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Clinical Safety of Fentanyl-Propofol Anesthesia on Haemato-Biochemical Parameters in Dogs

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Abstract

The aim of the study was focused to find out the effect on haematological and biochemical parameters following administration of propofol in combination with fentanyl citrate. The present study was carried out on six apparently healthy mongrel dogs of either sex weighing approximately 15-20 kg. Each dog was subjected to three different treatments, and each treatment lasted for one day. An interval of 8 days between two treatments was provided. The haematological parameters like PCV, haemoglobin, total erythrocyte and total leucocyte counts showed non-significant decrease in all the three treatment groups. Significant ($P<0.05$) increase in neutrophil count and decrease in lymphocyte count was noticed in all the treatment groups. The eosinophils and monocytes did not show any significant changes. The biochemical parameters like glucose and total bilirubin showed significant increase ($P<0.05$) in all the three treatment groups. The alanine aminotransferase, alkaline phosphatase and blood urea nitrogen were increased non-significantly while serum total protein and serum creatinine revealed no significant changes in all the three treatment groups. The haemato-biochemical changes induced by propofol and fentanyl citrate as pre-anaesthetics were of transient in nature and suggestive of least temporary alterations in liver and kidney functions. Thus, it is concluded that propofol in combination with fentanyl citrate as pre-anaesthetics can be used safely for induction of general anaesthesia in dogs with minimal or no side effects.

Keywords: Propofol, Fentanyl citrate, Haemato-biochemical parameters, Safety.

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Introduction

The modern surgical practice necessitates the use of effective analgesic and anesthetic agents. Greater demands on surgical facilities favour the application of short acting drugs without residual effect. Intravenous agents are popular in veterinary anesthesia for all species of animals and this is, undoubtedly, partly due to the speed and pleasantness with which they produce unconsciousness[7]. Canine patients, unlike those of the ruminants, require general anesthesia for safe and effective handling for almost all minor and major surgical procedures. The anesthetic should have a wide margin of safety and should not cause any long term physiological alteration.

Fentanyl citrate is a 4-acylanilino–piperidine compound, shorter acting and about 1000 times as potent as pethidine. It has been used to produce complete surgical analgesia in dogs. It is synthetic opioid related to phenylpiperidine derivates. It is popular drug in anaesthetic practice. Fentanyl is more lipids soluble than morphine, which contribute to its rapid onset and short of duration action[8]. The ideal anaesthetic technique in critically ill patient should be such that it should have least adverse effects on cardiopulmonary function and haemato-biochemical parameters of the patient in the course of induction and after surgery. Moreover it

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should have rapid and smooth recovery. Fentanyl citrate is a synthetic opioid and chemically it is 4-acylanilino–piperidine compound, having short duration of action and is about 100 times as potent as morphine. Fentanyl is distributed to the brain, heart, lungs, kidneys, and spleen followed by a slower redistribution to muscle and fat. Biotransformation is primarily hepatic, via dealkylation and hydroxylation into inactive metabolites. Elimination of fentanyl is primarily hepatic; less than 7% and 1% of the dose is excreted as unchanged fentanyl in the urine and feces, respectively[9].

Propofol (2, 6-di-isopropylphenol) is a novel non-barbiturate intravenously administered anaesthetic agent, which is having rapid induction and hepatic metabolism, short acting, smooth recovery, lack of cumulative effect even after prolonged administration and absence of excitatory effects on induction, maintenance or recovery[1]. The propofol has no untoward effects on liver and kidney function and can be safely used in patients with renal insufficiency and in cirrhotic patient[10,12]. Moreover, propofol had antiemetic and antiepileptic properties[2]. The total body clearance of propofol is rapid and exceeds hepatic blood flow, suggesting extra-hepatic metabolism. Propofol is metabolized to glucuronide and sulphate conjugates, and only trace amounts of other compounds are formed. It is primarily excreted in urine with only small amount in faeces[11].

Combination of fentanyl-propofol offers longer duration of action with smooth induction and recovery. So, the present study was conducted to evaluate the safety of hemato-biochemical parameters in Mongrel breed of dogs.

Materials and Methods

Experimental animals

The present study on Safety effects of fentanyl-propofol anaesthesia in haemato-biochemical parameters of dogs was carried out on 1-2 year old six apparently healthy mongrel dogs of either sex weighing approximately 15-20 kg. All dogs were kept on a similar diet and managemental conditions during the entire experiment. Deworming of all animals were done with Praziplus (Praziquantal 25 mg and Albendazole 300 mg) orally at the dose rate of one bolus/10 kg body weight. After a gap of one week and 10 days prior to commencement of the experiment all dogs were vaccinated against rabies using anti-rabies vaccine (Inj. Raksharab) 1 ml I/M.

