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Endoxin: a major factor regulating cardiovascular system[1]

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ABSTRACT

Endoxin is a factor with a digitalis-like biological activity. It is a Na^+ pump inhibitor and may be an endogenous medium of digitalis receptor. There are abnormal plasma levels of endoxin in some pathophysiologic states such as hypertension, acute myocardial infarction, arrhythmia, heart failure, *etc*. Some studies have demonstrated that the abnormal endoxin levels may be implicated in pathogenesis of these diseases or pathophysiologic process involved. Therefore, to clarify the effects of endoxin has much significance in understanding pathogenesis, prevention and treatment of hypertension and other cardiovascular diseases.

INTRODUCTION

It has been proved that Na^+-K^+-ATPase in membranes is a digitalis receptor. This finding makes people to search for its endogenous mate and antagonist. Fishman, Haupert *et al*[1,2] first separated endoxin from mammalian hypothalamus in 1979. Gruber *et al*[3] separated endoxin from plasma of dog in 1980. Endoxin was also known as endogenous digitalis-like factor or substance, endogenous ouabain-like factor or substance, endogenous digoxin-like factor or substance or third factor. This paper reviews the relationship between endoxin and some cardiovascular diseases, and effect of anti-digitalis antibody in prevention and treatment of these cardiovascular disorders.

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STRUCTURE, DISTRIBUTION, SECRETION, BIOSYNTHESIS, BIOLOGIC ACTIVITIES, AND PHYSIOLOGIC FUNCTION OF ENDOXIN

Endoxin has been extensively researched upon regarding its structure, secretion, biosynthesis, biologic activities, physiologic function and role in cardiovascular diseases in the last 20 years. Endoxin has a chemical structure of either ouabain or of one of its isomers in which the location and orientation of two or more steroidal hydroxyl groups differ.

Circulating levels of endoxin depend upon the adrenal cortex and metabolic events preceding and following pregnenolone formation involved in endoxin biosynthesis[4,5]. Within the adrenal gland, the stimulus-secretion mechanisms for endoxin are distinct from those for aldosterone. Laredo *et al*[6] found that angiotensin Ⅱ (AT Ⅱ) stimulated the secretions of endoxin, aldosterone, and cortisol from bovine adrenocortical cells. The nonselective AT Ⅱ receptor antagonist (Sar^1-Ile^8) blocked AT Ⅱ-stimulated secretion of all three steroids without affecting basal output. In the presence of the angiotensin type 1 receptor (AT_1R) antagonist DuP753, AT Ⅱ-stimulated secretions of aldosterone and cortisol were blocked while secretion of endoxin was unaffected. In the presence of the angiotensin type 2 receptor (AT_2R) antagonist PD123319, both basal and AT Ⅱ-stimulated secretions of aldosterone and cortisol were normal while stimulated secretion of endoxin was inhibited. The secretion of endoxin was activated maximally by the AT_2R agonist CGP42112 under conditions in which aldosterone secretion was unaffected. These results demonstrate that AT_2R stimulate secretion of endoxin from bovine adrenocortical cells. But, the second messenger mechanisms involved in this secretion are not known. Shah *et al*[7,8] investigated the effects of several pharmacologic agents that affect signaling pathways on the basal and stimulated secretions of aldosterone and endoxin from primary cell cultures of bovine adrenocortical cells. The AT_2R antagonist PD123319, blocked the effects of AT Ⅱ on secretion of endoxin but not aldosterone. Treatment of the cells with either dibutyryl cAMP, a membrane permeant

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analog, or the phorbol ester tetradecanoyl phorbol acetate stimulated aldosterone secretion but had no effect on the secretion of endoxin. On the other hand, the membrane permeant analog, 8BcGMP, maximally activated secretion of endoxin whereas incubation of cells with sodium orthovanadate blocked AT Ⅱ stimulated secretion of endoxin. Neither 8BcGMP nor sodium orthovanadate affected the basal or stimulated components of aldosterone secretion. These results show that the secretions of aldosterone and endoxin from bovine adrenocortical cells are mediated by different intracellular signaling mechanisms.

