Effect of Dibutyryl Cyclic AMP on Hemodynamics and Chemical Mediators in Dogs with Experimentally-Induced Endotoxic Shock

Shozo OKANO¹, Masahiro TAGAWA, Norimoto URAKAWA¹, and Ryo OGAWA²

Division of Veterinary Surgery, ¹Veterinary Pharmacology, Nippon Veterinary and Animal Science University, 1–7–1 Kyonan-cho, Musashino, Tokyo 180, and ²Department of Anesthesiology, Nippon Medical School, 1–1–5 Sendagi, Bunkyo-ku, Tokyo 113, Japan

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ABSTRACT. The therapeutic effect of dibutyryl cyclic AMP (DBcAMP) in endotoxic shock was evaluated, using 11 dogs with experimentally-induced endotoxic shock (5 in DBcAMP group and 6 in control group) under general anesthesia. The DBcAMP group was treated by single intravenous injection of DBcAMP (10 mg/kg) at 15 min before inoculation with endotoxin (3 mg/kg). After the inoculation of endotoxin, this group was given drip infusion of DBcAMP at a rate of 0.1 mg/kg/min over 180 min. Hemodynamic parameters and chemical mediators were measured until 360 min after endotoxin inoculation. The cardiac output and urinary volume, which were decreased in the control group, were significantly inhibited to decrease in the DBcAMP group (p<0.01). The increases in 6-keto-PGF₁α and thromboxane B₂, chemical mediators released in endotoxic shock, were significantly inhibited (P<0.05 and p<0.01, respectively). These results suggest that DBcAMP is useful for the treatment of endotoxic shock. — KEY WORDS: canine, dibutyryl cyclic AMP, endotoxic shock, thromboxane B₂, 6-keto-PGF₁α.


Endotoxic shock is triggered by injection of gramnegative microbes. A diversity of studies have been undertaken on its etiology, pathology and therapeutic management [7, 9, 17, 22]. An especially interesting finding obtained in studies of the pathology and therapy of endotoxic shock is the release of a variety of chemical mediators. Among them, lipid mediators such as prostaglandin I₂ (PGI₂) and thromboxane A₂ (TXA₂) have drawn much attention [4, 10, 11, 22].

It has been reported that unlike cyclic AMP (cAMP), dibutyryl cyclic AMP (DBcAMP), a derivative of cAMP, relatively easily permeates cell membranes [12], has a positive inotropic and a vasodilative action [1, 14, 15, 23, 30], and with these facts it is useful in the management of circulatory in sufficiency and shock.

In this study, the effects of DBcAMP were investigated from the behaviors of hemodynamic parameters and arachidonic acid cascade (6-keto-PGF₁α and thromboxane B₂), which are respectively the stable metabolites of PGI₂ and TXA₂ in dogs with endotoxic shock.

MATERIALS AND METHODS

Eleven clinically healthy Beagle dogs (9.5–14 kg) without friarea infection were randomly divided into a DBcAMP-treated group (n=5) and a control group (n=6). The dogs were anesthetized with atropine sulfate (0.05 mg/kg, i.m.) and pentobarbital sodium (30 mg/kg, i.v.), and were immobilized by injection of pancuronium bromide (0.1 mg/kg, i.v.). Then, the respiration was controlled with room air so that the arterial carbon dioxide tension was maintained at 30–40 mmHg. In a right lateral recumbent posture, an 7 french catheter was inserted and dwelt in the aorta via the femoral artery for measurement of the aortic pressure and sampling of blood and a 5 french Swan-Ganz catheter in the pulmonary artery via the femoral vein for measurement of the pulmonary arterial pressure and cardiac output (CO). During the experiment, anesthetics and a muscle relaxant were additionally used as necessary to stabilize the anesthesia.

On completion of all experimental preparation, pre-experimental data were collected after confirming the stabilization of hemodynamic parameters. The DBcAMP group was given 10 mg/kg of DBcAMP (Daichii Pharmaceutical Co., Ltd., Tokyo) and the control 5 ml of physiological saline, by single intravenous injection, respectively. At 15 min after this procedure, 3 mg/kg of endotoxin (Escherichia coli 055: B5, Difco, Detroit, U.S.A.) were inoculated intravenously over 5 min in the both groups. Furthermore, from immediately after inoculation of the endotoxin, the DBcAMP group had intravenous infusion of DBcAMP at a rate of 0.1 mg/kg/min over a period of 180 min, and the control group was given intravenously 10 ml of physiological saline over a period of 180 min.