Plan of experiment

The total duration of study was approximately 3 months. Each dog was given three different treatments, each of which lasted for one day. An interval of 8 days between two treatments was provided. Each experimental animal was starved for 24 hr and deprived of water for 6 hours prior to the treatment. In a day only one animal was used for the specific treatment.

Methodology

Atropine sulphate @ 0.04 mg/kg was administered I/M twenty minute prior to each treatment in all the dogs. Each animal was subjected to the following three treatments at an interval of 8 days.

Treatment I: - Fentanyl citrate @ 10 mcg/kg B.Wt. I/M, followed by propofol @ 5 mg/kg B.Wt. I/V (After ten minutes of first injection)

Treatment II: - Fentanyl citrate @ 10 mcg/kg B.Wt. I/M, followed by propofol @ 6 mg/kg B.Wt. I/V (After ten minutes of first injection)

Treatment III: - Fentanyl citrate @ 10 mcg/kg B.Wt. I/M, followed by propofol @ 7 mg/kg B.Wt. I/V (After ten minutes of first injection)

Collection of blood samples

Seven blood samples from each animal and for each treatment were collected at 0, 5, 10, 15, 30, 60 and 90 min intervals, respectively for haematological and biochemical studies.

Seven ml of blood was collected from each animal for haematological estimations out of this 2 ml of blood was poured in sterile vials containing EDTA @ 2 mg/ml of blood, while for estimation of blood glucose and total bilirubin 2 ml of blood was collected in centrifuge tubes containing heparin @ 0.2 ml (1% solution)/10 ml of blood as anticoagulant for plasma separation. Plasma glucose and plasma total bilirubin were estimated within 6 hr of collection. The remaining portion of blood was poured in sterile centrifuge tube and allowed to clot and serum was separated out by centrifugation at 2500 rpm for 10 min[3] for the estimation of total proteins, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen and creatinine.

Haematological Parameters

Haematological parameters: Total erythrocyte count (TEC %), Haemoglobin (Hb gm%), Packed cell volume (PCV %), Total Leucocyte count (TLC %) and Differential Leucocyte count (DLC %) were analyzed as per the method described by Jain[14].

Biochemical Parameters

Plasma glucose was estimated by Glucose Oxidase (GOD/POD) method using Merckotest reagent kit as described by Trinde.

Total proteins were estimated by refractrometer as described by Jain[14].

Alanine aminotransferase (ALT) was estimated by DNPH method using Acesure ALT reagent kit as described by Reitman and Frankel[13].

Alkaline phosphatase (ALP) was estimated using Qualigens Diagnostics kit as described by Kind and King[15].

Total bilirubin was estimated by Jendrassik and Grof' method using Merckotest reagent kit as described by Jendrassik and Grof[16].

Blood urea nitrogen (BUN) was estimated by DAM method using Acesure urea reagent kit as described by Marsh *et al.*[17].

Creatinine was estimated by Alkaline Picrate method using Qualigens Diagnostic kit as described by Bonses and Tausskay[4].

Statistical Analysis

The data was analysed by using simple analysis of variance to test the difference among the intervals under each treatment. The standard procedure as outlined by Snedecor and Cochran[18] was used.

Results

Hematological Parameters

Total erythrocyte count (millions/cu.mm.)

A non-significant decreasing trend of erythrocyte count was observed in all three treatments. The control values of 6.65 ± 0.05, 6.53 ± 0.04 and 6.53 ± 0.05 millions/cu.mm. decreased to minimum values of 5.48 ± 0.10, 5.31 ± 0.04 and 5.26 ± 0.03, at 10 min in all three treatments. Thereafter, the values increased toward the normal.

Hemoglobin concentration (g/dl)

A non-significant gradual decrease in hemoglobin concentration was observed in all three treatments. The control values of 12.46 ± 0.12, 12.60 ± 0.14 and 12.66 ± 0.15 g/dl decreased to minimum values of 10.81 ± 0.04, 10.66 ± 0.07 and 10.50 ± 0.06 at 15 min in all treatments, respectively. This was followed by gradual increase in values towards control values.

