Research Article

Hematology & Hemotherapy Journal

Camel-Derived Haemoglobin, A New Blood Substitute and Oxygen Therapeutic

Shokry M*, Prince AB, Attia M, Ibrahim I, Farghalli H, Tohamy A and Mostafa A

Departments of Surgery, Biochemistry, Physiology, Forensic Medicine abd Toxicology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract

Introduction

Haemoglobin-Based Oxygen Carriers (HBOCs) are being developed as substitutes to replace the oxygen-carrying functions of erythrocytes and thereby lessen the demand of donor blood during surgery and trauma situation [1]. The HBOCs are designed to increase the oxygen-carrying capacity while reducing the risk commonly associated with allogenic RBCs transfusion [2]. Bovine and human haemoglobin form the bases of many different types of HBOCs [3-5].

Aim of the study

The aim of this study was to develop an effective and safe acellular HBOC blood substitute from the blood of camels.

Materials and methods

The study was experimental and comprised 2 stages:

Stage I: Laboratory preparation of Camel Haemoglobin (CHBOC)

Stage II: Experimental application of the developed CHBOC on 10 normal adult dogs. The dogs were randomized into 2 groups (Test=7 dogs-14 trials and Control=3 dogs-6 trials). Both groups were subjected to exsanguination of 40% of estimated blood volume to achieve severe hypovolemia. The test group was infused with CHBOC (40 gm/L) dissolved in lactated Ringer's. The control group was infused with HES 200 (6 gm/L) (Hydroxyethyl starch) dissolved in Saline. Blood samples were collected from the dogs of both groups at base line Before Exsanguination (BE) and After Exsanguination (AE) by one and 24 hours for CBC and estimation of certain blood biochemical values reflecting the liver and kidney functions. Other clinical physiological parameters were also recorded.

Results and conclusion

The clinical, haematological and biochemical responses were normal. The CHBOC showed improved early survival and stabilized physiological and haemodynamic functions.

***Corresponding author:** Shokry M, Departments of Surgery, Biochemistry, Physiology, Forensic Medicine abd Toxicology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, E-Mail: mshokry@cu.edu.eg

Received Date: July 20, 2016

Accepted Date: January 31, 2017

Published Date: February 14, 2017

Citation: Shokry M, Prince AB, Attia M, Ibrahim I, Farghalli H, et al. (2017) Camel-Derived Haemoglobin, A New Blood Substitute and Oxygen Therapeutic. J Hematol Hemother 2: 003.

Introduction

Blood primarily functions transport oxygen to tissues. This function performed by Haemoglobin (Hb), a protein encapsulated inside the Red Blood Cells (RBCs) that is capable of binding and releasing oxygen [6]. Hb-Based Oxygen Carriers (HBOCs) are being developed as substitute to replace the oxygen-carrying functions of erythrocytes and thereby lessen the demand of donor blood during surgery and trauma situations [1]. Artificial blood substitutes present several advantages over the use of donor blood for blood transfusions because they have no antigenic blood groups on their surface, no possibility for transmitting infections, they have a longer storage lifetime and are cost efficient [7-10].

Bovine and human Hb form the bases of many different types of HBOCs ranging from chemically modified Hbs, including cross-linked, polymerized, polymerized conjugated to particle encapsulated [3-5,11].

Currently, bovine-derived HBOC has been successfully used in South Africa to support a patient with autoimmune hemolytic anemia and has approved by the FDA for routine use in canine anaemia [12,13]. Other medical applications include organ preservation to meet the requirements of patients who cannot receive donor blood because of religious beliefs [14]. However, over the past decades, the field of blood substitutes has been exciting due to the potential risk of Bovine Spongiform Encephalopathy (BSE), one of a number of prion blood borne pathogens affecting animals, with its possible link to CJD (Creutzfeldt-Jakob Disease) that can resist sterilization procedures employed in the production of blood substitutes [15,16]. In order to eliminate the risks associated with prion protein infection, Camel-derived HBOC (CHBOC) has attracted considerable attention as a potential safe alternative to bovine-derived HBOC. Compared to other animal species, camels have more RBCs and higher Hb and Mean Corpuscular Hb Concentration (MCHC) [17]. Camels express heavy-chain antibodies that can be used to clone nanobodies, which are antibody-derived therapeutic proteins. A major advantage of nanobodies is that they can be easily attached to other proteins and nanoparticles by a simple chemical procedure, with therapeutic applications in cancer [18]. In addition camel's blood is easily available from slaughter houses in countries where camel meat is consumed.

