Gelatine and hydroxyl ethyl starch hypervolemic hemodilution – Effect on hemorheology and retinal circulation in a pig model

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Abstract

The present study was designed to determine the effects of hemodilution with gelatine (GEL) and hydroxyethyl starch (HES) on hematocrit, blood viscosity, systemic hemodynamics, and central retinal arterial blood flow in anaesthetized pigs. 20 pigs were studied. Hypervolemic hemodilution was induced by 30 ml kg^{-1}h^{-1} GEL (n=10) or HES (n=10) infused over 30 min. The hematocrit decreased comparably in both groups. Plasma viscosity was higher after HES than after GEL. Systolic central retinal blood flow and resistance index increased and were higher after HES than after GEL. Despite a greater plasma viscosity HES increases retinal microcirculation during hypervolemic hemodilution.

Introduction

Monitoring the flow of the central retinal artery may be a promising tool for assessing the effects of different therapeutic strategies as well as protective measures on this specific vascular bed as well as indirectly on cerebral perfusion [1,2]. Besides being used for volume replacement [3,4] colloids are frequently used in the treatment of acute non-arteritic ischemic optic neuropathy and retinal perfusion impairments. The incidence of non-arteritic anterior ischemic optic neuropathy varies between 2.3 and 10.3 per 100,000 individuals, and increases to 34.6 six weeks and to 51.8 six months after cataract extraction, respectively [5-7].

Retinal perfusion disturbances are characterized by increased plasma viscosity, erythrocyte aggregation and low flow rates [8-10] in combination with arteriosclerotic vessel damage. Treatment strategies are aimed to prevent further damage of retinal cells and consecutive cell death with an irreversible loss of visual acuity in the acute stage. Furthermore, long term complications such as macular edema and retinal neo-

vascularizations as well as neovascular glaucoma due to ischemia should be diminished or prevented.

At present, hematocrit is the only parameter that is determined in daily practice in the early stage of the disease. Few data are available on the effects of hemodilution on blood and plasma viscosity as the only therapy of retinal perfusion disorders. Effects of this treatment have been compared with clinical and functional results (visual acuity, macular edema, neovascularization) [11]. The mechanism, i.e. the pathophysiological path of modifying retinal perfusion and saving retinal tissue is not known. Comparably, the extent of alteration of retinal perfusion that can be obtained has not been well investigated. Therefore, direct measurement of blood flow of the arteria centralis retinae may be a promising tool to evaluate the retinal circulation [12] and the effect of colloid treatment on retinal perfusion disorders.

Up to now only chronic models exist to describe the effects of hemodilution on retinal perfusion. No data are available on the acute effects of a hypervolemic dilution with colloids on retinal blood flow in

an acute experimental setting. The present study was designed to determine the effects of a hypervolemic dilution with different colloids on hematocrit, plasma viscosity and direct measurement of blood flow in the central retinal artery.

Material and methods

Animals

After the approval of the appropiate committee for animal protection (Ministerium für Räume und Natur des Landes Schleswig-Holstein, Kiel, Germany) 20 female pigs (median weight : 31 kg; 3 months old) were included in this investigation and randomly distributed to group HES or GEL. All animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institute of Health (NIH publication 85-23, revised 1985).

Anaesthesiological protocol

After intramuscular premedication and reaching an adequate level of sedation with 2 mg · kg^{-1} Xylazin (Rompun®, Bayer AG, Leverkusen, Germany), 1 mg · kg^{-1} ketamine hydrochloride (Ursotamin®, Serumwerke Bernburg, Germany) and 30 µg · kg^{-1} atropine sulphate (Atropinsulfat®, B.Braun, Melsungen, Germany) an intravenous catheter was inserted in the left ear vein. Anaesthesia was induced with the intravenous application of propofol (Disoprivan® 1%, AstraZeneca, Wedel, Germany) (3 mg · kg^{-1}) and further ketamine hydrochloride (1 mg · kg^{-1}). In deep anaesthesia muscle relaxation was performed with pancuronium bromide (Pancuronium ''Organon''®, Organon, Oberschleissheim, Germany) (0.1 mg · kg^{-1}) to facilitate endotracheal intubation. Normocapnic ventilation was performed and anaesthesia was maintained with isoflurane 0.8%, and an intravenous continuous infusion of ketamine (60 mg · kg^{-1} · h^{-1}).