Packed cell volume (per cent)

The values of packed cell volume showed non-significant decreasing trend in all the groups. The control values of 42.75 ± 0.06, 42.76 ± 0.05 and 42.61 ± 0.08 decreased to minimum values of 40.35 ± 0.06, 40.28 ± 0.08 and 40.13 ± 0.08 per cent at 30 min in all three treatments. Thereafter, gradual increase was observed.

Total leucocytes count (thousands/cu.mm.)

A non-significant decreasing trend was observed in all the three treatments. The minimum values of 6.41 ± 0.06, 6.38 ± 0.06 and 6.30 ± 0.06 thousands/cu.mm. were observed at 15 min in all three treatments I, II and III, respectively. Thereafter, gradual increase was observed till the end of observation period.

Differential leucocyte count

Neutrophil (per cent)

The number of neutrophils were significantly increased ($P<0.05$) from 10 min to 90 min having a maximum increase at 30 min with value of 67.78 ± 0.33 per cent, the control value being 59.96 ± 0.51 in treatment I. In treatment II, significant increase ($P<0.05$) was observed between 10 min and 90 min of observations. The maximum increase was recorded at 30 min where control value of 60.11 ± 0.41 increased to 68.13 ± 0.21 per cent. In treatment III also, a significant increase ($P<0.05$) was observed between 10 min and 90 min having a maximum increase of 68.20 ± 0.21 at 30 min as compared to control value of 60.13 ± 0.40.

Lymphocytes (per cent)

The number of lymphocytes were significantly decreased ($P<0.05$) from 10 min to 60 min having a maximum decrease at 30 min with value of 27.53 ± 0.40, the control value being 36.80 ± 0.32 per cent in treatment I. In

treatment II, significant decrease (P<0.05) was observed between 10 min to 90 min of observation. The maximum decrease was recorded at 30 min, where control value of 36.25 ± 0.50 decreased to 27.30 ± 0.39 per cent. In treatment III also, a significant decrease (P<0.05) was observed between 10 to 90 min having a maximum decrease of 27.10 ± 0.26at 30 min as compared to control value of 36.49 ± 0.56 per cent.

Eosinophil (per cent)

The eosinophil count did not show any significant change in treatment I, II and III and values recorded at different durations of observation ranged between 1.83 ± 0.01 to 2.85 ± 0.01, 1.85 ± 0.01 to 2.90 ± 0.03 and 1.86 ± 0.01 to 2.92 ± 0.03 per cent in treatments I, II, and III, respectively.

Monocyte (per cent)

There was no significant difference in monocyte count in either of the three treatments and values fluctuated between 1.51 ± 0.07 to 1.87 ± 0.01, 1.55 ± 0.06 to 1.88 ± 0.01 and 1.53 ± 0.07 to 1.88 ± 0.02 per cent in treatments I, II and III, respectively.

Biochemical Parameters

Plasma glucose (mg/dl)

The plasma glucose level was influenced significantly in treatments I, II and III. In treatment I, the values remained significantly high (P<0.05) between 5 to 15 min of observations, where the highest values of 99.75 ± 0.92 from the mean control value of 80.35 ± 0.42 mg/dl was recorded at 15 min of observation. In treatment II, the plasma glucose was significantly (P<0.05) elevated between 5 to 15 min and the highest value of 103.36 ± 0.49 mg/dl from the mean control value of 80.18 ± 0.40 was recorded at 15 min. Treatment III, also exhibited a significantly increased level (P<0.05) of plasma glucose between 5 to 15 min having maximum level of 103.61 ± 0.58mg/dl at 15 min from the control value of 79.65 ± 0.20.

Serum total protein (g/dl)

A non-significant change in the values of total protein was observed in all the three treatments where the values ranged between 11.35 ± 0.07 to 10.71 ± 0.07, 11.40 ± 0.09 to 10.66 ± 0.06 and 11.33 ± 0.08 to 10.59 ± 0.09 g/dl in treatments I, II and III, respectively.

Alanine aminotransferase (IU/L)

A gradual non-significant increase in the value of alanine aminotransferase was observed with maximum values of 33.20 ± 0.93, 33.65 ± 0.07 and 33.78 ± 0.04 IU/L from control values of 30.33 ± 0.08, 30.40 ± 0.08 and 30.50 ± 0.10 at 15 min in all treatments, respectively. Thereafter, the values decreased toward the normal.