The objective of this study was to develop an effective and safe acellular CHBOC blood substitute. In addition, the clinical,

haematological and biochemical evaluation of CHBOC administration were investigated in a dog model of haemorrhagic hypovolemia.

Materials and Methods

The tolerability of the developed product of camel's hemoglobin CHBOC was evaluated in 10 moderately exsanguinated (40%) experimental mongrel dogs that were randomized to receive either 40 g/I CHBOC dissolved in lactated Ringer's solution at a rate of -5 ml/kg (Test group-14 trials) or 6 g/l HES 200 solution - 6% hydroxyethyl starch (Fresenius Kabi-Germany) dissolved in saline at a rate of -5 ml/kg (Control group-6 trials).

All dogs were allergically assessed against the prepared CHBOC before administration [19].

All experimental dogs were sedated with Xylazine HCl 2% (Xylaject, Adwia, Egypt) (0.5 mg/Kg) and subjected to moderate jugular exsanguinations to approximately 40% of estimated blood volume (70-110 mlkg) (approximately 500 ml) [20]. According to Wolfensohn and Lloyd (2003) (approximately 500 ml blood). Jugular exsanguinations were conducted by using a large bore needle (2-3 mm). The needle was attached to a rubber tube of suitable length, coated with paraffin wax to prevent clotting. The tube was left hanging freely into 1 l graduated glass vessel. In addition, an intravenous cannula was introduced into the cephalic vein of the forearm of each dog, ready for subsequent infusion.

The exsanguinated experimental dogs were randomized into two groups:

- The test group (n= 7- 14 trials) was infused with CHBOC (40 g/l) dissolved in lactated Ringer's solution at a rate of (5 ml/kg).
- The control group (n=3- 6 trials) infused with HES 200 solution (6 g/l), 6% hydroxyethyl starch (Fresenius Kabi-Germany) dissolved in saline at a rate of (5 ml/kg).

Blood samples with and without the anticoagulant were collected from the dogs of both groups before exsanguinations (baseline-BE, after exsanguinations-AE, 1 hour after infusion-1 hA and 24 hour after infusion-24 hA) for analysis of the Complete Blood Count, CBC (Hb, Haematocrit-HCT, RBCs, MCV, MCHC and WBCs) using spectrophotometry, microcentrifugation and haemocytometer) and for the blood chemistry (glucose, cholesterol, triglycerides, urea nitrogen, creatinine, bilirubin, albumin, aspartate Aminotransferase-AST and alanine Aminotransferase-ALT) using chemistry autoanalyzer (Roche/Hitachi 912, Switzerland).

Results data and figures were presented as m±S.E. Comparison between mean values were made for each time point using two-tailed paired student's t test with $P<0.05$ considered statistically significant.

Results

Survival was not altered in the experimental dogs of both groups. It is interesting to note that, administration of CHBOC to the test group was associated with remarkable vitality, a high capacity for exercise and a great appetite for up to 2 days post-infusion. On the contrary, the control group infused with HES 200 solution exhibited weakness and reduced appetite for nearly one week post-infusion.

The recorded changes in certain physiological variables After Exsanguinations (AE) and 1 hour post infusion (1 hA) were slight hypothermia and marked tachycardia in both groups (Table 1 & Figures1-3).

Moderate exsanguinations were followed by significant reduction ($P<0.05$) in the measured values of Hb, HCT and RBCs, while calculated values of MCV showed significant increase ($P<0.05$) post-exsanguinations and infusion. The WBCs count showed mild changes in both groups at all time points (Table 2).