Takahashi and Bernini *et al*[9,10] found that endoxin was produced in the hypothalamus but not in the adrenals, and that the adrenal glands influence the turnover of the hypothalamic endoxin. Yamada *et al*[11] reported that endoxin neuronal somata were mainly localized in the paraventricular nucleus and the supraoptic nucleus and its accessory nuclei. A number of somata were also scattered in other hypothalamic areas. The processes of these neurons ran from the areas where the somata were located, through the lateral and basal area of the hypothalamus, to the infundibulum. These nerve fibers with varicosities were associated with the primary capillaries of hypophysial portal veins. A few immunopositive nerve fibers were also seen in the pituitary posterior lobe. Intensive immunoreactivities were observed in the subfornical organ and organum vasculosum laminae terminalis.

Endoxin is carried by albumin in plasma[12]. Volume load, especially hypernatremic load, stimulates its secretion[13]. Release of endoxin from cultured bovine pulmonary arterial endothelial cells and myocardial cells is increased under hypoxia[14,15]. Atrial natriuretic polypeptide (ANP) administration resulted in a significant elevation of sodium excretion and glomerular filtration rate and a fall in blood pressure. Endoxin concentrations in plasma rose significantly, as did urinary endoxin excretion. ANP administration resulted in a fall in aldosterone as well as ACTH[16].

The studies have shown that endoxin is an endogenous medium of digitalis receptor and can remarkably inhibit Na^+-K^+-ATPase activity in cell membrane, has competitive displacing activity against [^{3}H]-ouabain binding to the enzyme, inhibitory activity for ^{86}Rb uptake into intact human erythrocytes, cross-reactivity with anti-digoxin antibody. Therefore, it is a Na^+ pump inhibitor. Na^+ pump in cell membrane maintains intracellular ionic concentration and controls membrane potential. Its inhibition by endoxin enhances the intracellular Na^+ concentration. This in turn activates the Na^+-Ca^{2+} exchange mechanism, which induces intracellular Ca^{2+} increase, membrane depolarization, and noradrenaline release from perivascular adrenergic nerve endings. In addition, endoxin stimulated endothelin-1 (ET-1) secretion in a dose-dependent manner from cultured endothelial cells[17]. These are its mechanisms that promote vasoconstriction, positive inotropic effect, enhance renal tubular sodium excretion. It was considered to play a causative role in pathogenesis such as hypertension, heart failure *etc*.

ENDOXIN AND CARDIOVASCULAR DISEASES

Essential hypertension As a vasoactive substance, endoxin can participate in the regulation of blood pressure and play a role in the pathogenesis of arterial hypertension. Some studies have shown that the concentrations of plasma endoxin remarkably rise in essential hypertension. Sagnella *et al*[18] examined concentration of plasma endoxin in 22 patients with essential hypertension. It was found that concentrations of plasma endoxin in patients with essential hypertension were remarkably higher than that in healthy subjects, and had a positive correlation with systolic blood pressure (SBP). We have also observed similar results[19]. Baldoncini *et al*[20] evaluated the effect of ANP infusion on plasma and urinary endoxin levels, 18 essential hypertensive patients were randomly double-blind assigned to receive either ANP ($0.3\ g \cdot kg^{-1} \cdot min^{-1}$, $n = 10$) or its vehicle (50 mL isotonic saline, $n = 8$) over a period of 60 min in supine position after 1 week on a normal NaCl intake (120 mmol/24 h). Plasma and urinary endoxin levels were measured at −60, 0, 30, 60, 120, 180, and 240 min (infusion time from 0 − 60 min). During ANP infusion, plasma endoxin levels decreased [from $(25 \pm 7)\ ng \cdot L^{-1}$ at 0 min to $(12 \pm 6)\ ng \cdot L^{-1}$ at 60 min, $P < 0.01$], while urinary endoxin excretion increased [from $(60 \pm 26)\ ng \cdot L^{-1}$ at 0 min to $(246 \pm 34)\ ng \cdot L^{-1}$ at 30 min, $P < 0.01$; and $(402 \pm 44)\ ng \cdot L^{-1}$ at 60 min, $P < 0.01$]. After 3 h of ANP infusion, both plasma and urinary endoxin returned to baseline levels. The data show that ANP infusion increased urinary endoxin excretion and decreased its circulating levels. This different response of ANP may be explained due to an acute increase of extracellular fluid volume (ECFV) which might simultaneously inhibit the increase in circulating endoxin levels by promoting the urinary excretion of this substance.

