Br. J. Exp. Path. (1989) 70, 463–468

Increased chemiluminescence of polymorphonuclear leucocytes in dogs with volume overload heart failure

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Received for publication 16 December 1988
Accepted for publication 31 March 1989

Summary. Polymorphonuclear leucocyte (PMN) stimulation is known to generate oxygen free radicals. Exogenous oxygen free radicals, generated by xanthine and xanthine oxidase, have been implicated in the decrease of cardiac contractility. It is possible that PMN have increased capacity to release oxygen free radicals in failing heart. It was, therefore, decided to investigate PMN chemiluminescence (oxygen free radicals) from blood in dogs with heart failure due to chronic volume overload. The dogs were divided into two groups: (A) normal, six dogs; (B) dogs with mitral insufficiency (MI) of 6–9 months' duration, six dogs. Haemodynamic parameters were recorded to assess cardiac failure. Mixed venous blood was collected to measure PMN chemiluminescence. Stimulation of PMN was initiated by addition of opsonized zymosan and chemiluminescence was monitored using a luminometer. The haemodynamic parameters in dogs with MI showed that these dogs had left ventricular failure. The peak chemiluminescent activity of PMN in blood of dogs with left ventricular failure was approximately four times that in the blood from normal dogs. This increase in chemiluminescence reflects an increase in the generation of oxygen free radicals from PMN in dogs with chronic heart failure. The decrease in the myocardial contractility in cardiac failure might be due to an increase in the oxygen free radicals produced by the PMN.

Keywords: heart failure, oxygen free radicals, polymorphonuclear leucocyte chemiluminescence, myocardial contractility, superoxide dismutase

It has been reported that a decrease in the myocardial contractility was associated with a decrease in Ca^{2+} binding and uptake by sarcoplasmic reticulum (SR) and Ca^{2+} ATPase of SR from failing heart due to chronic volume overload (Prasad *et al.* 1986). We have recently shown that oxygen free radicals generated by exogenous administration of xanthine and xanthine oxidase produced a marked decrease in the cardiac function and index of myocardial contractility (Prasad *et al.* 1989). Hess *et al.* (1982) have reported that oxygen free radicals depressed the Ca^{2+} accumulation by SR and

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Ca^{2+} ATPase of SR. There are various factors that could lead to an increase in the oxygen free radicals during volume overload including xanthine–xanthine oxidase, leukotrienes and prostaglandin synthesis and an increase in circulating catecholamines. One possible source is the polymorphonuclear leucocytes (PMN). Stimulation of these cells is known to generate oxygen free radicals (Fantone & Ward 1982).

During volume overload, there is a relative myocardial ischaemia due to increased intraventricular pressure and increased oxygen demand due to increased intramyocardial tension. During ischaemia there is an increase in the xanthine and xanthine oxidase, and a decrease in the superoxide dismutase and glutathione peroxidase, activity leading to an increase in the oxygen free radicals (Jennings & Reimer 1982; Chambers *et al.* 1985; Guarnieri *et al.* 1980). During phagocytosis there is a release of a variety of mediators potentially deleterious to the myocardium, one of these being highly reactive oxygen free radicals (Fantone & Ward 1982). Leukotriene LTB_4, has been found to be a potent inducer of leucocyte chemotaxis, aggregation and degranulation (Ford-Hutchinson *et al.* 1980). Leukotriene LTB_4, a potent chemotactic and leucoagglutinating agent, is known to activate neutrophil that would result in secretion of oxygen free radicals.

It is proposed that volume-overload heart failure might be due to a decrease in the myocardial contractility because of increased activity of polymorphonuclear leucocytes (PMN). It was therefore decided to investigate the phagocytic activity of PMN in the failing heart due to induced chronic mitral insufficiency (volume overload) in dogs. Luminol-dependent chemiluminescence provides a sensitive indicator of the rate of production of oxygen free radicals by resting and stimulated PMN *in vitro* (Allen & Loose 1976). Therefore, oxygen free radicals production of PMN were measured by luminol-dependent chemiluminescence.

