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Full Length Research Paper

Effects of various forms of lipopolysaccharide on the expression of inflammatory mediators and cardiac biomarkers in human cardiac fibroblasts and human coronary smooth muscle cells

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Inflammation is an important event in the development of vascular diseases such as hypertension, atherosclerosis, and restenosis. The stimulation of lipopolysaccharide (LPS) from bacteria induces the release of critical proinflammatory cytokines that activate potent immune responses which may cause injury of cells *in vivo* and *in vitro*. Upon cardiac cell death caused by inflammation, the apoptotic cardiac cells express higher amount of cardiac markers. In this study, the effect of various LPS on human cardiac fibroblasts (HCFs) and human coronary smooth muscle cells (HCSMCs) were evaluated. Various forms of LPS were applied to HCFs and HCSMCs for 24, 48, 72 and 96 h. Proliferation rate of these cells was evaluated after stimulation. The levels of lactate dehydrogenase (LDH), N-terminal pro B-type natriuretic peptide (pro-BNP) and the MB isoenzyme of creatine kinase (CK-MB) were measured by an automation system. Cytokine levels in culture supernatants and extracted protein of cells were mixed and measured with IL-1β, IL-6 and IL-10 ELISA kits. Significant increase in the proliferation of two cardiac cells ($P<0.05$) after incubation for 48 and 72 h was noted but not for 24 and 96 h ($P>0.05$). Cardiac markers and inflammatory cytokines were significantly higher than control at 48 and 72 h ($P<0.05$), which demonstrated that HCFs and HCMSCs were under inflammation leading to cell injury between 48 and 72 h. LPS is one of the factors giving rise to periodontal diseases, it is also involved in *in vitro* cardiac cell injury. Therefore, LPS may be used as a bio-marker to monitor local or systemic inflammation.

Key words: Lipopolysaccharide, human cardiac fibroblasts, human coronary smooth muscle cell, inflammatory cytokines, cardiac bio-marker.

INTRODUCTION

The heart tissues consist of cardiomyocytes, fibroblasts and vascular cells (smooth muscle, endothelium), the fibroblast cells are diverse and experimental evidence has shown that substantial heterogeneity exists between fibroblasts and different tissues (Brown et al., 2005). The human cardiac fibroblast (HCF) is one of the two major cell types of the heart; combination of the cardiomyocytes regulates the normal cardiac function. They are the main

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components of extracellular matrix that regulate the function of the heart including the chemical, physical and electrical signals (Kamkin et al., 2003; Camelliti et al., 2005; Porter and Turner, 2009). HCFs are also responsible for the synthesis of vital extracellular matrix products in the heart, including fibrillar collagen types I and type III (Myllyharju and Kivirikko, 2001; Lijnen et al., 2003). The human coronary smooth muscle cells invent from progenitors in the proepicardial organ, a transitory organization formed by mesothelial cells overlying the sinus venous, they are responsible for the inward and departing blood flow (Lam et al., 2009).

Lipopolysaccharide (LPS) is an eminently effective inflammatory immune activator, a key constituent of the various bacteria cell walls especially in Gram-negative strains (Ulevitch and Tobias, 1999). It functions to steady the general membrane structure and to prevent it from certain chemical attack. It can stimulate the production of endogenous pyrogen IL-1 and tumor necrosis factor (Beutler, 2002; Stawowy et al., 2003). LPS is the most powerful bacterial product in terms of its pro-inflammatory activities. Stimulation of innate immune system or immune cells *in vitro* by LPS, could lead to uncontrollable cytokines and other mediators production, which may cause various inflammatory diseases in the human body (Lu et al., 2009).