Experimental hypertension Many studies had been publicized in this regard. Yamada *et al*[21] determined the levels of plasma endoxin to assess the role of endoxin in volume-expanded, reduced renal mass (RRM)-saline (S) hypertension model in rats. In the first stage of the experiment, at 3 weeks after subtotal nephrectomy and after drinking 1 % saline solution, SBP of 18 rats with RRM-S was significantly higher than in 17 sham-operated saline-drinking control (C-S) rats [(154 ± 4) *vs* (132 ± 2) mmHg, $P < 0.01$]. Plasma endoxin levels were seven folds higher in RRM-S rats than in C-S rats [(355 ± 68) *vs* (54 ± 4) pmol·L^{-1}]. In the second stage of the experiment, plasma endoxin levels of 10 RRM-S, 12 C-S, and 10 subtotally nephrectomized rats drinking distilled water (RRM) were measured. Concomitant with a marked increase in blood pressure, RRM-S rats showed significantly higher plasma endoxin levels compared with C-S and RRM rats. In both experiments, plasma endoxin levels correlated significantly with SBP. These findings suggested that plasma endoxin may play a role in hypertensive mechanisms in rats with RRM and excess Na^+ intake. Takada *et al*[22] evaluated the urinary excretion of endoxin to investigate its pathophysiologic roles in sodium metabolism and blood pressure in 5/6 RRM rats, a model-expanded hypertension. About five-sixths of kidney mass (5/6 RRM, $n = 9$) was removed from male Sprague-Dawley rats, or the rats were sham operated (control, $n = 10$). Both groups were fed regular diets with tap water for 1 week as a control period, followed by 1 % saline solution for 4 weeks. SBP, urine volume (UV), urine Na volume (UNaV), and endoxin were measured on the last 2 d of every week throughout the experiment period. SBP and UNaV were higher in 5/6 RRM rats than in control rats. Urinary endoxin significantly increased, reaching peak value in the first week. There was a significant positive correlation between the change in endoxin and the changes in UNaV and SBP. Other authors[23,24] also found that tissue and plasma levels of endoxin were higher in Milan hypertensive rats than in Milan normal rats and were not influenced by exogenous endoxin sources. Fedorova *et al*[25] found that the intramuscular ACTH treatment (0.5 mg·kg^{-1}·d^{-1}) for 8 d in male Fisher 344NB rats resulted in increased SBP [(151 ± 12) *vs* (121 ± 4) mmHg, $P < 0.01$], inhibition of Na^+-K^+-ATPase in aortic sarcolemma [(2.99 ± 0.35) *vs* (5.43 ± 0.17) mol ADP·mg^{-1} Protein·h^{-1}]. These findings suggested that endogenous Na^+-K^+-ATPase inhibitor was increased after 8 d of treatment of rats with ACTH. We[26] found that the myocardial tissue activities of Na^+-K^+-ATPase in spontaneously hypertensive rats (SHR) were remarkably lower than in WKY rats; the level of endoxin in SHR was remarkably higher than in WKY rats.

These results demonstrate the importance of endoxin in various forms of experimental hypertension.