Methods and materials

The experiments were carried out on mongrel dogs of either sex weighing between 20 and 32 kg. The dogs were divided into two groups: (A) control, six dogs; (B) mitral insufficiency (MI) of 6–9 months' duration, six dogs.

Haemodynamic measurements

The dogs were anaesthetized with Pentothal sodium (25 mg/kg intravenously). The trachea was intubated and the dog was ventilated with room air using a Harvard respiratory pump with a volume of 20 ml/kg and a respiratory rate of 20/min. A satisfactory plane of anaesthesia was maintained with halothane via a closed-cuff endotracheal tube. A 7 French gauze Cournand (Cordis GF) catheter was positioned at the aortic arch through the femoral artery to record aortic pressure. The same catheter was pushed into the left ventricle to record left ventricular pressure. A Swan–Ganze balloon-tipped flow-directed catheter was positioned in the pulmonary artery to determine the cardiac output by the thermodilution technique using Edward cardiac output computer model 9510-A. This catheter was also used for collecting blood samples for measuring oxygen free radicals producing activity of polymorphonuclear leucocytes.

The measurement of haemodynamic parameters was similar to that described by Prasad & Bharadwaj (1987). The first derivative (dp/dt) of left ventricular pressure was recorded with a differentiating device coupled to left ventricular pressure at a frequency response of 100 Hz. Left ventricular pressure and the corresponding dp/dt and lead II ECG were recorded simultaneously on a Beckman R411 dynograph recorder. The pressures were recorded with an Ailtech microdot pressure transducer. Integrated isovolumic pressure (IIP) was measured (Yang *et al.* 1972). The ratio of $(dp/dt)/IIP$ was used as one index of myocardial contractility because it is not affected by pre-load and

by a small change in the heart rate (Yang *et al.* 1972). Cardiac index was calculated by previously described method of Prasad *et al.* (1977). For cardiac index the body surface area of the dog was determined according to the method of Ettinger & Suter (1970).

Surgical procedure for mitral insufficiency

Mitral insufficiency was produced by the previously described method of Prasad *et al.* (1977). Briefly, the heart was exposed by opening the chest through the fifth left intercostal space under anaesthesia. A small incision was made into the left atrium. The index finger was passed through this incision and through the mitral valve to detach two or more chordae tendinae from the anterior cusp of the valve to raise the left atrial pressure to 2.5–3 times the normal. The atriotomy was repaired, the lungs were inflated and the chest was closed. The murmurs and thrill were present throughout the period of observation.

The haemodynamic parameters were measured after 6 to 9 months of mitral insufficiency. Left ventricular failure was considered to be present when there was a decrease in the index of myocardial contractility (dp/dt)/IIP, cardiac index (CI), and an increase in the left ventricular end-diastolic pressure (LVEDP).

PMN counts and chemiluminescence measurement

Mixed venous blood samples were collected from both the normal dogs and dogs with mitral insufficiency in EDTA-containing blood collection tubes for measuring oxygen free radicals producing activity of polymorphonuclear leucocytes. Total nucleated blood cell counts were made using a Coulter S plus IV and differential counts were done from smears stained with Wright's Giemsa stain.

For measurements of luminol-dependent chemiluminescence, 0.1 ml (approximately 5×10^5 PMN) of blood were added to a counting vial containing 2.6 ml of Hanks' balanced salt solution (HBSS), pH 7.4, and luminol at a final concentration in the reaction vial of 10^{-4} M. Samples were placed in the luminometer and the background chemiluminescence were recorded for 10 min. Phagocytosis was initiated by the addition of 0.3 ml (10 g/l) of opsonized zymosan. The chemiluminescence activity was monitored for 70 min using a Picolyte model 6500 luminometer. In some experiments chemiluminescence was measured in the presence of superoxide dismutase (100 U/ml). The counts in the reaction vials were made for 5 s every 5 min for a period of 70 min. The results were finally expressed as counts per min (c.p.m.) per 10^5 PMN.