Porphyromonas gingivalis (P. gingivalis), a Gram-negative bacterium strongly associated with periodontitis, which is a chronic inflammatory disease of the tissue surrounding the tooth root surface (Paquette, 2002). It is suspected that both the bacterial factors, such as *P. gingivalis* and host factors have effects on such disease (Al-Qutub et al., 2006). Studies also suggest that there is an association between periodontitis and systemic diseases such as coronary heart disease and preterm birth (Paquette, 2002). LPS obtained from *P. gingivalis* is atypical, as it exhibits bizarre quantities of lipid A heterogeneity including both tetra- and penta-acylated lipid A structures (Bainbridge et al., 2002; Darveau, 2004; Yi, 2000; Lu et al., 2009). Designated *P. gingivalis* LPS_{1690}, a major monophosphoryl penta-acylated lipid A cluster is observed, when incubated at 1 µg/ml haemin which is a microenvironmental component for *P. gingivalis* that can modulate the lipid A structure found in a bacterium (Al-Qutub et al., 2006). *P. gingivalis* LPS_{1435} is observed at higher haemin concentrations (Al-Qutub et al., 2006). Penta-acylated lipid A structures have been demonstrated to assist E-selectin expression, but tetra-acylated lipid A structures do not present similar function (Lu et al., 2009). Multiple lipid A structures that may stand for an alteration made by *P. gingivalis* in reaction to the modification of systemic or local host micro-environments (Lu et al., 2009).

At present, little information is available on how these isoforms of *P. gingivalis* LPS could differentially affect host innate immune responses. LDH is an isoenzyme not only specific for measuring cardiac cells damage, but also assesses the presence and severity of chronic tissue damage in systemic human body (Wang et al., 2007; Totlandsdal et al., 2008). An association with high levels of LDH and changes in the ratio of LDH usually indicates some type of tissue damage or death (Wang et al., 2007; Totlandsdal et al., 2008). CK-MB is an enzyme possessed by several tissues and cell types useful for the determination of cardiac problems (Wallimann and Hemmer, 1994), which is expressed in cardiac muscles and BB-CK is expressed in smooth muscles and in most non-muscle tissue. When levels of this enzyme is high, the individual is susceptible to cardiac damage as in troponin T, it is commonly found to be relatively low in systemic circulation in humans (Wallimann and Hemmer, 1994). The pro-BNP acts as a cardiac marker that is an indicator of a decline in systemic vascular resistance and central venous pressure; therefore, it is an indicator to a decrease in cardiac output (Atisha et al., 2004). This cardiac marker is used in the diagnosis of congestive heart failure, and has been proved practical in the projection of heart failure when the pro-BNP level is found to be higher in patients exhibiting worse outcomes.

Interleukin is a generic term for a group of multi-functional cytokines produced by a large variety of body cells responsible for the regulation of the immune system, by facilitating communication between immune cells used to contest against detrimental substances such as bacteria through chemical signals (Razelle, 1996). Due to the large number of different interleukins, it is clear that each one is unique to its function yielding many different purposes (Razelle, 1996). IL-1β, a cytokine responsible for the stimulation of inflammation, is involved in the proliferation, differentiation and apoptosis of cells (Gurantz et al., 2005). IL-6 is responsible for programming cytokine designed for inflammation and the maturation of B cells most commonly produced at sites of severe and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor alpha (Du et al., 2005; Turner et al., 2008). IL-10, a type of cytokine known for its anti-inflammatory properties, counteracts inflammatory cytokines such as “IL-1B and IL-6 by activated macrophages (Berlato et al., 2002).

The purpose of this study was to investigate how the application of various lipopolysaccharide (LPS): *P. gingivalis* wild type LPS, *P. gingivalis* LPS_{1690}, *P. gingivalis* LPS_{1435} and *Escherichia coli* LPS affect the activity, cytokines expression and cardiac bio-markers release of human cardiac fibroblasts and human coronary smooth muscle cells which are key characters for normal heart functions.

MATERIALS AND METHODS

LPS Preparation

P. gingivalis LPS was isolated from *P. gingivalis* ATCC 33277 and prepared by the cold $MgCl_2$-ethanol procedure, based upon the protein digestion of whole-cell extracts with proteinase K and

successive solubilisation and precipitation procedures (Lu et al., 2009). The LPS was supplementarily purified to eliminate trace amounts of endotoxin protein; the final protein contamination was less than 0.1%. The fatty acids present in the *P. gingivalis* LPS were analyzed by gas chromatography-mass spectroscopy (Lu et al., 2009). Three separate extractions of *P. gingivalis* LPS were obtained, including *P. gingivalis* wild type LPS (m/z 1690) (*P. gingivalis* wild type LPS), *P. gingivalis* 1587KO (m/z 1690) (*P. gingivalis* LPS_{1690}) and *P. gingivalis* 1773KO (m/z 1435/50) *(P. gingivalis* LPS_{1435}). *E. coli* O55:B5 LPS was purchased from Sigma (St Louis, MO, USA). All types of LPS were diluted by Hanks' Balanced Salt Solution (HBSS) (St Louis, MO, USA) in the stimulation tests before use.