Blood biochemistry parameters reflecting renal function (BUN and creatinine) and liver function (bilirubin, AST and ALT) showed minor changes post-infusion from the values at base line. Other parameters reflecting metabolic activities (glucose, cholesterol, triglycerides and albumin) showed significant elevation of the glucose value ($P<0.05$) in the test group received CHBOC 1hr-.post-infusion (Table 3).

Discussion

In the present study, the dog model of moderate exsanguinations has been designed to clinically evaluate the newly prepared CHBOC as a blood substitute and compare its effects on various haemodynamic measurements with colloid HES 200 (hydroxyethyl starch 6%). The results showed that CHBOC improved early survival and stabilized physiological and haemodynamic functions. Nevertheless, further studies on the developed product aimed at improving its properties by ultra-purification and liposomal encapsulation may enforce its safety and eliminate any possible side effects.

Dogs resuscitated with CHBOC exhibited marked vitality, high exercise capacity and increased appetite that continued for 2 days post-infusion compared with dogs resuscitated with HES 200. Increased exercise capacity had been observed in human subjects administered HBOC of bovine origin resulting from greater O_2 uptake and CO_2 production and lower lactate levels [21]. Additionally, no adverse effects were recorded in terms of the clinical systemic response, CBC and blood biochemistry analysis. Nearly identical results were reported when HBOC of bovine origin was used for restoring muscular tissue oxygenation after profound isovolaemic haemodilution in dogs and for resuscitation following haemorrhagic shock in a swine model [22]. Bovine HBOC- 201 (Hemopure) has been used to manage autoimmune haemolytic anaemia in critically ill Jehovah's Witness patients and for treatment of acutely anaemic surgical patients in South Africa [12-14]. The beneficial effects of cell-free Hb were also demonstrated in the presence of the animal's red blood cells in maintaining physiological viscosity and limiting vasoconstriction a result of the pharmacological properties of cell free Hb [23]. Although HBOC can function as a bridge to spontaneous haematopoiesis, it may also accelerate the haematopoiesis process as serum erythropoietin level-increased by two-fold to six-fold over baseline at 24 hours after HBOC infusions [20]. Therefore, if further infusions of HBOC are administered as HBOC is metabolized, all banked red cell transfusion could be eliminated [24].

The demonstrated low HCT values 24 hr post-CHBOC infusion suggests repeat infusion of CHBOC to maintain tissue oxygenation. Similarly, HBOCs agents persist for a relatively short time in the circulation with a half-life of 24-48 hr while, the RBCs have a half-life of 28-36 days [25]. Therefore, frequent monitoring of CBC is necessary

	Before Exsanguination		After Exsanguination		1 hour After Administration		24 hours After Administration	
	Test N=14 BET	Control N=6 BEC	Test N=14 AET	Control N=6 AEC	Test N=14 AAT	Control N=6 AAC	Test N=14 AAT	Control N=6 AAC
B. temp. Co	39.5±0.1	39.5±0.3	39.0±0.2	38.6±0.4	39.2±0.1	38.3±0.2	39.5±0.2	38.8±0.1
Heart R/min	99.4±3.6	93.3±2.8	121.0±4.4	120.0±4.9*	115.0±2.8	111.0±0.8*	100.0±1.4	103.0±1.8
Resp. R/min	15.1 ±1+0.8	9.3±0.4	18.6±1.7	14.7±1.1	18.0±1.1	14.0±0.0	13.7±0.7	12.0±0.7

Table 1: Physiological variables before and after exsanguination and 1hour and 24 hours after administration of CHBOC (Test) and HES 200 (Control) (Mean±SE).