Cardiac hypertrophy Many patients with essential hypertension exhibit increased left ventricular mass, the latter may be in relation to endoxin. An electrocardiogram and an echocardiogram of patients with severe hypertension showed severe left ventricular hypertrophy and elevated plasma aldosterone levels[27]. Magnetic resonance imaging (MRI) revealed a small mass in the right adrenal gland. Before removal of the tumor, plasma endoxin levels were elevated. After removal of the tumor, endoxin levels quickly returned to the normal level. A series of echocardiograms and electrocardiograms over a 6-year period after removal of the tumor showed marked regression of cardiac hypertrophy. These findings suggest that endoxin may be closely related to the development of concentric cardiac hypertrophy in primary aldosteronism. Manunta *et al*[28] investigated relationships among plasma endoxin, left ventricular mass, and cardiac function in patients with essential hypertension. Plasma endoxin was determined in 110 normotensive subjects and 128 patients with essential hypertension. Echocardiographic parameters and humoral determinants were measured in essential hypertension. Plasma endoxin levels were increased in patients with essential hypertension versus normotensive subjects. The distribution of plasma endoxin was unimodal in normotensives, whereas it was bimodal in essential hypertension. Twenty-four-hour diastolic ambulatory blood pressure was slightly higher in essential hypertension with high endoxin compared with essential hypertension with normal endoxin ($P < 0.05$). Left ventricular mass index and stroke volume in essential hypertension with high endoxin were greater than in essential hypertension with normal endoxin ($P < 0.01$, $P < 0.05$, respectively), although heart rate was slower ($P < 0.01$). Multiple regression analysis that tested the influence of body mass index, age, gender, 24-hour blood pressure, and endoxin on left ventricular mass revealed independent contributions of SBP (13.2 %), diastolic blood pressure (DBP) (12.4 %), and plasma endoxin (11.6 %). They concluded that approximately 50 % of patients with uncomplicated essential hypertension had elevated-high circulating endoxin level, that later affected cardiovascular function and struc-

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ture and thus may be considered as a factor that contributes to the risk of morbid events. Hayashi *et al*[29] investigated plasma endoxin concentrations and right ventricular endomyocardial biopsy specimen in 40 patients with hypertrophic cardiomyopathy. An increase in endoxin of more than 0.2 $\mu g \cdot L^{-1}$ in plasma was found in six out of 27 patients with non-obstructive hypertrophic cardiomyopathy (22.2 %) and five out of 13 with obstructive hypertrophic cardiomyopathy (38.4 %). Under light microscopy, positive staining against digoxin antibody was observed heterogeneously on some cardiocytes. In non-obstructive hypertrophic cardiomyopathy, endoxin in plasma correlated positively with the left atrial dimension and inversely with the cardiac index. In obstructive hypertrophic cardiomyopathy, plasma and myocardial endoxin were positively correlated; they also correlated with left ventricular end-diastolic pressures. Under electron microscopy, endoxin was detected in the sarcolemma of the free wall, T-tubules, intercalated disc and Z-bands of cardiocytes. We[26] also found that concentration of endoxin in myocardial tissue was positively correlated with transverse diameter of myocardial cell in SHR.

These results suggest that endoxin may be taking part in the pathophysiologic processes of hypertrophic cardiomyopathy or ventricular hypertrophy.

Acute myocardial infarction In 1990, we[30] first found that the concentrations of serum endoxin in patients with acute myocardial infarction (AMI) were higher than those of normal subjects during 1 – 4 d after infarction. The concentrations of serum endoxin gradually dropped with time after infarction. The concentrations of serum endoxin were not different between patients with anterior and anteroseptal MI and patients with inferior MI. The concentrations of patients with arrhythmia were higher than those of patients without arrhythmia in 1st and 2nd day after AMI. The concentrations were positively correlated with SBP, DBP, heart rate (HR), serum Na^+, blood sugar (BS), and number of red blood cells (RBC). Because high levels of serum endoxin or use of exogenous digitalis preparation (with higher plasma concentration of digitalis) during acute stage of AMI make areas of myocardial infarction extension, and cause arrhythmias, the reasons for not using digitalis preparation during the early period of AMI are elucidated by this study. Bagrov *et al*[31,32] also found similar results showing that plasma concentrations of endoxin in patients on the 1st day after AMI were increased as compared with both healthy controls and patients with unstable angina pectoris. On the 1st day after AMI, the plasma levels of endoxin in seven patients with primary ventricular fibrillation were higher than in 47 patients without ventricular fibrillation. In 14 patients with AMI and congestive heart failure (class III, Killip), plasma concentrations of endoxin were lower than in 40 patients with AMI without congestive heart failure. The results show that changes in plasma endoxin may be involved in myocardial ischemia-induced arrhythmogenesis and may participate in the pathogenesis of congestive heart failure after AMI. Kohn *et al*[33] found that concentration of serum endoxin in patients afflicted with AMI increased in comparison with health subjects. An increase of endoxin in patients with AMI probably coincides with a decreased cardiac output, with the activation of the stress axis and retention of sodium and fluids. But no significant relation of endoxin with the activity of creatinekinase, AMI localization, occurrence of arrhythmias, heart insufficiency and AMI mortality have been observed.