Alkaline phosphatase (IU/L)

The values of alkaline phosphatase increased non-significantly in all three treatments. The maximum value of 43.43 ± 0.14, 43.48 ± 0.13 and 43.51 ± 0.11 from control value of 41.36 ± 0.12, 41.40 ± 0.11 and 41.41 ± 0.10 at 15 min, in all three treatments were recorded. After maximum increase, the values declined toward near to the control values.

Plasma total bilirubin (mg/dl)

Significant increase (P<0.05) in total bilirubin was observed in treatment I, between 15 min to 90 min, having maximum increased value of 0.42 ± 0.04 at 30 min with control value being 0.28 ± 0.02 mg/dl. In treatment II, significant increase (P<0.05) was recorded between 15 to 90 min with the peak increased value of 0.42 ± 0.06 mg/dl from the control value of 0.28 ± 0.02, at 30 min. In animals of treatment III, also, there was a significant rise (P<0.05) between 10 min to 90 min having maximum increased level of 0.43 ± 0.06 from control value of 0.29 ± 0.02 mg/dl, at 30 min. However, the values remained within physiological limits.

Blood urea nitrogen (mg/dl)

In blood urea nitrogen also a non-significant increase was observed in all three treatments. The control values of 9.79 ± 0.04, 9.81 ± 0.03 and 9.84 ± 0.02 mg/dl, increased to maximum of 12.67 ± 0.07, 12.72 ± 0.10 and 12.79 ± 0.14 mg%, at 30 min in all three treatments I, II and III respectively. Thereafter, a gradual decline was observed and values returned toward near to control values.

Serum creatinine (mg/dl)

No significant differences in serum creatinine level in either of the three treatments were observed but the values fluctuated between 0.71 ± 0.09 to 0.90 ± 0.003, 0.72 ± 0.09 to 0.90 ± 0.07 and 0.73 ± 0.09 to 0.90 ± 0.01 mg/dl in treatments I, II and III, respectively.

Discussion

Haematological parameters

A non-significant decreasing trend of total erythrocyte count was observed in all the three treatments. Minimum values were recorded at 10 min in all the three treatments. A non-significant gradual decrease in haemoglobin concentration was observed in all the three treatments. The control values decreased to minimum at 15 min in all the three treatments. The packed cell volume values showed non-significant decreasing trend in all the groups. The control values decreased to minimum at 30 min in all the three treatments. The similar effects were also reported during evaluation of propofol in combination with preanaesthetic in canine[19,20]. A non-significant reduction in mean haemoglobin value and PCV are in agreement with the observation of Nikhate[21] in which the dog was anaesthetized by using either thiopentone sodium or propofol in combination with premedicant. Sharma *et al.*[22] recorded non-significant decrease in TEC, PCV and Hb concentration by using propofol in combination with triflupromazine or midazolam in dogs. Bayan *et al.*[6] and Venugopal *et al*[23] also reported decrease in haemoglobin after propofol anaesthesia with and without premedication in dogs.

Decrease in the haematological parameters could be due to sequestration of blood cells in the spleen and lungs during anaesthesia[9]. The reduction in haemoglobin and pack volume in all the groups of anaesthesia could be due to enalargement of spleenic sinusoids accommodating more amount of blood thereby increasing spleenic pooling of blood. The decrease in haematological parameters could also be due to increased plasma volume during anaesthesia on account of vasodilation resulting in vascular pooling [23].

A non-significant decreasing trend in total leucocyte count was observed in all the three treatments. The number of neutrophils were significantly increased (P<0.05) from 10 min to 90 min in all; the three treatments. Whereas number of lymphocytes decreased significantly (P<0.05) from 10 min to 60 in treatment I, 10 min to 90 min in treatment II, and III. The non-significant alterations were observed in eosinophil and monocyte counts in all the three treatments. The similar effects were observed in previous publications during evaluation of propofol in combination with preanaesthetic in canine[19,20]. Sharma *et al.*[22] recorded significant increase in neutrophil with a significant decrease in lymphocyte counts by using propofol with triflupromazine or midazolam in dogs. Similar changes in neutrophil and lymphocyte counts were observed with non-significant changes in total leucocyte count by using propofol-eth in dogs by Jain[14]. The release of adrenal corticoids and subsequent leucocytosis, neutrophilia and lymphocytopaenia have been reported by Pandey and Patel[24]; Wilkinson[25]; following anesthesia. In the present study the reported leucocytosis, neutrophilia and lymphocytopaenia might be due to possible release of adrenal corticoids to combat stress induced by administration of propofol.