Cardiac cells culture and pilot study of LPS

Human cardiac fibroblasts (HCF, adult ventrical, Catalog# 6310) were purchased from ScienCell Research Laboratory (San Diego, CA, USA) and Human Coronary Smooth Muscle Cells (HCSMCs) were purchased from Cascade Biologics (Cascade Biologics Inc., OR, USA). HCFs and HCSMCs were cultured in Dulbecco's Modified Eagle Medium and Dulbecco's Modified Eagle Medium/ Ham's F12 (1:1), respectively, both containing 10% fetal bovine serum, 100 U/ml penicillin G and 100 mg/ml streptomycin (Invitrogen, Hong Kong) at 37°C in a humidified atmosphere of 95% air, 5% CO_2. The cells used in this study were from the early passages 2 to 6, to limit the possible variations in LPS stimulation (Li et al., 2009).

The two cardiac cells (between passages 2 and 6) were seeded in 96-well culture plates (Nalge Nunc International Corp., IL, USA) for 24, 48, 72 and 96 h in the presence or absence of various LPS (*P. gingivalis* wild type LPS, *P. gingivalis* LPS_{1435} and *P. gingivalis* LPS_{1690} at 1, 10, and 100 ng/ml; *E. coli* LPS at 100 µg/ml, 10 µg/ml, 1 µg/ml, 100 ng/ml, 10 ng/ml and 1 ng/ml) to conduct a pilot study to determine the optimal stimulating time and LPS concentrations. After preliminary experiments, 100 ng/ml has been found to induce cardiac markers and cytokines expression in both cell lines for *P. gingivalis* wild type LPS, *P. gingivalis* $LPS_{1435,}$ LPS_{1690} and *E. coli* LPS at 48 and 72 h.

Proliferation rate measured by WST-1 for cardiac cells after stimulation with LPSs

The activity of LPSs were assessed with cell proliferation reagent WST1 (Roche, Mannheim, Germany) using HCF and HCSMC cell lines in the pilot and final experiments. Cells were cultured at a density of 4000 cells per well into 96-well microtitre plates (Nalge Nunc International Corp., IL, USA). At the designated time-points being 48 and 72 h after inoculation, 10 µl WST1 was applied to each well and incubated for 60 min at 37°C. The optical density was read at 450 nm by Universal Microplate Reader (Sunrise, TECAN, Austria). Each sample was analysed in triplicates.

Cardiac markers measurement

The cells were plated at a density of 5 x 10^5 cells/well into a 6-well plate (Nalge Nunc International Corp., IL, USA) in different groups as the aforestated condition. At harvest time points, the culture medium had been collected and the cells were applied by standard trypsinization (0.5 mg/ml trypsin and 0.2 mg/ml EDTA in phosphate buffered saline (PBS)), then the cells were washed with PBS, and cell pellets were digested, isolated and purified to get proteins by a Total Protein Extraction Kit (Millipore Corp., MA, USA).

The cultured cell lysate and culture medium of these two kinds of cells were used to measure the levels of lactate dehydrogenase (LDH), N-terminal pro B-type natriuretic peptide (pro-BNP) and the MB isoenzyme of creatine kinase (CK-MB) by using an automation closed kit system in COBAS INTEGRA 400 PLUS and ELECSYS 2010 immunoassay analysers (ROCHE, Germany). The marker levels were obtained by comparing with the standard curve generated from the standards provided by the manufacturer.

Enzyme-linked immunosorbent assay (ELISA) measurement on soluble IL-1β, IL-6 and IL-10 from cells

The cytokine levels were measured by using cell cultured medium and isolated protein from cardiac cells to determine the ability of different types of LPS to stimulate HCFs and HCSMCs. Cytokine levels in culture supernatants and extracted protein of cells were mixed and measured with IL-1β, IL-6, IL-10 ELISA kits (Diaclone, France), following the manufacturer's instructions from the kits. 100 µl mix of the soluble protein was incubated into a micro-plate provided. The plate was incubated for 2 h at 350 rpm and washed with washing buffer. Then the wells were dried and 200 µl of substrate tetramethylbenizidine was added into each well in the dark at room temperature. The plates were then read at 450 nm wavelength using microplate reader (Sunrise, TECAN, Austria). The levels of cytokines in the samples were obtained by comparison with the standard curve generated from standards supplied by the manufacturer. Each sample was analysed in triplicates.