Congestive heart failure Pathogenesis of congestive heart failure is not clear yet. Now that it has been observed that Na^+-K^+-ATPase in membranes is a digitalis receptor, heart failure may be correlated with functioning of digitalis receptor and its endogenous medium. We[34,35] first put forward the hypothesis that relative and/or absolute insufficiency of endoxin secretion takes part in the pathogenesis of heart failure. This is also the basis of treatment of congestive heart failure using exogenous digitalis. We found that the concentrations of serum endoxin in patients with congestive heart failure were lower than those of normal subjects. The concentrations of serum endoxin correlated inversely with the severity of heart failure. A significantly positive correlation between concentrations of serum endoxin and left ventricular ejection fraction (LVEF) were observed. Bagrov *et al*[31,32] also found similar results in patients with AMI. Hemodynamic abnormalities induced by an antagonist of endoxin also sustained our previous hypothesis. However, there are also contradictory reports about changes in serum endoxin in patients with heart failure[36,37]. The difference may be related with etiology, duration of heart failure, history of using exogenous digitalis, hepatic and renal function, *etc*.

Cor pulmonale The patients with cor pulmonale have a lower digitalis tolerance. This may be related to multiple factors including endoxin. We[38-40] investigated the concentrations of serum endoxin in patients with acute attack of chronic cor pulmonale (CCP) and normal subjects. The results showed that serum endoxin in pa-

tients with acute attack of CCP were markedly lower than those of normal subjects, but were markedly increased during the remission stage. The concentrations of serum endoxin had a significant negative correlation with the severity of heart dysfunction and p_{aCO_2}, and a significantly positive correlation with p_{aO_2}. The course of the disease, the status of hepatic and renal function, and blood pH obviously affected the concentrations of serum endoxin. Meanwhile, we found that difference between serum digitalis concentrations and serum endoxin concentrations in patients with digitalis poisoning was beyond 1.0 g·L^{-1}, and the difference was higher than that in patients without digitalis poisoning, latter within 1.0 g·L^{-1}. Therefore, calculation of absolute increase in values of serum digitalis concentrations have major significance in prognosis of digitalis poisoning. This may elucidate why serum digitalis concentrations overlap between digitalis poisoning and no poisoning. The reason is that there are different levels of endoxin in each subject, which cause different serum digitalis concentrations (Fig 1). However, there is a reverse report about change in serum endoxin in patients with cor pulmonale[41].

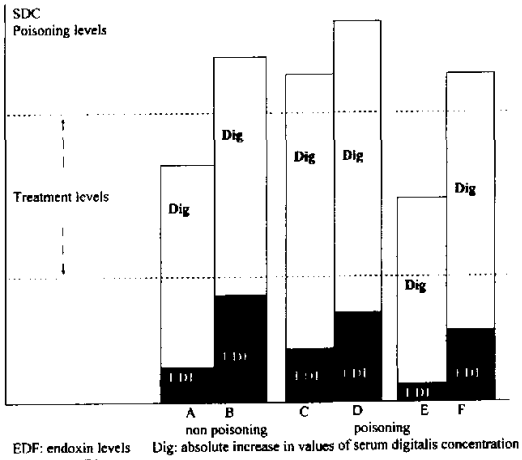

Fig 1. Difference in endoxin levels in different subjects causing different SDC.