Luminol (5-amino-2,3-dihydrol-1,4-phthalazine dione), zymosan and superoxide dismutase (from bovine erythrocytes, 3050 U/mg protein) were purchased from Sigma Chemical Co. (St Louis, MO). All other chemicals were of reagent grade. Student's unpaired t-test was used in the statistical analysis. Statistical significance was considered as $P<0.05$.

Results

Haemodynamics

The haemodynamic data for normal and mitral insufficiency dogs of 6–9 months' duration are summarized in Fig. 1. The cardiac index, left ventricular end-diastolic

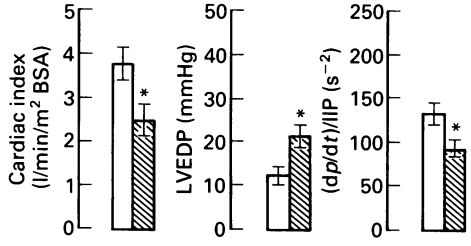

Fig. 1. Cardiac index, left ventricular end-diastolic pressure (LVEDP), and index of myocardial contractility (dp/dt)/IIP in □, six normal dogs and ▨, six dogs with 6–9 months of mitral insufficiency. The results are expressed as mean ± s.e. *$P<0.05$.

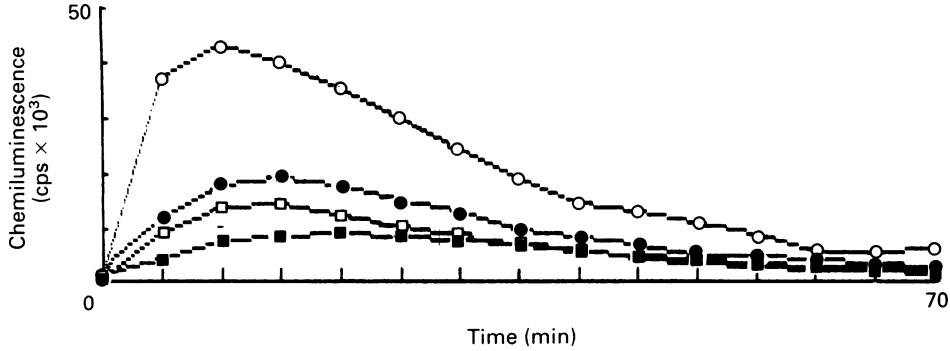

Fig. 2. A typical tracing of zymosan-stimulated chemiluminescence in blood from dogs with normal and failing heart due to mitral insufficiency (MI) in the presence and absence of superoxide dismutase (SOD). Abscissa shows the time and the ordinate indicates the chemiluminescence (counts per second). Note the peak value between 10 and 15 min after addition of zymosan. □, Normal; ■, normal+SOD; MI,○; MI+SOD, ●.

pressure, and index of myocardial contractility (dp/dt)/IIP for normal dogs were 3.8 ± 0.4 l/min/m^{2}BSA, 12.6 ± 2.0 mmHg and $132 \pm 10\ s^{-2}$ respectively. Six–9 months of mitral insufficiency produced a significant decrease in the cardiac index and index of myocardial contractility; and an increase in the left ventricular end-diastolic pressure. These haemodynamic data suggested that the left ventricle was in failure.

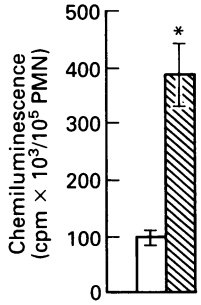

Fig. 3. Zymosan-stimulated polymorphonuclear leucocytes chemiluminescence in blood from □, normal dogs and ▨, dogs with left ventricular failure due to mitral insufficiency. The results are expressed as mean ± s.e. *$P < 0.05$.

Chemiluminescence

Zymosan-stimulated PMN chemiluminescence was recorded for 5 s every 5 min for 70 min. The results were obtained as counts per second. A typical graph of PMN chemiluminescence in blood from dogs with normal and failing heart in the presence or absence of superoxide dismutase (SOD) is shown in Fig. 2. The chemiluminescence increased rapidly with the addition of zymosan and reached a peak value within 10–15 min, after which it decreased slowly for the observation period. The peak value of chemiluminescence was higher in the dogs with failing heart than in the dogs with normal heart. Also, the zymosan-stimulated chemiluminescence was lower in the presence of SOD in both groups of dogs. This shows that the chemiluminescence was due to oxygen free radicals.