Biochemical parameters

The plasma glucose level was influenced significantly in treatments I, II and III. In treatment I, the values remained significantly high (P<0.05) between 5 to 15 min of observation, where the highest value was recorded at 15 min of observation. In treatment II also, the plasma glucose was significantly (P<0.05) elevated between 5 to 15 min with the highest value recorded at 15 min. Treatment III, also exhibited a significantly increased level (P<0.05) of plasma glucose between 5 to 15 min having maximum increase at 15 min. The similar effects were observed during evaluation of propofol in combination with pre-anaesthetic in canine[19,20]. The hyperglycemia was noticed in dogs of all the groups and is in concurrence with the findings reported by David[26] and Nikhate[23]. Similar significant increase in glucose levels were also observed by David[26] and Bayan *et al.*[6] following administration of propofol-triflupromazine and propofol alone in dogs, respectively. The hyperglycemia in anaesthetized dog could be attributed to release of adrenocorticoid in response to stress as reported by Sharma *et al.*[22] where in dogs were anaesthetized with the use of barbiturate. Through the hyperglycemia was observed in all the groups of dog, the increase in serum glucose values fluctuated within normal physiological range and is in concurrence with the findings of Breziski *et al.*[5]. A non-significant change in the values of total protein was observed in all the three treatments and the values ranged between 10.71 ± 0.07 to 11.41 ± 0.11, 10.66 ± 0.06 to 11.40 ± 0.09 and 10.59 ± 0.09 to 11.33 ± 0.08 g/dl in treatments I, II and III, respectively. The similar effects were observed by Mukati and Chauhan during evaluation of propofol in combination with pre-anesthetic in canine[19,20].

A gradual non-significant increase in the value of alanine aminotransferase was observed in all the three treatments with maximum values at 15 min in all the three treatments. The values of alkaline phosphatase increased non-significantly in all the three treatments. The maximum increase at 15 min in all the three treatments was recorded. Propofol is rapidly distributed and eliminated from the body. It is rapidly metabolized in the liver and eliminated through urine[27]. Parikh *et al.*[28] mentioned that increase in the ALT activity during anesthesia might be due to alteration in cell membrane permeability in response to hemodynamic changes induced by anesthetic drugs. Kelly[29] indicated that there is a possibility of leakage of serum alkaline phosphatase through plasma membrane of hepatic cells during the liver cell damage. Therefore, in the present study, there is a possibility that during the process of metabolism of these drugs there might have been some disruption in the liver parenchymal cells. This in turn has increased the cell membrane permeability leading to elevation in the level of ALT in the blood.

Significant increase ($P<0.05$) in total bilirubin was observed in treatment I and II, 15 min to 90 min, having maximum increased value at 30 min. In treatment III, significant increase ($P<0.05$) was recorded between 10 to 90 min with the peak increased value at 30 min. The increase was remained within physiological limits. The bile pigment, mainly bilirubin, represents the final excretory product of haemoglobin degradation in liver following its release from the non-functional erythrocytes. Damage to the hepatocyte and/or the duct system together with impaired renal function might be expected in acute chemical toxicity. This could produce elevated circulatory levels of the bile pigments because of diminished excretion[30].

Blood urea nitrogen and serum creatinine

In blood urea nitrogen also a non-significant increase was observed in all three treatments. The control values increased to maximum at 30 min in all the three treatments. No significant difference in serum creatinine level in any of the three treatments was observed. The decrease in the glomerular filtration rate could result into increased concentration of creatinine in blood and any retardation in the glomerular filtration rate will cause high concentration of blood urea nitrogen[32]. Kelawala *et al.*[31] attributed increase in BUN to fasting of animals and mild depression of kidney function and mentioned that the temporary decrease in renal blood flow during anesthesia leads to increased BUN and creatinine in blood. Therefore, it is presumed that the administration of propofol alone or in combination with fentanyl citrate caused some alteration in glomerular filtration rate thereby causing an increase in the level of blood urea nitrogen and creatinine in the present study.

Conclusion

The haematological and biochemical changes induced by propofol aned fentanyl citrate as pre-anaesthetics were of transient in nature and suggestive of least temporary alterations in haematology, liver and kidney functions. Thus, it is concluded that propofol in combination with fentanyl citrate as preanaesthetics can be used safely for induction of general anaesthesia in dogs with minimal or no side effects.

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Conflict of Interest

None Declared

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