Diabetes mellitus Endoxin abnormality may also take part in the pathophysiologic process of diabetes mellitus. Martinka *et al*[42] assessed relationship of endoxin to glucose tolerance and insulin levels in pregnant women. They found that endoxin levels in hyperinsulinemic pregnant women were higher than in those with normal insulin levels; endoxin thus significantly correlated with insulin levels as well as with insulinogenic index; the increase in plasma glucose and insulinemia during oral glucose tolerance test (OGTT) was accompanied by a decrease in endoxin. These findings suggest that endoxin does not respond only to changes regarding sodium-retention and volume-expansion, but also to changes in glucose and insulin metabolism. Pamnani *et al*[43] examined the role of plasma endoxin in the hypertensive streptozotocin-induced insulin dependent diabetic (IDDM) RRM rats. The increase in blood pressure associated with an increase in ECFV, and endoxin and a decrease in myocardial Na^+-K^+-ATPase activity, suggested that increased endoxin, which inhibits cardiovascular muscle cell Na^+-K^+-ATPase activity, might be involved in the mechanism of IDDM-hypertension. In a second study, the heart prolonged impulse *in vitro* resulted in a decrease in cardiac Na^+-K^+-ATPase activity, suggesting that cardiovascular deconditioning might in part result from insufficient endoxin. In order to elucidate the causal relationship between Na^+-K^+-ATPase and diabetic nephropathy, Mimura *et al*[44] studied the erythrocyte Na^+-K^+-ATPase activity in NIDDM. Na^+-K^+-ATPase activity in microalbuminuric patients was reduced compared with those without microalbuminuria and control subjects. Microalbuminuric patients had higher SBP and greater frequency of parental hypertension than those without microalbuminuria. Na^+-K^+-ATPase activity in diabetic patients with hypertension was reduced compared with those in patients without hypertension. Moreover, Na^+-K^+-ATPase activity in diabetic patients with parental hypertension was significantly reduced compared with those in patients without parental hypertension. There was no difference in erythrocyte Na^+ content in diabetic patients with and without microalbuminuria. So did in diabetic patients with and without hypertension, with and without parental hypertension. But plasma endoxin showed no correlation with Na^+-K^+-ATPase activity in diabetic patients with microalbuminuria or hypertension or parental hypertension. Straub *et al*[45] compared the intensity of typical late complications in diabetic patients who were not on glycoside drugs with low *vs* high levels of serum endoxin. Patients with high endoxin levels showed better test results in vibratory perception, had better percentile localization concerning maximal pupillary area in darkness, contraction velocity at 1 s, and dilation velocity at 6 s, had less retinopathy, and better percentile localization in the respiratory sinus arrhythmia test. There was no difference concerning nephropathy, blood pressure, coronary

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heart disease and peripheral vascular disease. Nishimura *et al*[46] investigated whether urinary excretion of free dopamine was related with the humoral factors which affect Na^+-K^+-ATPase activity in kidney. They found that urinary excretion of free dopamine and endoxin were and positively correlated with urinary excretion of C-peptide in normotensive subjects, but not in hypertensive subjects. In normotensive subjects, urinary excretion of free dopamine and endoxin may be regulated at least in part by insulin secreted endogenously. In hypertensive subjects, however, this regulatory mechanism of the diuretic factors, such as insulin, endoxin and dopamine, was thought to be disturbed, which resulted in decompensation of a diuretic and antidiuretic balance leading to blood pressure elevation. Graves *et al*[47] explored serum endoxin in IDDM women with normotensive pregnancies or pregnancy-induced hypertension and compared it to nondiabetic pregnant women. The results demonstrated that nondiabetic women with preeclampsia had increased serum endoxin levels as compared to normotensive pregnant women. Women with transient hypertension of pregnancy had intermediate values. Pregnant normotensive IDDM women had higher serum endoxin than nondiabetic counterparts. These results suggest that endoxin may be taking part in pathogenesis of IDDM related complications and pregnancy-induced hypertension.

