Changes in angiotensin converting enzyme, electrocardiograms and histology during sedation and general anaesthesia in dogs

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ABSTRACT

In present study, 16 dogs were divided into 4 equal groups to study the status of angiotensin converting enzyme, electrocardiograms and histomorphology in sedated and anaesthetized dogs. All the dogs were premedicated with atropine sulphate (0.045 mg/kg). After 20 min, detomidine (0.2 mg/kg) in groups 1 and 3 and xylazine (2 mg/kg) in groups 2 and 4 were intramuscularly injected, followed by thiopental sodium after 15 min, intravenously, till-effect for anaesthetic induction in animals of groups 3 and 4. The angiotensin converting enzyme (ACE) activity was found significantly increased at 15–45 min in group 1 and at 30 min in group 2. Whereas, significant increase in ACE in animals of groups 3 and 4 was recorded at 5–15 min and 5 min, respectively. The significant hypertension persisted for 60 min in animals of groups 1 and 3, and for 30 min in animals of groups 2 and 4. In groups 1 and 2, sino-atrial block in all dogs and second degree A V block in 1 dog of each group were seen within 2–15 min of drug administration. Ventricular premature contractions at 15–30 min were recorded in 1 dog of group 2 and group 4. The plasma electrolytes were nonsignificantly altered. Microscopically, haemorrhages with engorgement of blood vessels was seen in the parenchymatous organs. In lungs, the haemorrhages along with mild edema were seen in the perivascular areas and alveoli. Both xylazine and detomidine with and without thiopental sodium had increased the ACE activity, which resulted in proportionate hypertension, along with changes in alveolar epithelium and pulmonary endothelium during peak effect.

Key words: Angiotensin converting enzyme, Anaesthesia, Arterial blood pressure, Dogs, Electrocardiogram

Carp et al. (1994) suggested that renin-angiotensin system plays important role in maintaining blood pressure after epidural anaesthesia in animals. The angiotensins are peptides that act as vasoconstricting agents. Narrowing of the diameter of the blood vessels sends up the blood pressure. Angiotensin converting enzyme is an exopeptidase, which is found mainly in lung capillaries. Xylazine and detomidine had produced significant hypoxaemia with nonsignificant change in PaCO₂ and blood pH in sheep (Celly et al. 1997). Sinus arrhythmia and second degree A-V conduction block have been reported after subarachnoid administration of romifidine and lidocaine in goats (Kinjavdekar et al. 2006).

Present study was planned to observe the ACE enzyme activity, electrocardiographic (ECG) abnormalities and the histopathological changes in anaesthetized dogs using detomidine, xylazine premedication for thiopental sodium anaesthesia in dogs.

MATERIALS AND METHODS

Healthy dogs (16), 1.5–2 years of age of both sex, were divided into 4 equal groups (1, 2, 3 and 4). Atropine sulphate @ 0.045 mg/kg, intramuscular (i/m), was given 20 min prior to the administration of alpha₂-adrenoceptor agonist in all the animals of different groups. Detomidine (0.2 mg/kg) in groups 1 and 3; and xylazine (2 mg/kg) in groups 2 and 4, were injected intramuscularly. After 15 min of detomidine and xylazine medication thiopental sodium was intravenously administered till-effect for anaesthetic induction in dogs of groups 3 and 4.

Parameters: The ACE activity in plasma was estimated before medication and at 5, 15, 30, 45, 60 and 180 min post-medication (Cushman and Cheung 1971). Arterial blood pressure (ABP) was recorded from femoral artery using aneroid manometer at the time mentioned earlier for ACE activity. ECG was recorded before medication (time=0) and 2, 5, 10, 15, 30, 45, 60, 75, 90, 120, 150 and 180 min of induction of anaesthesia with thiopental sodium at limb lead II at 1 mV and 25 cm/s paper speed. Chloride [Cl⁻] (mercuric nitrate method) using diagnostic kits and sodium (Na⁺) and...
potassium (K⁺) by flame photometric method, were estimated in plasma before medication (time=0) and at 1, 24 and 72 h. Two dogs of all the groups were euthanized just after recovery for histopathological studies. The tissues were processed by conventional method and stained with haematoxylin and eosin (H&E).