Statistical analysis

Data are reported as mean±SD and all statistical analyses were performed using SPSS 15.0. Data were analyzed using the one way ANOVA and student's *t* test. Differences between the experimental and control groups were regarded as statistically significant when $P<0.05$.

RESULTS

The influence of different isoforms of LPS on the proliferation of two types of cardiac cells in the pilot study

In the pilot study, we found that the optimal concentration of *P. gingivalis* wild type LPS, *P. gingivalis* $LPS_{1435,}$ LPS_{1690} and *E. coli* LPS was 100 ng/ml. This was determined as they were the optimal concentration demonstrating a statistical difference between the control and test groups ($P<0.05$) (Figures 1 and 2).

The influence of various LPS on the proliferation of human cardiac fibroblasts (HCFs) and human coronary smooth muscle cells (HCSMSs)

After determining the optimal concentration of different types of LPS in the pilot study, the influence of *P. gingivalis* wild type LPS, *P. gingivalis* $LPS_{1435,}$ LPS_{1690} and *E. coli* LPS on the proliferation of two cardiac cells were also evaluated. There was a significant increase in the two cardiac cells proliferation ($P<0.05$) after incubation for 48 and 72 h as shown in the final experiments (Table 1). However, the cell proliferation rate of the test

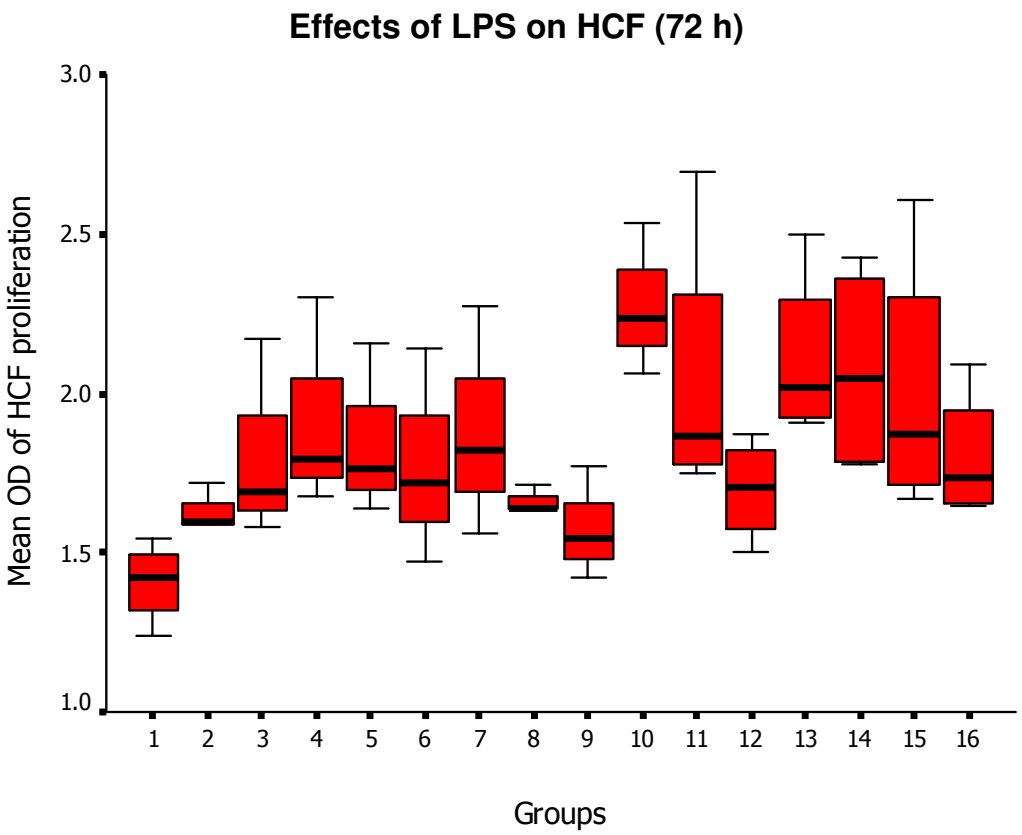

Figure 1. The cell proliferation of human cardiac fibroblast after stimulation with various LPS for 72 h. Groups: (1) control;(2) Wild type LPS 1 ng; (3) Wild type LPS 10 ng; (4) Wild type LPS 100 ng; (5) *P. gingivalis* LPS_{1435} 1 ng; (6) *P. gingivalis* LPS_{1435} 10 ng; (7) *P. gingivalis* LPS_{1435} 100 ng; (8) *P. gingivalis* LPS_{1690} 1 ng; (9) *P. gingivalis* LPS_{1690}10 ng; (10) *P. gingivalis* LPS_{1690} 100 ng; (11) *E. coli* LPS 100 µg; (12) *E. coli* LPS 10 µg; (13) *E. coli* LPS 1 µg; (14) *E. coli* LPS 100 ng; (15) *E. coli* LPS 10 ng; (16) *E. coli* LPS 1 ng.