Zymosan-stimulated peak chemiluminescence activity of polymorphonuclear leucocytes in blood from normal dogs and dogs with left ventricular failure due to mitral insufficiency is summarized in Fig. 3. The chemiluminescence in the normal dog was found to be $(98.2 \pm 15.7) \times 10^3$ counts $min^{-1}\ 10^5\ PMN^{-1}$. The peak chemiluminescence activity of polymorphonuclear leuco-

cytes in the blood of dogs with left ventricular failure was 3.9-fold greater than that in the blood from normal dogs.

Discussion

The results showed that there was approximately a fourfold increase in the zymosan-stimulated polymorphonuclear leucocytes (PMN) chemiluminescence in dogs with left ventricular failure due to mitral insufficiency as compared to that in the normal dogs. It is to be noted that whole blood was used to investigate the PMN chemiluminescence in this study. No attempt was made to isolate PMNs. However, the chemiluminescence of the blood was expressed in terms of the PMN content of the blood. Whole blood has been used by various investigators in the past for chemiluminescence studies (DeChatelet & Shirley 1981; Tono-oko *et al.* 1983; Selvaraj *et al.* 1982).

Oxygen free radicals have been implicated in cardiac toxicity induced by pharmacological agents (Jackson *et al.* 1984) or ischaemia-reperfusion interventions (Jolly *et al.* 1984). Free radicals can be produced both intracellularly or from extracellular components such as polymorphonuclear leucocytes (Mullane *et al.* 1984). PMN stimulation is accompanied by production of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) which may react to form hydroxyl radical ($\cdot OH$) and singlet oxygen ($^1O_2$) (Fantone & Ward 1982; Del Maestro 1980). Subsequent involvement of leucocyte-associated myeloperoxidase generates hypochlorous acid (HOCl) from H_2O_2 and Cl^- (Del Maestro 1980). In the present study, the phagocytic activity of PMN was found to be increased in the dogs with failing heart. An increased amount of oxygen free radicals are produced during increased phagocytic activity of PMN. The increased amount of oxygen free radicals would then depress the myocardial contractility leading to left ventricular failure. It has been shown by Hess *et al.* (1982) that exogenous generated oxygen free radicals depressed the Ca^{2+} accumulation by sarcoplasmic reticulum (SR) and Ca^{2+} ATPase of SR. The studies of Prasad *et al.* (1989) have also shown that the exogenous oxygen free radicals decreased the cardiac function and index of myocardial contractility.

There was a fourfold increase in the PMN chemiluminescence in the dogs with left ventricular failure as compared to that in the normal dogs. The mechanism for this increase in the PMN chemiluminescence in the dogs with failing heart is not known. Various factors might lead to an increase in the PMN chemiluminescence. An increased chemiluminescence might be due to an inherent change in the property of leucocytes in failing heart. Infection can be ruled out as a cause of increased chemiluminescence because it is unlikely that only the dogs with heart failure were infected. Another important mechanism for an increased activation of PMN and hence increased secretion of oxygen free radicals ('respiratory burst') in the failing heart might be ischaemia-induced release of arachidonic acid from phospholipids. The increase in the chemiluminescent activity of PMN in dogs with failing heart indicates an increase in the production of oxygen free radicals which would lead to a decrease in the contractility by depressing Ca^{2+} accumulation by SR and Ca^{2+} ATPase of SR. Xanthine and the xanthine oxidase system may also be involved in the left ventricular failure. Oxygen free radicals are produced by all the above-mentioned procedures. However, the present study deals only with the role of PMN in left ventricular failure due to volume overload.

Acknowledgements

This work was supported by the Saskatchewan Health Research Board and Saskatchewan Heart Foundation. We wish to thank Mr P.K. Chattopadhyay for technical assistance and Ms Karen Bader for preparing the manuscript.

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