Data were analyzed using one-way analysis of variance (ANOVA) for comparison of means between the groups and paired t-test to compare the means at different intervals to their base values. The data were presented as the mean ± SE. The level of significance was P<0.05.

RESULTS AND DISCUSSION

The ACE activity gradually increased up to 30 min in animals of groups 1 and 2, after the injection of alpha₂-adrenoceptor agonistic drug (Table 1). The significant (P<0.05) increase in the ACE activity was observed at 15–45 min in group 1 and at 30 min in group 2. The values of ACE were nonsignificantly higher in animals of group 1 than that of group 2, at respective time period. In anaesthetized animals, the significant increase persisted for a short duration, where it was for 15 min and 5 min in groups 3 and 4, respectively. Alifimoff et al. (1985) did not observe the inhibition of ACE activity due to enflurane, halothane and isoflurane. However in previous study, a significant role for the maintenance of blood pressure, by the renin-angiotensin system, during halothane and enflurane anaesthesia has been demonstrated (Miller et al. 1978).

The renin-angiotensin-aldosterone controls normal blood pressure and is critically involved in the development of such clinical states as arterial hypertension and congestive heart failure. More recently, it has become clear that angiotensin generated outside the kidney contributes to blood pressure control by local as well as systemic effects. Renin cleaves α₂-globulin angiotensinogen (from the liver) to form a relatively vasoactive decapeptide, angiotensin I. ACE (dipeptidyl carboxypeptidase) helps in the conversion of angiotensin I to angiotensin II by removing 2 amino acid residues from carboxy terminus of angiotensin I. Angiotensin II causes vasoconstriction and increases the cardiac contractility, cardiac output, and total peripheral resistance. In addition, angiotensin II plays a significant role in blood pressure maintenance under stress conditions, through its vasoconstrictor and aldosterone-stimulating actions (Reece 2005). In mammals the vasoconstrictor effect of angiotensin II works directly on receptors of the arterial smooth muscle. It also potentiates alpha adrenergic effects.

There was greater increase in the ABP in detomidine administered animals (groups 1 and 3) than those animals that received xylazine (groups 2 and 4) at different test intervals (Table 2). Significant (P<0.05) hypertension was recorded following the injection of detomidine and xylazine for 60 min and 30 min, respectively. In present study, the pattern of arterial blood pressure matched with the activity of ACE at all test intervals in each group. Thus, the ACE activity in plasma directly reflected the state of ABP at a particular time. The increase in ABP, after administration of alpha₂-adrenoceptor’s agonistic drug, may be due to their direct vasoconstrictive effect on the alpha₂-adrenoceptors of the smooth muscle of the blood vessels (Vainio 1990). Besides the stimulation of alpha₂-adrenoceptors, the hypertension in the present study may also be due to angiotensin II induced vasoconstriction in response to increased ACE activity. Therefore, ACE inhibitors may be used to control the hypertensive effect of sedatives and general anaesthetics.