groups was slightly higher than the control group after 24 and 96 h ($P > 0.05$), the data was not shown in this manuscript.

The influence of various LPS on the release of different markers of HCFs and HCSMSs

To explore the specific effect of LPS on the two cardiac cells, we also evaluated the various cardiac markers in the two types of cells. Cardiac markers (LDH, NT pro-BNP and CK-MB) and cytokine levels (IL-6 and IL-1β) of HCFs from 48 to 72 h for different types of LPS ($P<0.05$) (Table 2). The same trend was observed in HCSMs, the levels of cardiac markers and pro-inflammatory cytokines at 72 h were significantly higher than that at 48 h ($P<0.05$) (Table 3).

DISCUSSION

It is known that heart diseases such as atherosclerosis, hypertension and sepsis-induced myocardial dysfunction, result from the stimulation of host cells by bacterial LPS (Morrison and Ulevitch, 1978; Aoyagi and Izumo, 1993; Heo et al., 2008). LPS stimulation leads to the expression and release of a portfolio of markers and proinflammatory cytokines and lipid mediators, which in turn can initiate a chain of events. In addition, it was reported recently that LPS can induce HCSMCs proliferation and inflammation which contributes to the development of atherosclerosis (Yang et al., 2005; Li et al., 2007).

However, little is known about the effect of various types of LPS in HCFs and HCSMCs. Robert et al. (1997) reported that *P. gingivalis* LPS and *E. coli* LPS could induce white blood cells proliferation, cytokine and CD14 production, they may act as a regulatory factor in the chronic periodontal inflammatory response (Roberts et al., 1997). Some isoforms of *P. gingivalis* LPS and *E. coli* LPS could demonstrate different effects on host immune responses in the human body (Lu et al., 2009). One study reported that the *P. gingivalis* LPS_{1435} does not initiate endothelial cell by using Toll-like receptor 4 (TLR4); however, *P. gingivalis* LPS_{1690} and *E. coli* LPS were both