*Significant at P<0.05

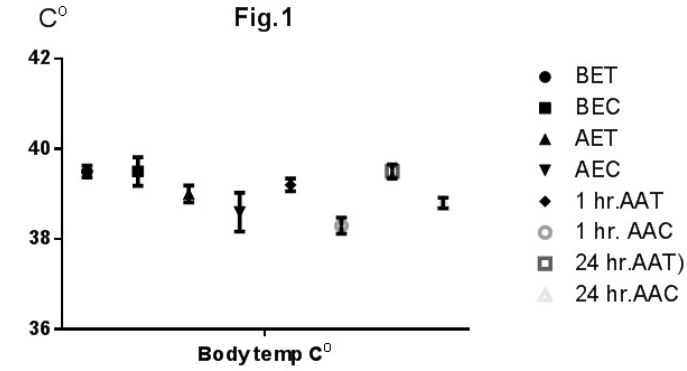
CHBOC (Camel-derived Haemoglobin O2 Carrier)

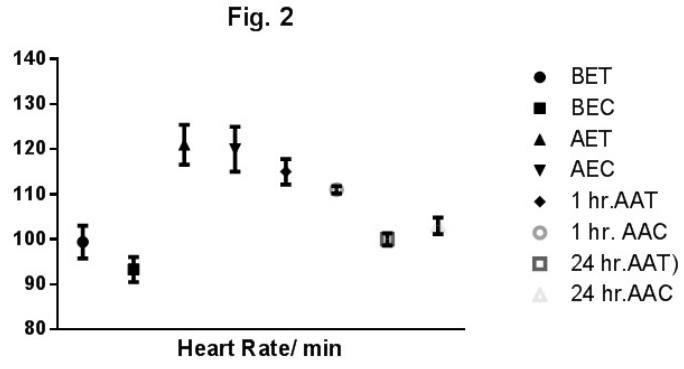
HES 200 (6% Hydroxyethyl Starch)

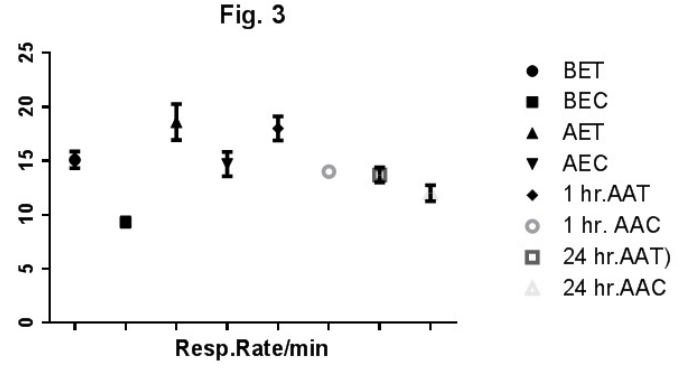
BET (Before Exsanguination Test), **BEC** (Before Exsanguination Control)

AET (After Exsanguination Test), **AEC** (After Exsanguination Control)

AAT (After Administration Test), **AAC** (After Administration Control)







Figures 1-3: Some physiological parameters, **BET:** Before Exsanguination Test; **BEC:** Before Exsanguination Control; **AET:** After Exsanguination Test; **AEC:** After Exsanguination Control; **AAT:** After Administration Test; **AAC:** After Administration Control.

	Before Exsanguination		After Exsanguination		1 hour After Administration		24 hours After Administration	
	Test N=14 BET	Control N=6 BEC	Test N=14 AET	Control N=6 AEC	Test N=14 AAT	Control N=6 AAC	Test N=14 AAT	Control N=6 AAC
Hb								
g/dl	12.3±0.3	13.2±0.7	9.4±0.2*	9.6±0.3*	10.5±0.3	9.5±0.3	10.8±0.3	9.6±0.3
HCT								
%	38.7±1.2	43.0±0.6	30.7±0.7*	33.6±0.2*	32.3±0.9	32.6±0.2	33.6±0.2	33.6±0.2
RBCs 10^6/ml	4.4±0.2	4.9±0.6	3.3±0.1*	3.6±0.1*	3.4±0.1	3.5±0.1	3.4±0.1	3.4±0.1
MCV								
fl	88.1±1.4	87.1±0.5	89.4±1.0	92.7±2.0*	93.6±1.2*	95.6±3.5*	94.5±1.7*	102.3±2.8*
MCHC %	31.4±0.5	30.7±1.3	30.7±0.4	28.7±1.2	33.0±0.4	29.3±1.1	32.9±0.3	28.7±0.8
WBCs 10^3/ml	8.4±0.3	6.4±0.3	5.8±0.3	7.3±1.2	7.1±0.5	8.3±0.8	8.7±0.6	8.8±0.5

Table 2: Haematological variables before and after exsanguination and 1hour and 24 hours after administration of CHBOC (Test) and HES 200 (Control) (Mean±SE).