**Table 1. Angiotensin converting enzyme activity (micro unit/ml) in plasma after administration of detomidine, xylazine, detomidine-thiopental sodium and xylazine-thiopental sodium in atropinized dogs (mean±SE)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>5</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine + detomidine</td>
<td>0.46</td>
<td>0.49</td>
<td>0.53*</td>
<td>0.58*</td>
<td>0.55*</td>
<td>0.51</td>
<td>0.48</td>
</tr>
<tr>
<td>(group 1)</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.01</td>
</tr>
<tr>
<td>Atropine + xylazine</td>
<td>0.44</td>
<td>0.47</td>
<td>0.50</td>
<td>0.51*</td>
<td>0.48</td>
<td>0.44</td>
<td>0.41</td>
</tr>
<tr>
<td>(group 2)</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.03</td>
<td>±0.03</td>
<td>±0.01</td>
</tr>
<tr>
<td>Atropine + detomidine</td>
<td>0.51</td>
<td>0.61*</td>
<td>0.59*</td>
<td>0.56</td>
<td>0.51</td>
<td>0.45</td>
<td>0.40</td>
</tr>
<tr>
<td>(group 3)</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.03</td>
<td>±0.02</td>
</tr>
<tr>
<td>Atropine + xylazine-thiopenol</td>
<td>0.44</td>
<td>0.52*</td>
<td>0.50</td>
<td>0.48</td>
<td>0.47</td>
<td>0.45</td>
<td>0.41</td>
</tr>
<tr>
<td>(group 4)</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.01</td>
</tr>
</tbody>
</table>

* Significant at 5% level from pre-administration value.

**Electrocardiographic studies**

In animals of groups 1 and 3, significant (P<0.05) increase in P wave duration and reduction in P wave amplitude were recorded up to 75–90 min. In one dog of group 3, increased P wave amplitude was also observed at 150–180 min. However, in animals of groups 2 and 4, the deviation in atrial depolarization time and atrial conduction area was nonsignificant (P>0.05). Tall P waves are seen in conditions causing right atrial hypertrophy and/or dilation and various congenital heart defects. In present study, the deviation in P wave duration and amplitude reflected the increased atrial depolarization time and decreased atrial conduction area due to the depressive effect of drugs on sympathetic nervous system. The QRS amplitude nonsignificantly (P>0.05) increased at 10 min in all the animals of different groups. Although no significant abnormality in either T wave or S-T segment was observed during observation period but a nonsignificant (P>0.05) increase in T wave in all the animals...
Table 2. Arterial blood pressure (mm Hg) after administration of detomidine, xylazine, detomidine-thiopental sodium and xylazine-thiopental sodium in atropinized dogs (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>5</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine + detomidine (group 1)</td>
<td>120.00</td>
<td>177.66*</td>
<td>201.33*</td>
<td>201.53*</td>
<td>187.66*</td>
<td>173.66*</td>
<td>145.66*</td>
</tr>
<tr>
<td>Atropine + xylazine (group 2)</td>
<td>121.66</td>
<td>151.66*</td>
<td>160.66*</td>
<td>162.00*</td>
<td>135.00*</td>
<td>118.33*</td>
<td>115.33*</td>
</tr>
<tr>
<td>Atropine + detomidine+thiopental (group 3)</td>
<td>122.66</td>
<td>186.00*</td>
<td>183.00*</td>
<td>170.00A*</td>
<td>159.33</td>
<td>151.33A*</td>
<td>118.00*</td>
</tr>
<tr>
<td>Atropine + xylazine+thiopental (group 4)</td>
<td>124.00</td>
<td>175.66*</td>
<td>164.00*</td>
<td>143.66B*</td>
<td>137.33B</td>
<td>135.00B</td>
<td>107.00*</td>
</tr>
</tbody>
</table>

* Significant at 5% level from pre-administration value; a,b significantly differ at 5% level at corresponding time interval; A,B significantly differ at 5% level at corresponding time interval.

of different groups was seen during peak effect of drugs, attributable to myocardial hypoxia and hyperkalemia. A nonsignificant increase in plasma potassium level was recorded after medication in the present study. Tilley (1985) has reported the larger T wave in animals with heart diseases during bradycardia, myocardial infarction and electrolyte imbalance. In groups 1 and 2, sino-atrial (S-A) block (Fig. 1) in all dogs and second degree atrioventricular (A V) block (Fig. 2) in one dog of each group were seen within 2–15 min of drug administration. A V block after detomidine and SA block after detomidine-butorphanol have also been reported in donkeys (Joubert et al. 1999). These changes were due to parasympathomimetic action of alpha 2 -adrenoceptor agonistic drugs.

