Case Report

Study of Intrinsic Cardiac Dysfunction in Septic Shock Conditions by Isolated Working Heart: A Primary Approach before New Therapeutic Proposals?

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Abstract

Septic shock is an inflammatory response to an infection leading to cardiovascular dysfunction. Today, only few studies have evaluated whether cardiac dysfunction in septic shock is due to load modifications, neuroendocrine influence or intrinsic cardiac alterations. The isolated working heart approach allows measurement of cardiac function under controlled conditions. Our study demonstrated for the first time that in septic shock rat, alteration of cardiac function is characterized by both systolic and diastolic dysfunctions beside load conditions. This observation might help to apprehend the particular dysfunction associated with sepsis.

Introduction

Sepsis can be defined as deregulated host response to an infection leading to a circulatory and organ dysfunction [1]. Cardiac dysfunction is present in 40% of patients and plays a critical role in the patient outcome. It increases mortality by 40% and is a major predictor of morbidity and mortality in sepsis [2].

The main goals of these past decades have been to increase our knowledge about cardiac function and its pathophysiology to reduce septic shock burden with low success rate. It remains controversial whether the cardiac alteration associated with sepsis is a consequence of a drastic drop in blood pressure, capillary leakage and neuroendocrine system activation or an intrinsic alteration of the cardiac function or regulation [3]. In fact, the intrinsic contractile function of the heart and the intrinsic myocardial response to sepsis has been poorly investigated. As recently reported, the characterization of sepsis-induced cardiomyopathy and its mechanisms is incomplete and needs to be examined [4–6].

Using echocardiography and isolated working heart techniques, this study allows the investigation of the cardiac function in septic shock in the presence or absence of pre-load, after-load and neurohumoral modifications.

Methods

Endotoxemic model

All animal protocols were approved by the ethic committee in charge of animal experimentation of Pays de la Loire and were performed in accordance with French law on animal welfare, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, revised 2011).

Ten-weeks-old male Sprague–Dawley rats (Janvier, Le Genest St, France) were housed under standard conditions of room temperature and 12 h light/dark cycle. Food and water were available *ad libitum*. Control rats (CTRL) received physiologic serum injection where as endotoxemic shock (LPS) was induced by intravenous injection of 5 mg/kg LPS from *Escherichia coli* O111:B4 (Sigma, St Quentin Fallavier, France), as previously described [5]. Rats were studied 3 hours after shock induction.

In vivo evaluation

Transthoracic echocardiography was performed as previously described [8]. Hemodynamic arterial pressure was performed using a 2F catheter (Millar, Houston, USA) in isolated right carotid

Table 1: Evaluation of the septic shock model by measurement of hemodynamic arterial pressure, metabolic and echocardiographic parameters from CTRL and LPS rats 3 hours after shock induction by LPS.

		CTRL (n=7)		LPS (n=9)	
Hemodynamic parameters	Mean arterial pressure (mmHg)	87.17	± 1.30	72.75	± 4.69 *
Plasmatic parameters	Lactatemia (mmol.L^{-1})	2.80	± 1.23	4.74	± 1.41 *
	Troponin T (mmol.L^{-1})	6.17	± 1.03	88.44	± 14.40 *
Cardiovascular function	Heart rate (beats.min^{-1})	373.29	± 7.78	419.78	± 5.32 *
	Cardiac output (mL.min^{-1})	92.96	± 9.14	59.68	± 7.37 *
	Ejection fraction (%)	80.71	± 2.24	65.11	± 3.06 *
	E/A ratio	1.10	± 0.02	0.85	± 0.01 *
	IVRT	20.57	± 0.57	26.00	± 0.96 *

Values are expressed as mean ± SEM of 5-9 rats, * p<0.05 *vs* CTRL.
E: Early left ventricular filling velocity; A: late left ventricular filling velocity; IVRT: Interval of isovolumic relaxation time.

Table 2: Main parameters of LV functions were recorded in basal conditions 3 hours after shock induction after a 20 minute stabilization period by isolated working hearts from CTRL and LPS rats.