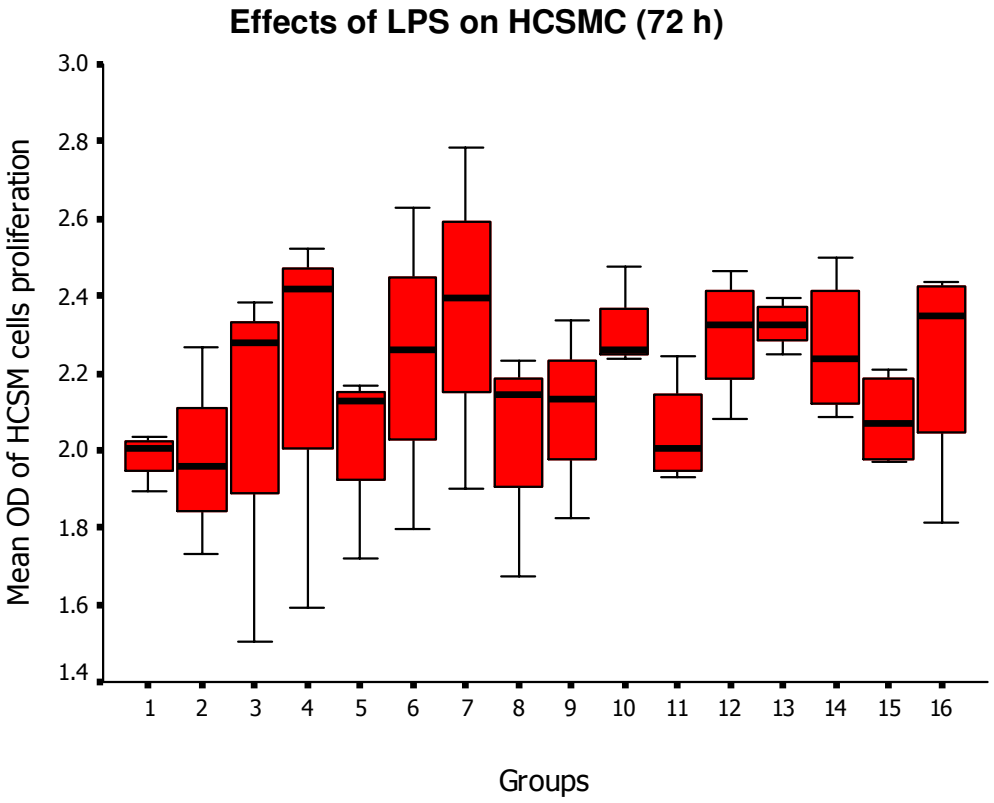

Figure 2. The cell proliferation of human coronary smooth muscle cells after stimulation with various LPS for 72 h. Groups: (1) control; (2) Wild type LPS 1 ng, (3) Wild type LPS 10 ng; (4) Wild type LPS 100 ng; (5) *P. gingivalis* LPS_{1435} 1 ng; (6) *P. gingivalis* LPS_{1435} 10 ng; (7) *P. gingivalis* LPS_{1435} 100 ng; (8) *P. gingivalis* LPS_{1690} 1 ng; (9) *P. gingivalis* LPS_{1690}10 ng; (10) *P. gingivalis* LPS_{1690} 100 ng; (11) *E. coli* LPS 100 µg; (12) *E. coli* LPS 10 µg; (13) *E. coli* LPS 1 µg (14) *E. coli* LPS 100 ng; (15) *E. coli* LPS 10 ng; (16) *E. coli* LPS 1 ng.

Table 1. Proliferation rate (mean±SD) of human cardiac fibroblasts (HCF) and human coronary smooth muscle cells (HCSMC) after stimulation with various LPS for 48 and 72 h.

	Group	48 h	72 h
HCF	Control	1.1345(±1.41)	0.12620(±0.06)
	Wild type LPS 100 ng/ml	1.2511(±1.92)*	0.33178(±0.08)
	P. gingivalis LPS_{1435} 100 ng/ml	1.2559(±1.88)*	0.35985(±0.05)
	P. gingivalis LPS_{1690} 100 ng/ml	1.2883(±1.67)*	0.18848(±0.04)
	E. coli LPS 100 ng/ml	1.3715(±2.09)*	0.29732(±0.02)
HCSMC	Control	1.1173(±1.98)	0.06232(±0.12)
	Wild type LPS 100 ng/ml	1.2027(±2.18)*	0.50960(±0.03)
	P. gingivalis LPS_{1435} 100 ng/ml	1.1292(±2.36)*	0.44312(±0.03)
	P. gingivalis LPS_{1690} 100 ng/ml	1.2017(±2.22)*	0.27231(±0.03)
	E. coli LPS 100 ng/ml	1.1677(±2.12)*	0.14220(±0.03)

*Significant difference from the control, P<0.0.

shown to activate the NF-kB pathway in human endothelial cells (Chen et al., 2007*),* and they could also induce E-selectin protein expression to a significant level (Darveau et al., 1995). In this study, we applied the *P.*

Table 2. Expression levels (mean±SD) of cytokines and cardiac biomarkers.

