentially causes severe tissue injury and subsequent multiple organ dysfunction [1, 4].

Sivelestat sodium hydrate, sodium N-{2-[4-(2,2-dimethylpropionyloxy) phenylsulfonylamino] benzoyl} aminoacetate tetrahydrate (Elaspol, ONO-5046-Na [$C_{20}H_{21}N_2NaO_7S$-Na-$4H_2O$, molecular weight, 528.51]; Ono Pharmaceutical Co., Osaka, Japan), is a synthetic, specific, low-molecular weight neutrophil elastase inhibitor. The inhibitory activity (Ki value) of sivelestat against human neutrophil elastase is 46 nmol/L. Its 50% inhibitory concentration values (IC_{50}) against elastin and neutrophil elastase activity in human plasma are 1.7 μmol/L and 22.8 μmol/L, respectively [5]. The drug is intravenously active, and competitively inhibits the activity of neutrophil elastase in humans, hamsters, and dogs, but does not affect other proteases such as plasmin, thrombin, kallikrein, cathepsin B, or collagenase I [5]. The eliminating phase of the drug is biphasic, and the half-life of the first and second phases are constant at about 2 and 3 hours, respectively [6]. Intrinsic macromolecular antiproteases, which inhibit neutrophil elastase in an ordinary state, are immediately inactivated by superoxide radicals and blocked from close contact with neutrophils during the onset of an inflammatory state [4, 7]. A low-molecular weight antiprotease, such as sivelestat, achieves close contact with neutrophils without rapid inactivation by superoxide and therefore is advantageous [8]. The efficacy of sivelestat on postperfusion lung, ischemia-reperfusion and endothelial cell injuries have been demonstrated in several investigations [9–11]. The use of the drug in humans has been approved in Japan for cases of acute lung injury [12], although there have been conflicting conclusions regarding its benefits [13].

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Address reprint requests to Dr Hiramatsu, Department of Cardiovascular Surgery, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan; e-mail: yuji3@md.tsukuba.ac.jp.

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Table 1. Measured Blood and Plasma Constituents and Microchannel Transit Time During Experiments[a]

Variable	Group	Donor	0 Minutes	30 Minutes	60 Minutes	120 Minutes	SC	*p* Value (Analysis of Variance)
Neutrophil count (% of donor)	Control	100		98.2 ± 3.1	92.6 ± 3.0	90.1 ± 3.5[d]	84.8 ± 1.7[f]	0.97
	Sivelestat	100		96.1 ± 1.1	90.5 ± 3.9[d]	88.0 ± 3.5[e]	85.3 ± 3.0[f]	
Neutrophil elastase (ng/mL)	Control	247.7 ± 54.4	346.5 ± 75.5	1171.1 ± 150.5[f]	1712.5 ± 206.7[b,f]	2744.4 ± 188.4[c,f]	1301.3 ± 239.0[b,f]	0.0006
	Sivelestat	238.4 ± 64.1	377.5 ± 83.8	742.0 ± 151.7[d]	1166.6 ± 198.0[b,f]	1727.1 ± 215.3[c,f]	728.8 ± 201.8[b,d]	
Interleukin-8 (pg/mL)	Control	10.5 ± 3.2	8.5 ± 3.8	16.6 ± 4.7	25.9 ± 4.8	174.0 ± 46.7[c,f]	22.4 ± 3.9	< 0.0001
	Sivelestat	10.5 ± 3.3	8.9 ± 3.8	11.7 ± 3.7	10.6 ± 4.0	45.7 ± 7.0[c]	20.2 ± 6.4	
CD11b (% of donor)	Control	100	102.7 ± 5.3	553.4 ± 52.1[f]	584.3 ± 29.4[f]	606.1 ± 43.9[f]	189.1 ± 30.8[d]	0.52
	Sivelestat	100	97.4 ± 5.5	523.5 ± 41.0[f]	600.3 ± 48.6[f]	703.8 ± 73.0[f]	209.1 ± 43.2[d]	
L-selectin (% of donor)	Control	100	108.3 ± 3.0	48.4 ± 8.7[f]	28.0 ± 6.2[f]	18.2 ± 4.0[f]	93.3 ± 7.2	0.15
	Sivelestat	100	101.6 ± 1.8	44.3 ± 6.9[f]	23.4 ± 5.2[f]	12.0 ± 3.8[f]	68.5 ± 11.7[f]	
F-actin (% of donor)	Control	100	108.5 ± 11.6	227.2 ± 44.6[c,f]	167.5 ± 29.0[c,d]	182.7 ± 16.8[c,f]	129.9 ± 23.0[b]	0.0037
	Sivelestat	100	104.3 ± 17.9	109.0 ± 12.9[c]	78.0 ± 15.5[c]	67.6 ± 16.7[c]	69.7 ± 9.7[b]	
Transit time (% of donor)	Control	100		110.6 ± 5.3	136.8 ± 10.2[d]	160.1 ± 19.2[f]	120.0 ± 14.1	0.017
	Sivelestat	100		106.9 ± 5.9	113.0 ± 3.4	110.5 ± 4.9[c]	134.5 ± 17.5	

[a] Changes in measured blood and plasma constituents and microchannel transit time during experiments. Values are expressed as the mean ± standard error of the mean, [b] $p < 0.05$, [c] $p < 0.01$ by two-way analysis of variance with Bonferroni correction as compared between the Sivelestat group and the Control group, [d] $p < 0.05$, [e] $p < 0.01$, [f] $p < 0.001$ by one-way analysis of variance as compared with the Donor value.

SC = standing control.