The following hemodynamic parameters were measured; heart rate (HR), mean aortic pressure (MAP), mean pulmonary arterial pressure (MPAP), CO by thermal dilution, and urinary volume (UV). From the values obtained, the cardiac index (CI), systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated. The hemodynamic parameters were recorded using a Life-Scope 11 (OMP-7201, Nihon Koden, Tokyo). For measurement of CO, a thermal dilution cardiometer (MTC-6210, Nihon Koden, Tokyo) was used.

Plasma concentrations of 6-keto-PGF₁α and thrombox-
ane B₂ were measured by using 6-keto-PGF₁α kit and thromboxane B₂ kit (Daichi RI, Tokyo).

The data were expressed as mean ± standard deviation. Statistical analysis was carried out by Student's t test and p<0.05 was defined as statistically significant.

RESULTS

Administration of DBcAMP had almost no effect on the HR and MPAP. The endotoxin-induced depression of the MAP was suppressed in the DBcAMP group. At 60 min after endotoxin inoculation, the MAP in the DBcAMP group was significantly higher (p<0.05) compared with the control group. The endotoxin-induced decrease in CI was also suppressed in the DBcAMP group, and the value at 180 min after endotoxin inoculation was significantly high (p<0.01) against the value in control group. The SVR and PVR underwent no significant changes until 180 min after endotoxin inoculation. However, from 180 min after endotoxin inoculation, both of these levels tended to increase in the control group, while the elevation of the resistance was inhibited in the DBcAMP group. On examining the UV, an anuric state was induced by endotoxin inoculation in the control group. In the DBcAMP group, on the other hand, the UV tended to gradually increase and showed a significantly high level (p<0.01) against the control group from 240 min after endotoxin inoculation (Table 1).

The endotoxin-induced increase in 6-keto-PGF₁α was significantly inhibited (p<0.01) by administration of DBcAMP at 60 and 180 min after endotoxin inoculation. At 360 min after endotoxin inoculation, the increase in 6-keto-PGF₁α remained still inhibited significantly (p<0.05) compared with the control group (Fig. 1).

The endotoxin-induced increase in thromboxane B₂ was significantly inhibited (p<0.05) by administration of DBcAMP at 60 min after endotoxin inoculation. At 180 min after endotoxin administration, the increase in thromboxane B₂ remained still inhibited significantly (p<0.01) compared with the control group (Fig. 2).

![Fig. 1. Effects of dibutyryl cyclic AMP on 6-keto-PGF₁α of dogs with experimentally-induced endotoxic shock. Data represent mean ± SD. •: DBcAMP; ○: control; Stars: p<0.05 versus control; ★: p<0.01 versus control.]

Table 1. Effects of dibutyryl cyclic AMP on hemodynamics of dogs with experimentally-induced endotoxic shock

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
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<tr>
<td>H R (1)</td>
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<tr>
<td>DBcAMP control</td>
<td>140.4±21.1</td>
<td>161.4±28.0</td>
<td>165.6±17.2</td>
<td>180.2±30.4</td>
<td>167.8±17.1</td>
<td>157.6±21.2</td>
<td>152.2±15.6</td>
<td>151.0±23.9</td>
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<td>MAP (2)</td>
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<tr>
<td>DBcAMP control</td>
<td>114.3±12.7</td>
<td>68.1±19.5</td>
<td>72.7±13.3</td>
<td>73.5±20.7</td>
<td>82.3±18.9</td>
<td>79.2±12.1</td>
<td>86.9±11.9</td>
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<tr>
<td>DBcAMP control</td>
<td>11.7±2.0</td>
<td>11.0±2.2</td>
<td>9.8±1.7</td>
<td>13.1±6.4</td>
<td>14.4±4.1</td>
<td>11.6±4.7</td>
<td>11.5±2.0</td>
<td>14.1±3.1</td>
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<td>C R (3)</td>
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<tr>
<td>DBcAMP control</td>
<td>3.44±0.44</td>
<td>3.2±0.69</td>
<td>2.56±0.75</td>
<td>2.68±0.85</td>
<td>3.08±0.60</td>
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<td>2.38±0.30</td>
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<td>SVR (3)</td>
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<td>PVR (3)</td>
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<tr>
<td>DBcAMP control</td>
<td>469±57</td>
<td>720±285</td>
<td>566±200</td>
<td>668±218</td>
<td>660±173</td>
<td>609±299</td>
<td>675±108</td>
<td>751±146</td>
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<tr>
<td>DBcAMP control</td>
<td>1.3±1.0</td>
<td>N D (3)</td>
<td>1.0±0.5</td>
<td>0.4±0.2</td>
<td>0.7±0.4</td>
<td>2.1±2.2</td>
<td>2.4±2.6</td>
<td>3.1±1.5</td>
</tr>
</tbody>
</table>