Time (h)	Group	Biomarker					
		LDH	NT pro-BNP	CK-MB	IL-1β	IL-6	IL-10
48	Control	60.18(±1.48)	70.01(±1.72)	0.18(±0.004)	45.46(±0.64)	101.38(±1.42)	15.61(±0.22)
	Wild type LPS 100 ng/ml	76.55(±1.88)*	218.16(±5.36)*	0.53(±0.013)*	92.07(±1.29)*	239.96(±15.19)*	16.89(±0.41)
	P. gingivalis LPS_{1435} 100 ng/ml	109.51(±2.69)*	117.48(±2.89)*	0.23(±0.007)*	53.15(±0.61)*	205.46(±2.88)*	15.68(±0.22)
	P. gingivalis LPS_{1690} 100 ng/ml	98.88(±2.43)*	120.88(±2.97)*	0.29(±0.007)*	54.17(±0.99)*	291.59(±6.40)*	16.64(±0.23)
	E. coli LPS 100 ng/ml	78.23(±1.92)*	287.35(±7.06)*	0.69(±0.017)*	138.33(±2.51)*	396.33(±6.16)*	17.19(±0.47)
72	Control	63.19(±1.55)	82.55(±2.03)	0.20(±0.005)	50.87(±0.71)	106.72(±1.49)	15.62(±0.22)
	Wild type LPS 100 ng/ml	138.20(±3.39)*	200.30(±4.92)*	0.48(±0.012)*	96.10(±1.35)*	269.79(±25.26)*	17.24(±0.31)
	P. gingivalis LPS_{1435} 100 ng/ml	122.27(±3.003)*	140.55(±3.45)*	0.34(±0.008)*	54.85(±0.64)*	208.52(±2.92)*	17.64(±1.28)
	P. gingivalis LPS_{1690} 100 ng/ml	96.75(±2.38)*	215.08(±5.28)*	0.52(±0.013)*	55.70(±0.86)*	257.42(±9.41)*	16.67(±0.23)
	E. coli LPS 100 ng/ml	75.17(±1.36)*	318.75(±7.83)*	0.76(±0.019)*	175.60(±3.23)*	572.25(±8.79)*	20.61(±0.29)
	Normal range/Unit	240-480 U/L	140-320 pg/ ml	<6.73 ng/ml	15.6-500 pg/ml	2-200 pg/ml	5-400 pg/ml

*Significant difference from the control, P<0.05. LDH, Lactate dehydrogenase; CK-MB, the MB isoenzyme of creatine kinase; NT pro-BNP, N-terminal pro B-type natriuretic peptide; *E. coli, Escherichia coli;* LPS, lipopolysaccharides.

gingivalis wild type LPS, *P. gingivalis* $LPS_{1435,}$ LPS_{1690} and *E. coli* LPS to two cardiac cells, the results showed that LPSs could promote the cell proliferation of HCFs and HCSMCs. LPS induces a higher level of LDH, pro-BNP and CK-MB expression after 48 and 72 h of incubation which indicated that both HCFs and HCSMCs are under cell injury (Tables 2 and 3). In this study, we applied pro-BNP, a sensitive cardiac marker to monitor cardiac cells damage (Heeschen et al., 2004; Tello-Montoliu et al., 2007; Kaski et al., 2010). A combination of pro-BNP, LDH and CK-MB may be useful to check for cardiovascular diseases, as these levels are often increased in those diseases (Heeschen et al., 2004; Tello-Montoliu et al., 2007; Kaski et al., 2010). The pro-BNP appears to be a unifying feature that is independent of other biochemical markers (myocardial necrosis, inflammation) and is a powerful and independent determinant of the short-term cardiac risk in patients with acute coronary syndromes (Heeschen et al., 2004; Tello-Montoliu et al., 2007; Kaski et al., 2010). Serial measurements of pro-BNP in patients with cardiac diseases may be used for more rapid identification in early stages (Heeschen et al., 2004; Tello-Montoliu et al., 2007; Kaski et al., 2010).

In addition, the results indicated that LPS could induce IL-1β and IL-6 in a time dependent fashion except for IL-10 (Tables 2 and 3). Therefore, these results matched with the findings of Roberts et al. (1997) that LPS could markedly induce inflammatory responses in HCFs and HCSMCs along with a proliferation state, but they may cause cardiac cell death leading to an expression of higher amount of cardiac markers (Roberts et al., 1997). Cardiac markers are biomarkers to evaluate cardiac function. Our results demonstrated that the two kinds of cardiac cells had been in a phase of impaired condition (Tables 2 and 3). The level of LPS-induced CK-MB and LDH increased from 48 to 72 h compared with the control group. In addition, the L-lactate dehydrogenase (LDH) was also applied to determine cardiac cell injury in the current *in vitro* experiments.