Alitalo et al. (1986) reported that atropine could not abolish the AV block in detomidine administered horses and therefore, suggested the role of other factors on heart apart from the vagal tone. Similarly, SA and AV blocks were observed in present study in atropinized dogs. In contrast, Gastroys et al. (1990) did not find any heart block after the injection of romifidine in atropine premedicated horses and described the significance of atropine in prevention of cardiac abnormalities associated with alpha2-agonistic drugs. In animals of groups 3 and 4 the sino-atrial and second degree A V blocks, which were seen after the administration of detomidine and xylazine, however, disappeared after IV injection of thiopental sodium. Sinus arrhythmias at 90–180 min (one dog) and ventricular premature contractions at 15–30 min (one dog) were recorded in group 2 (Fig. 3) as well as in group 4 (Fig. 4). The ventricular premature contractions and atrial premature contractions were also reported during carfentanil-xylazine anaesthesia in dears (Caulkett et al.)

Figs 1–4. Electrocardiogram showing 1. sino-atrial block at 5 min after atropine-detomidine administration; 2. second degree atrioventricular block at 15 min after atropine-detomidine administration; 3. ventricular premature contraction at 15 min after atropine-xylazine administration; 4. ventricular premature contractions at 15 min after atropine-xylazine-thiopental sodium administration.
2000). In addition, the thiopental and halothane in combination also produced arrhythmogenic effect in dogs (Duer 2007).

Electrolytes

There was nonsignificant (P>0.05) increase in potassium and chloride levels and decrease in sodium in plasma at 1 h in all the animals of different groups. The values of these electrolytes, however, reduced at 24 h and returned to baseline at 72 h.

Histopathological studies

Microscopic examination in the animals those were injected with detomidine revealed the presence of centrilobular passive hyperaemia in liver and haemorrhages with engorgement of blood vessels in the lungs, brain and kidneys. Liver also showed central degenerated area with healthy peripheral hepatic cells. Central vein and sinusoids were dilated and engorged heavily with erythrocytes, along with the degeneration and atrophy of hepatic parenchyma (Fig. 5). Brain exhibited the congestion of capillaries and the areas of haemorrhages (Fig. 6). Red pulp of spleen was engorged (Fig. 7). In kidney, cortical and medullary intertubular haemorrhages were present (Fig. 8). Similar changes in parenchymatous organs have also been described with xylazine in rats, rabbits and dogs (Oh and Lee 1984). Myocardial hypoxia and hypotension observed during anaesthesia may be attributed to stagnation of blood in great veins and thereby resulting in general passive hyperaemia. Blood vessels of lungs were engorged and the haemorrhages along with edema were seen in the perivascular areas and alveoli (Fig. 9). Similarly, Celly et al. (1999) reported the massive peribronchial, perivascular and alveolar edema, and the presence of erythrocytes in many alveoli with extensive damage to endothelial cells at 10 min with normal sedative doses of xylazine in sheep. Altered capillary permeability, increased pulmonary capillary pressure and decreased oncotic pressure are the causes of pulmonary edema. Permeability changes in the pulmonary capillaries can arise in response to various vasoactive mediators produced by activation of different cells such as mast cells, platelets, macrophages and neutrophils (Sibille and Reynolds 1990). Whereas, increased mean arterial pressure and pulmonary artery wedge pressure can lead to hemodynamic edema.

The histopathological changes observed in different organs in xylazine premedicated animals (group 2) were more or less identical to the animals of group 1. Whereas, in animals of groups 3 and 4, the extent of changes observed in different organs was slightly greater than that of animals of groups 1 and 2, attributable to the additional medication of thiopental sodium. However, microscopically no significant difference was observed among different groups of animals in present study.

Results of the study indicated that the angiotensin converting enzyme activity is related to the arterial blood pressure in anaesthetized patients and suggested the role of renin-angiotensin-aldosterone system on patient’s haemodynamics during anaesthesia. The changes observed at cellular level in different organs would be responsible for their compromised functions for transient period.

REFERENCES