Arrhythmias Pathogenesis of arrhythmia are related with abnormal transportation of ions. Endoxin, serving as an inhibitor of Na^+-K^+-ATPase in membranes, may be taking part in production and development of arrhythmias. Kieval *et al*[48] used standard microelectrode techniques to study the direct effects on the cellular electrophysiology of canine Purkinje fibers of endoxin. They found that endoxin reduced maximal diastolic potential, action potential amplitude and duration and maximal rate of rise of phase 0, and produced delayed afterdepolarizations and triggered activity. We[49,50] found that concentrations of serum endoxin in patients with ventricular premature beats were markedly higher than in those of normal cases; in patients with rapid atrial fibrillation were markedly lower than in those of normal cases; in patients with atrial premature beats were slightly lower than in those of normal cases. The concentrations of serum endoxin in patients with ventricular premature beats dropped and with atrial fibrillation rose as the arrhythmia were controlled or improved. Bagrov *et al* and we[30-32] also found that endoxin might be a major cause of arrhythmia induced by AMI. Therefore, endoxin may be participating in the pathogenesis of arrhythmias.

CLINICAL APPLICATION OF ANTI-DIGITALIS ANTIBODY OR ANTISERUM: ANTAGONIST OF ENDOXIN

Antibodies, long used as discriminating tools in immunoassay, are now being used *in vivo*, both in diagnosis and therapy. In cardiovascular medicine, applications that have reached the stage of clinical trial include the reversal of digitalis intoxication by digoxin-specific antibodies and the imaging of cardiac necrosis with monoclonal myosin-specific antibodies. Now that endoxin has major effect in pathogenesis of these diseases, so it can be likely that antagonist of endoxin — anti-digitalis antibody or antiserum may be used to prevent and treat these diseases. Some studies have demonstrated their excellent values.

Hypertension In order to evaluate the role of endoxin in the genesis of DOCA-salt hypertension, Kojima *et al*[51] in 1982 observed the effects of intravenous anti-digoxin antibody on the blood pressure response in male Wistar rats that underwent heminephrectomy followed by treatment with DOCA and saline. Administration of anti-digoxin antibody caused a marked decrease in the blood pressure which continued for about an hour. Such a change in the blood pressure was not observed in pertinent control animals. Li *et al*[52] observed hemodynamic effects of digibind (anti-digoxin antibody) in ACTH-induced hypertension. ACTH increased SBP from (118 ± 2) mmHg to (132 ± 3) mmHg on treatment day 10. SBP was unchanged in sham treated rats. The acute administration of digibind decreased mean artery pressure (MAP) (14 ± 3) mmHg in ACTH hypertensive rats, but not in ACTH sham control normotensive rats. Blood pressure reached a minimum after (14 ± 3) min and the effect lasted for more than 30 min. No significant change in blood pressure was found in ACTH treated rats receiving sham (0.9 % NaCl) digibind injection. Huang *et al*[53] evaluated the effects of blockade of brain endoxin on MAP and renal sympathetic nerve activity (RSNA) in conscious Dahl salt-resistant (Dahl R) and Dahl salt-sensitive (Dahl S) rats on a regular or high sodium diet from 4 to 7 weeks of age. Intracerebroventricular injection of digoxin-specific antibody Fab (DAF) fragments did not change basal MAP and RSNA during the first 4 h after administration in Dahl S rats on a high sodium diet for 3 weeks. However, 18 h after the injection of DAF fragments, basal MAP and RSNA were significantly decreased, reaching values for Dahl S rats on a regular sodium diet. Huang *et al*[54] found that a marked lowering of arterial blood pressure was observed