Our finding is similar to the previous reports that the cardiac cells damage or death is associated with the release of a panel of cardiac markers (Atisha et al., 2004; Wallimann and Hemmer, 1994; Wang et al., 2007; Totlandsdal et al., 2008). Even though there are some clinical or experimental surveys on the heart injury correlated with several cardiac bio-markers along with 1 or 2 cytokines (Kofidis et al., 2008; Nesher et al., 2006; Serrano et al., 2010; Zhou et al., 2010), our study was the first to combine a panel of cardiac markers and a group of inflammatory cytokines (IL-1b, IL-6 and IL-10) to assess LPSs' effect on HCFs and HCSMCs. Some previous

Table 3. Expression levels (mean±SD) of cytokines and cardiac biomarkers.

Time (h)	Group	Biomarker					
		LDH	**NT pro-BNP**	**CK-MB**	**IL-1β**	**IL-6**	**IL-10**
48	Control	39.18(±0.96)	44.16(±1.09)	0.11(±0.003)	106.37(±1.25)	208.28(±6.61)	15.87(±0.22)
	Wild type LPS 100 ng/ml	93.56(±2.30)*	59.88(±1.47)*	0.14(±0.004)*	410.79(±5.75)*	280.94(±3.93)*	17.85(±1.39)
	P. gingivalis LPS_{1435} 100 ng/ml	78.68(±1.93)*	76.97(±1.89)*	0.18(±0.005)*	118.25(±2.07)*	224.65(±2.94)*	16.81(±0.32)
	P. gingivalis LPS_{1690} 100 ng/ml	85.05(±2.09)*	87.96(±2.16)*	0.21(±0.005)*	366.12(±6.40)*	238.85(±3.91)*	16.04(±1.36)
	E. coli LPS 100 ng/ml	58.17(±1.43)*	72.69(±1.79)*	0.17(±0.004)*	475.58(±6.66)*	293.84(±11.74)*	16.43(±0.23)
72	Control	40.12(±0.99)	40.92(±1.01)	0.10(±0.002)	104.31(±1.49)	148.89(±3.72)	16.26(±0.23)
	Wild type LPS 100 ng/ml	74.42(±1.83)*	65.93(±1.62)*	0.16(±0.004)*	488.05(±6.83)*	167.69(±4.14)*	16.71(±0.20)
	P. gingivalis LPS_{1435} 100 ng/ml	77.61(±1.91)*	89.43(±2.20)*	0.21(±0.005)*	130.34(±1.63)*	216.71(±3.03)*	17.03(±0.52)
	P. gingivalis LPS_{1690} 100 ng/ml	62.73(±1.54)*	90.46(±2.22)*	0.22(±0.005)*	397.57(±4.60)*	214.40(±3.00)*	17.70(±0.79)
	E. coli LPS 100 ng/ml	44.13(±1.08)*	78.02(±1.92)*	0.19(±0.005)*	488.78(±6.84)*	225.27(±13.44)*	16.13(±0.23)
	Normal range/Unit	240-480 U/L	140-320 pg/ ml	<6.73 ng/ml	15.6-500 pg/ml	2-200 pg/ml	5-400 pg/ml

*Significant difference from the control, P<0.05; LDH, lactate dehydrogenase; CK-MB, the MB isoenzyme of creatine kinase; NT pro-BNP, N-terminal pro B-type natriuretic peptide; *E. coli: Escherichia coli;* LPS, lipopolysaccharides.

reports showed that different forms of LPS have different effects on cells (Darveau et al., 1995; Roberts et al., 1997; Chen et al., 2007; Lu et al., 2009) but our results presented here did not demonstrate differences among the LPSs treating the cardiac cells *in vitro*.

It is arguable that the presence of the LPS is beneficial to fibroblasts and smooth muscle cells as elevated levels of IL-1β cause increased levels of cell proliferation and increased levels of IL-6 cause increased production of B cells to fight off disease with increased levels of IL-10, to condone the increased level of inflammation. In sum-mary, various isoforms of LPS including *P. gingivalis* wild type LPS, *P. gingivalis* $LPS_{1435,}$ LPS_{1690} and *E. coli* LPS affect the activity, cytokines expres-sion and cardiac bio-markers of HCFs and HCMSCs at a time dependent pattern that im-plicated the LPSs may cause certain damage to cardiac tissues *in vivo.*

Abbreviations

LPS, Lipopolysaccharide; **HCFs,** human cardiac fibroblasts; **HCSMCs,** human coronary smooth muscle cells; **pro-BNP,** N-terminal pro B-type natriuretic peptide; **CK-MB,** MB isoenzyme of creatine kinase.

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