*significant at P<0.05

CHBOC (Camel-derived Haemoglobin O2 Carrier)

HES 200 (6% Hydroxyethyl Starch)

BET (Before Exsanguination Test), **BEC** (Before Exsanguination Control)

AET (After Exsanguination Test), **AEC** (After Exsanguination Control)

AAT (After Administration Test), **AAC** (After Administration Control)

	Before Exsanguination		After Exsanguination		1 hour After Admin		24 hours After Admin	
	Test N=14 BET	Control N=6 BEC	Test N=14 AET	Control N=6 AEC	Test N=14 AAT	Control N=6 AAC	Test N=14 AAT	Control N=6 AAC
Glucose								
mg/dl	88.0±7.0	91.6±5.1	97.7±4.5	103.3±2.8	176.0±13.6*	98.6±2.3	94.1±5.9	90.6±4.0
Cholesterol mg/dl	221.4±14.1	158.7±21.9	219.1±14.1	162.7±9.2	177.7±11.5	151.0±8.4	179.9±8.3	141.7±12.3
Triglycerides mg/dl	55.7±5.9	44.6±8.8	61.8±4.9	46.6±13.5	65.0±4.0	47.3±3.3	53.4±4.2	45.0±4.5
Urea nitrogen								
mg/dl	28.2±2.1	23.3±0.9	32.0±2.8	23.6±1.7	33.8±2.5	23.0±1.3	29.5±1.2	22.6±2.0
Creatinine mg/dl	0.8±0.1	1.0±0.0	1.0±0.1	1.6±0.4	1.3±0.2	1.3±0.2	1.1±0.1	1.4±0.2
Bilirubin								
mg/dl	0.5±0.4	0.7±0.1	0.8±0.5	0.9±0.7	0.8±0.1*	0.7±0.6	0.7±0.0	0.7±0.1
Albumin								
g/dl	3.5±0.0	3.5±0.1	2.8±0.1	2.5±0.1	2.8±0.1	2.3±0.1	3.1±0.1	2.3±0.1
ALT								
U/L	20.3±3.1	16.7±0.4	20.6±3.6	14.7±0.9	23.6±2.5	16.3±1.1	19.1±1.9	15.0±1.0
AST								
U/L	25.6±3.2	15.0±0.6	25.3±3.6	14.6±1.3	21.6±2.5	13.7±0.8	20.9±2.2	14.7±0.4

Table 3: Blood chemistry variables before and after exsanguinations and 1 hour and 24 hour after administration of CHBOC (Test) and HES 200 (Control) (Mean±SE).

Significant at P<0.05

CHBOC (Camel-derived Haemoglobin O2 Carrier)

HES 200 (6% Hydroxyethyl Starch)

BET (Before Exsanguination Test), **BEC** (Before Exsanguination Control)

AET (After Exsanguination Test), **AEC** (After Exsanguination Control)

AAT (After Administration Test), **AAC** (After Administration Control)

to decide whether the patient needs repeat dosing of HBOC or has resumed a normal haemogram.