in chronic aortic coarctate hypertensive rats after intravenous administration of anti-digoxin antiserum. The hypotensive effect lasted for about 30 min after dosing. Yuan *et al*[55] explored the effects of ouabain antibody on the blood pressure in renovascular hypertensive rats and the relationship between endoxin and the development of hypertension. The results showed that the ouabain antibody could decrease the blood pressure of rats with 1K1C and 2K1C-salt, lasted 30 – 60 min, but little change in blood pressure was observed in 2K1C rats. The normal rabbit IgG did not decrease blood pressure in any renovascular hypertensive models. Goodlin[56] found that some pregnant patients with toxemia, especially those with thrombocytopenia, liver or renal dysfunction had elevated serum endoxin. So it was proposed that anti-digoxin antibody may be tried in the treatment of these selected patients with toxemia of pregnancy and the results were successful. Mann *et al*[57] compared the effects of intravenous injection of intact digoxin antibody (0.3 mg/rat) and its Fab fragment (40 mg/rat) on blood pressure, cardiac output and total peripheral resistance in conscious SHR and deoxycorticosterone hypertensive rats. *In vitro* findings showed that Fab fragment bound radio-labeled digoxin, digitoxin and ouabain more efficiently than did intact antibody. *In vivo*, Fab fragment prevented the increase in total peripheral resistance induced by intravenous injection of digoxin. However, Fab fragment did not alter blood pressure, cardiac output or total peripheral resistance in normal and salt-loaded SHR and in deoxycorticosterone hypertensive rats. But intact digoxin antibody lowered blood pressure in SHR and in deoxycorticosterone hypertensive rats.

These experimental data show that anti-digitalis antibody may be one of drugs that may effectively prevent and treat hypertension. Meanwhile, these findings also certify that endoxin is one of main causes for induction of hypertension.

Arrhythmias Bagrov *et al*[58] demonstrated that myocardial infarction was marked by a negative linear correlation between the intensity of ventricular fibrillation and the activity of Na^+-K^+-ATPase of intact erythrocyte that mirrored the content of circulating endoxin in rats. Administration of digoxin antibodies to the animals resulted in an antiarrhythmic effect and in the recovery of enzyme activity. They[59] further studied the effect of anti-digoxin antiserum on the ventricular fibrillation threshold (VFT) after coronary ligation in cats and on ventricular arrhythmias caused by myocardial infarction in rats and chloroform-induced hypoxia in mice. Intravenous administration of anti-digoxin antiserum (5 $mg \cdot kg^{-1}$) enhanced VFT in cats with myocardial infarction from (11.3 ± 1.6) to (53.3 ± 8.1) V and reduced ventricular arrhythmias in rats and mice.

Hypoxia-reoxygenation damage Intracellular Ca^{2+} overload is the molecular biological basis of myocardial ischemic reperfusion injury. Intracellular Ca^{2+} overload is related to some factors inhibiting ATPase activity in cell membranes. Endoxin can remarkably inhibit Na^+-K^+-ATPase activity in cell membranes, and may be associated with progression of myocardial ischemic reperfusion damage. Our lab has evaluated the protective effect of anti-digoxin antiserum on hypoxia-reoxygenation induced injured myocardium[60]. The results showed that the level of endoxin was remarkably higher, ATPase activities in cell membrane were remarkably lower in hypoxic group and hypoxia-reoxygenation injury group than those in normal group; anti-digoxin antiserum could resume ATPase activity in a concentration-dependent manner. This experiment demonstrated that anti-digoxin antiserum could lessen myocardial injury and may have a protective effect on hypoxia-reoxygenated myocardium by antagonizing the effects of endoxin.

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内源性洋地黄因子：调节心血管系统的一个重要因子[1]

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关键词 高血压；心肥大；心肌梗死；肺心病；充血性心力衰竭；糖尿病；心律失常

内源性洋地黄因子是一种具有洋地黄样生物活性的因子，是钠泵抑制剂和洋地黄受体的内源性递质．在某些病理生理状态下如高血压、急性心肌梗死、心律失常、心力衰竭等，血浆内源性洋地黄因子水平发生异常变化．一些研究证明，内源性洋地黄因子的分泌异常参与这些疾病的发病机制和病理生理过程．纠正这种异常可以预防和治疗这些疾病．因此，阐明内源性洋地黄因子的作用对明确高血压等心血管疾病的发病机制和防治具有重要意义．

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