Standard procedures for monitoring heart frequency by electrocardiogram and transcutaneous oxygen saturation were employed. An arterial line was placed in the superficial femoral artery for measuring systolic, middle and diastolic artery pressure and for drawing the blood samples. After the preparations a wait of 30 minutes was allowed to achieve stable con-

ditions before the measurements and interventions started.

Infusion protocol

The colloids gelatine succinat (Gelafundin 4%, B.Braun, Melsungen, Germany) for group GEL or hydroxylethyl starch 10 %, molecular weight 200000, degree of substitution 0.5 (Serag-Haes 10%, Serag Wiessner KG, Naila, Germany) for group HES were infused over a period of 30 min with the amount of 30 ml · kg^{-1}.

Determination of parameters

Immediately before and after the infusion of the colloid, blood samples were drawn for the determination of the hematocrit and the plasma viscosity. Parallel systolic and diastolic blood flow in the central retinal artery were measured. The resistance index was calculated using the following equation :
$(Flow_{syst} - Flow_{diast}) / Flow_{syst}$ [13].

Hematocrit and plasma viscosity

EDTA samples were drawn (Sarstedt, Nümbrecht, Germany) for the determination of hematocrit and plasma viscosity. The hematocrit was obtained by an ADVIA 120 hematology analyzer (Bayer, Fernwald, Germany).

For the determination of the plasma viscosity the EDTA samples were centrifugated for 5 min. with 3000 rpm to separate the plasma. A commercial plasma capillary viscometer (Fresenius, Bad Homburg, Germany) was used. The plasma viscosity was measured with an established procedure three times [14].

Measurement of blood flow

The measurement of the retinal blood flow was performed using a MultiDop-T ultrasound device (DWL, Sipplingen, Germany) three times; details were described elsewhere [15]. A 2.0 Hz Doppler probe was attached to the left eye to measure the systolic and diastolic blood flow of the central retinal artery of each animal. The following system settings were kept constant throughout the experiments: power 34 mW, TIC

0.3, sample volume length 5 mm, filter 100 Hz, scale 3000 Hz. Doppler signals were recorded during surgery, simultaneously stored on digital audio tape and evaluated offline by a blinded observer using the TCD7r software (DWL, Sipplingen, Germany).

Statistical analysis

Mean values of repeated measurements were determined before and after hemodilution for each animal to reduce variability.

The data within and between groups were compared by the non-parametric Wilcoxon test and Mann-Whitney U Test (SPSS, Version 14, Chicago). The level of significance was set at 5%. Values are presented as mean +/- SD (standard deviation).

Results

Blood flow measurements could be accomplished in all animals. Due to the fact that the arterial line could not be placed in one animal in the HES group, the data for hematocrit, plasma viscosity and blood pressure in the HES GROUP are based on 9 animals. No adverse effects attributable to the colloid infusion were observed.

Systemic hemodynamic parameters are presented in table 1. The study groups did not differ before or after hemodilution. Heart rate decreased and arterial blood pressure increased comparably in both groups.

Further results are presented in figures 1 - 5. There was no difference between either group concerning hematocrit, plasma viscosity, diastolic and systolic blood flow and resistance index before hemodilution.

Hematocrit decreased after hemodilution significantly in GEL from 26.1% to 16.1% (p=0.005) and in HES from 26.6% to 17.7% (p=0.007), respectively (figure 1).

Plasma viscosity differed significantly between both groups after hemodilution with an increase of 1.14 mPas for GEL and 1.22 mPas for HES respectively (p=0.008) (figure 2).