In the present study, the blood biochemical parameters reflecting the liver function (AST & ALT) showed no significant changes in both groups. Nevertheless, a marked elevation of bilirubin was observed one hour post-infusion of CHBOC. This is consistent with the results obtained in monkeys that received vesicular Hb (HbV) [26]. Such elevation is related to the liver, which is the main organ for Hb metabolism, thus Hb particles captured by the mononuclear phagocytes system such as kupffer cells, results in an excess load on the liver during the metabolism of massive amounts of Hb [27]. Hb derived from haemolysis can cause renal toxicity by the dissociation of tetramic Hb subunits into two dimmers, by extravasation and precipitation in the renal tubules [28]. However, in the present study blood biochemical parameters reflecting renal function (BUN and creatinine) showed slight changes in both groups that were within the normal range. Additionally, other blood metabolic biochemical parameters

(cholesterol, triglycerides and albumin) were unaffected but, the glucose levels increased significantly at 1 hr post-CHBOC infusion and regained normality at 24 hr post- CHBOC-infusion.

In spite of previous benefits of HBOC solutions, most of them have been reported to increase systemic and pulmonary vascular resistance in clinical and preclinical settings, thus limiting the range of the therapeutic applications for these solutions [29,30]. Furthermore, acellular type HBOCs are associated with significantly increased risk of death and myocardial infarction which could be a result of Nitric Oxide (NO) scavenging by cell-free Hb [31]. The reduction in NO levels in myocardial lesions is an important factor in inducing histological damage in cases of myocardial lesions [32]. To overcome the risks evolved from HBOCs agents, many safe HBOC products have been generated via either chemical or genetic modifications of Hb, using liposomes entrapping of Hb or nanocapsules adsorbing Hb or by generating Polyethylene Glycol (PEG) conjugated liposomes [33].

Limitations of the current study model include the lack of means for assessment of blood gases and cardiac output, oncotic pressure and toxicity studies.