Data represent mean±standard deviation.

a) heart rate (beat/min)  b) mean aortic pressure (mmHg)  c) mean pulmonary arterial pressure (mmHg)  d) cardiac index (l/min/m²)  e) systemic vascular resistance (dyne·sec·cm⁻⁵)  f) pulmonary vascular resistance (dyne·sec·cm⁻⁵)  g) urinary volume (ml/kg/hr)  h) not determined  i) p<0.05 versus control  j) p<0.01 versus control.
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Fig. 2. Effects of dibutyryl cyclic AMP on thromboxane B2 of dogs with experimentally-induced endotoxic shock. Data represent mean ± SD. •: DBcAMP; ○: control; ★: p<0.05 versus control; ★★: p<0.01 versus control.

DISCUSSION

DBcAMP is known to have, through mediation of cyclic AMP, hemodynamic effects with its cardiotoxic action, peripheral vasodilative action and action to increase the blood flow in the kidney [1, 14, 15, 23, 30]. Its action to increase hepatic energy charge has also been reported [20]. In addition, its therapeutic effect in endotoxic shock has been described [18, 21]. In this study, besides the already documented cardiotoxic action, the effect of DBcAMP on the release of 6-keto-PGF1α and thromboxane B2 was newly investigated.

The decrease in CO and UV following endotoxin inoculation were significantly inhibited by administration of DBcAMP as effective as the results reported previously [18, 21]. DBcAMP demonstrates its cardiotoxic action not through mediation of β-adrenergic receptor but directly as cyclic AMP by permeation through cell membranes [12, 30]. For this reason, it acts effectively even when the sensitivity to catecholamine is depressed. The increase in UV by DBcAMP seems to be attributable to the increase in CO, the increase in renal blood flow due to its action to dilate renal blood vessels, and the increase in Na+ clearance due to its action on distal renal tubules [8, 15, 19].

The participation of tumor necrosis factor (TNF) and platelet activating factor (PAF) in hemodynamic disturbance seen during endotoxin-induced shock has attracted much attention [3, 11]. TNF and PAF possess a variety of actions [3, 5, 29]. Changes induced experimentally by administration of TNF and PAF include hemodynamic disturbance which is similar to that occurring during endotoxin-induced shock [2, 28]. Administration of a PAF-antagonist against endotoxin-induced shock improves the inhibited hemodynamics [27]. DBcAMP controls the endotoxin-initiated release of TNF and PAF in vitro by augmenting the concentration of cyclic AMP in cells [6, 24]. These suggest the possibility that the action of DBcAMP to inhibit the release of TNF and PAF related to the improvement of the hemodynamic disturbance in this experiment.

PGI2 is produced in intravascular endothelial cells. It regulates the blood stream by its antithromboplastic and vasodilative actions. TXA2 is produced by platelets and has thromboplastic, vasoconstrictive and bronchoconstrictive actions. It is homeostatically balanced with PGI2 in vivo [16]. Administration of DBcAMP significantly inhibited the elevation of PGI2 and TXA2. The elevation of the concentration of intracellular cyclic AMP seems to be involved in the mechanism by which these mediators were inhibited. When the balance between cyclic AMP and cyclic GMP in cells is collapsed, the stability of cell membrane is deteriorated [13]. This induces the release of lysosomes or activation of phospholipase A2. DBcAMP stabilizes cell membranes and inhibits the activation of phospholipase A2 by entering easily into cells and augmenting the concentration of intracellular cyclic AMP. This may result in inhibition of the production of arachidonic acid and hence inhibition of the production of PGI2 and TXA2 that are produced by arachidonic acid. Apart from such a direct effect, DBcAMP also seems to suppress the production of arachidonic acid by improving the peripheral anemic condition through improvement of the hemodynamics.

DBcAMP significantly inhibited the decrease in CO and UV that are seen to occur in endotoxic shock. It also significantly inhibited the increase in arachidonic acid cascade, a factor involved in deterioration of shock. These results suggested that DBcAMP is useful for the treatment of endotoxic shock.

REFERENCES

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