		CTRL (n=7-12)	LPS (n=8-12)
	Heart rate (beats.min^{-1})	261.99 ± 10.05	297.32 ± 9.64 *
	Cardiac output (mL.min^{-1})	72.09 ± 6.44	44.60 ± 4.06 *
Systolic function	Systolic ejection period (msec)	60.82 ± 1.82	65.50 ± 1.79
	Contraction time (msec)	37.83 ± 5.91	46.17 ± 6.04
	LVP max (mmHg)	140.58 ± 4.77	106.21 ± 4.05 *
	dP/dt max (mmHg.sec^{-1})	5823 ± 502	3030 ± 194 *
Diastolic function	Relaxation time (sec)	55.14 ± 1.66	56.91 ± 2.33
	Diastolic filling period (sec)	84.09 ± 10.17	46.13 ± 5.59 *
	LVP min (mmHg)	-9.29 ± 3.07	0.84 ± 2.41 *
	dP/dt min (mmHg.sec^{-1})	-4495 ± 368	-2416 ± 133 *

Values are expressed as mean ± SEM of 7-12 hearts, * p<0.05 *vs* CTRL.
LVP: Left ventricular pressure; dP/dt: Derivative pressure, max: maximal or min: minimal.

artery. Pressure signal was recorded and analyzed using an A/D converter (EMKA Technologies, Paris, France).

Arterial blood samples were collected before the sacrifice at the abdominal aortic level and plasmatic parameters (troponin T and lactate) were analyzed using a GEM®4000 (Instrumentation Laboratory, Paris, France).

Working heart study

Isolated working hearts were perfused as previously described [9] at physiological pre- and after-load pressures (respectively 12.5 and 80 mmHg) for 20 minutes. The following functional parameters were monitored continuously during the perfusion experiments: cardiac output and coronary flow with calibrated electromagnetic flow probes (Scisense ADV500, Transonic, Elsloo, The Netherlands), Heart Rate (HR) and cardiac cycle, Left Ventricular Pressure (LVP), and values for the first derivative of LVP (dP/dt) with a pressure transducer (Perfused isolated heart system, EMKA Technologies).

Statistical analysis

Results are expressed as the mean ± SEM of n experiments and values were compared using Mann-Whitney test. A *p*-value <0.05 was considered statistically significant.

Results

LPS rats presented hypotension (87.17 *vs* 72.75 mmHg in LPS, p<0.05), tachycardia (353.8 *vs* 440.7 beat.min^{-1} in LPS, p<0.01) and an increase of plasmatic parameters such as troponin T (6.17 *vs* 88.44 mmol.L^{-1} in LPS, p<0.05) and lactate (2.80 *vs* 4.74 mmol.L^{-1} in LPS, p<0.05). Echocardiographic evaluation of LPS rats also showed a 36% decrease in cardiac output (p<0.05) and an impairment of systolic (-20% of ejection fraction) and diastolic (-23% of E/A ratio and +26% of isovolumic relaxation time) functions which indicate an alteration of cardiac function concordant with septic shock disease (Table 1).

The study of intrinsic cardiac function with isolated working heart at physiological load conditions also demonstrated a tachycardia (261.9 *vs* 297.3 beats.min^{-1} in LPS, p<0.05) specifically due to a reduction of the diastolic filling period (45% in the LPS group, p<0.05) without any other alteration of the cardiac cycle (Table 2). In the LPS group, an alteration of the systolic function was also observed, with a 24% reduction of the end systolic pressure (p<0.001) and a 48% reduction of the contractility (dP/dt max, p<0.001). Diastolic function was impaired in LPS rats with an increase in left ventricular minimal pressure (-9.3 mmHg in CTRL to 0.8 mmHg in LPS, p<0.05) and a reduced cardiac relaxation (dP/dt min of -4495.7 *vs* -2415.7 mmHg. sec^{-1} in LPS, p<0.001). Altogether these alterations led to a significant reduction in cardiac output (-38%, p<0.01).

Discussion

We demonstrate for the first time that alteration of the cardiac function at the early phase of septic shock is independent of the load conditions or the neurohumoral status and persist after a stabilization period indicating that the cardiac function is drastically altered by sepsis condition.

Hypotension observed in septic shock creates a drop of pre- and after-load and is associated with cardiac dysfunction shown by both systolic (low ejection fraction) and diastolic (acute ventricle dilatation) dysfunctions [10]. To correct pre-load alterations, therapy by fluid resuscitation is widely used. Nevertheless, despite a recovery of nearly physiological load conditions, patients still present with cardiac dysfunction [11]. In this context, isolated working heart allowed us to evaluate cardiac function under physiological load conditions on heart from control and animal suffering from septic shock. Interestingly, in these conditions, both systolic and diastolic dysfunctions are observed with an alteration of intraventricular pressures and their derivative values independently of pre- or after-load modifications. In parallel, tachycardia caused by septic shock is observed *in vivo* during echocardiography but also *ex vivo* indicating a persisting remodeling process.

The association of *in vivo* studies - like echocardiography - and isolated working heart brings new insight and open bring important information to develop therapeutic targets and to test new cardiac therapies in a pre-clinical setting.

Aknowledgement

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