References

1. Chang TMS (2000) Red blood substitutes. In: Contreras M (ed.). Best practice and research. Clinical haematology 13: 651-667.
2. Kim HW, Greenburg AG (2006) Toward 21st century blood component replacement therapeutics: artificial oxygen carriers, platelet substitutes, recombinant clotting factors, and others. Artif Cells Blood Substit Immobil Biotechnol 34: 537-550.
3. Jahr JS, Moallempour M, Lim JC (2008) HBOC-201, hemoglobin glutamer-250 (bovine), Hemopure (Biopure Corporation). Expert Opin Biol Ther 8: 1425-1433.
4. Chen JY, Scerbo M, Kramer G (2009) A review of blood substitutes: examining the history, clinical trial results, and ethics of hemoglobin-based oxygen carriers. Clinics (Sao Paulo) 64: 803-813.
5. Taguchi K, Miyasato M, Ujihira H, Watanabe H, Kadowaki D, et al. (2010) Hepatically-metabolized and -excreted artificial oxygen carrier, hemoglobin vesicles, can be safely used under conditions of hepatic impairment. Toxicology and Applied Pharmacology 248: 234-241.
6. Lieberman M, Marks A (2009) Marks' Basic Medical Biochemistry: A Clinical Approach. Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, USA.
7. Standl T (2004) Artificial Oxygen Carriers: Hemoglobin-Based Oxygen Carriers-Current Status. Transfus Med Hemother 31: 262-268.
8. Alayash AI (2004) Oxygen therapeutics: can we tame haemoglobin? Nat Rev Drug Discov 3: 152-159.
9. Kjellström BT (2003) Blood substitutes: where do we stand today? J Intern Med 253: 495-497.
10. Chang TMS (2004) A New Red Blood Cell Substitute. Crit Care Med 32: 612-613.
11. Smani Y (2008) Hemospan: a hemoglobin-based oxygen carrier for potential use as a blood substitute and for the potential treatment of critical limb ischemia. Curr Opin Investig Drugs 9: 1009-1019.
12. Jahr JS, Nesargi SB, Lewis K, Johnson C (2002) Blood substitutes and oxygen Therapeutics: An overview and current status. Am J Ther 9: 437-443.
13. Stowell CP (2002) Hemoglobin-Based Oxygen Carriers. Current Opinion in Hematology 9: 537-543.
14. Agrawal YP, Freedman M, Szczepiorkowski ZM (2005) Long-term transfusion of polymerized bovine hemoglobin in a Jehovah's Witness following chemotherapy for myeloid leukemia: a case report. Transfusion 45: 1735-1738.
15. O'Brien C (1996) Mad cow disease. Scant data cause widespread concern. Science 271: 1798.
16. Collinge J (1999) Variant Creutzfeldt-Jakob disease. Lancet 354: 317-323.
17. Aichouni A, Jeblawi R, Dellal A, Hammou A, Aggad H (2010) Breed variation in blood constituents of the one-humped camel (Camelus dromedaries) in Algeria. Journal of Camelid Science 3: 19-25.
18. Deffar K, Shi H, Li L, Wang X, Zhu X (2009) Nanobodies - the new concept in antibody engineering. African J of Biotechnology 12: 2645-2652.
19. James T (2002) Allergy testing. Am Fam Physician 15: 621-625.
20. Welfensohn S, Lloyed M (2003) Handbook of laboratory animal management and welfare. Blackwell Publishing Lts.
21. Hughes GS Jr, Francome SF, Antal EJ, Adams WJ, Locker PK, et al. (1995) Hematologic effects of a novel hemoglobin-based oxygen carrier in normal male and female subjects. J Lab Clin Med 126: 444-451.
22. Standl T, Burmeister MA, Horn EP, Wilhelm S, Knoefel WT, et al. (1998) Bovine haemoglobin-based oxygen carrier for patients undergoing haemodilution before liver resection. Br J Anaesth 80: 189-194.
23. Arnaud F, Hammett M, Asher L, Philbin N, Rice J, et al. (2005) Effects of bovine polymerized hemoglobin on coagulation in controlled hemorrhagic shock in swine. Shock 24: 145-152.
24. Rochon G, Caron A, Toussaint-Hacquard M, Alayash AI, Gentils M, et al. (2004) Hemodilution with stroma-free [correction of stoma-free] hemoglobin at physiologically maintained viscosity delays the onset of vasoconstriction. Hypertension 43: 1110-1115.
25. Sprung J, Popp H, O'Hara P, Woletz J (2001) The successful use of hemoglobin-based oxygen carrier as a primary blood substitute during abdominal aneurysm repair with large blood loss. Anesth Analg 92: 1413-1415.
26. Klein HG (2008) Hemoglobin substitutes. Clin Adv Hematol Oncol 6: 562-564.
27. Taguchi K, Watanabe H, Sakai H, Horinouch H, Kabayashi K, et al. (2012) A Fourteen-Day Observation and Pharmacokinetic Evaluation after a Massive Intravenous Infusion of Hemoglobin-Vesicles (Artificial Oxygen Carriers) in Cynomolgus Monkeys. J Drug Metab Toxicol 3: 128.
28. Sakai H, Horinouchi H, Tomiyama K, Ikeda E, Takeoka S, et al. (2001) Hemoglobin-vesicles as oxygen carriers: influence on phagocytic activity and histopathological changes in reticuloendothelial system. Am J Pathol 159: 1079-1088.
29. Parry E (1988) Blood substitutes: preparation, physiology and medical applications. VCH, The University of Michigan, USA.
30. Doherty DH, Doyle MP, Curry SR, Vali RJ, Fattor TJ, et al. (1998) Rate of reaction with nitric oxide determines the hypertensive effect of cell-free hemoglobin. Nat Biotechnol l16: 672-676.
31. Alayash AI (1999) Hemoglobin-based blood substitutes: oxygen carriers, pressor agents, or oxidants? Nat Biotechnol 17: 545-549.
32. Burhop K, Gordon D, Estep T (1999) Review of hemoglobin-induced myocardial lesions. Artif Cells Blood Substit Immobil Biotechnol 32: 353-374.
33. Natanson C, Kern SJ, Lurie P, Banks SM, Wolfe SM (2008) Cell-free hemoglobin-based blood substitutes and risk of myocardial infarction and death: a meta-analysis. JAMA 299: 2304-2312.
34. Mozzarelli A, Ronda L, Faggiano S, Bettati S, Bruno S (2010) Haemoglobin-based oxygen carriers: research and reality towards an alternative to blood transfusions. Blood Transfus 8: 59-68.