Systolic retinal blood flow increased significantly after hemodilution in GEL from 38.0 cm · s^{-1} to 44.8 cm · s^{-1} (p=0.005) and in HES from 36.6 cm · s^{-1} to
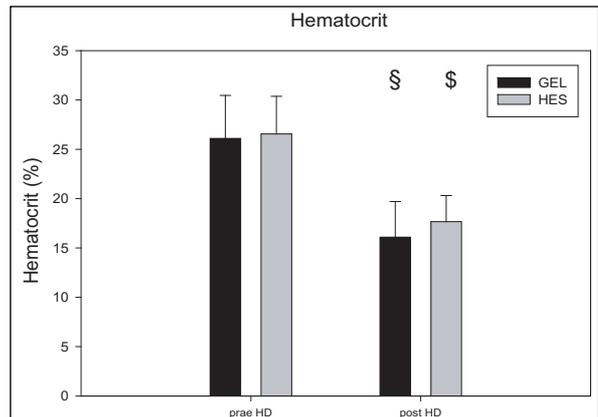

Figure 1. Hematocrit before and after hemodilution. prae HD: before hemodilution, post HD: after hemodilution. §: p = 0.005 for GEL and $: p = 0.007 for HES after hemodilution.
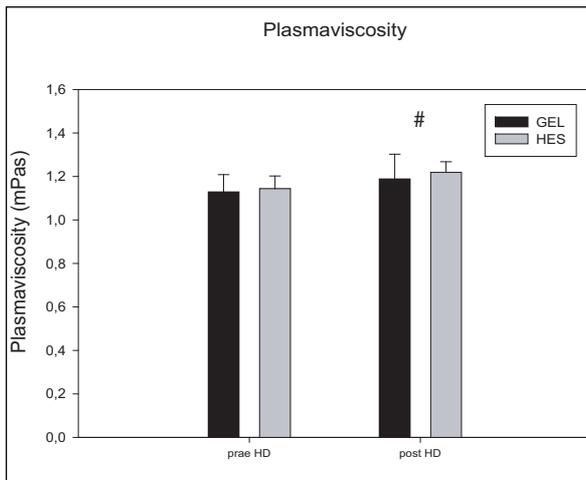
Table 1. Haemodynamic parameters

Parameter	Unit	Group	prae HD		post HD		p
			Mean	SD	Mean	SD	before / after HD
HR	1/min	GEL	129,7	4,7	109,7	7,5	0.005
	1/min	HES	130,8	4,5	106,3	6,1	0.005
sAP	mmHg	GEL	128,6	10,0	141,8	7,7	.007
	mmHg	HES	125,0	3,6	136,8	5,1	.007
mAP	mmHg	GEL	113,9	9,6	122,2	9,6	.005
	mmHg	HES	106,8	3,9	115,2	4,8	.008
dAP	mmHg	GEL	101,5	9,9	106,3	9,0	.011
	mmHg	HES	92,8	5,1	101,0	3,7	.007

HD: hemodilution; HR: heart rate; sAP: systolic arterial pressure; mAP: mean arterial pressure; dAP: diastolic arterial pressure
There was no significant difference between the two groups (Mann - Whitney U test).

Figure 2. Plasma viscosity before and after hemodilution. prae HD: before hemodilution, post HD: after hemodilution. #: p = 0.008 between GEL and HES after hemodilution.
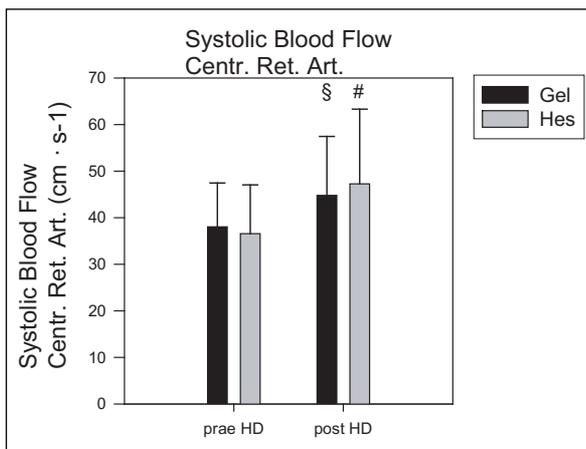

Figure 4. Diastolic blood flow in the central retinal artery before and after hemodilution. prae HD: before hemodilution, post HD: after hemodilution. Centr. Ret. Art.: Central retinal artery; No significant differences were observed between or within the groups before and after hemodilution.

Figure 3. Systolic blood flow in the central retinal artery before and after hemodilution. prae HD: before hemodilution, post HD: after hemodilution. Centr. Ret. Art.: Central retinal artery; #: p = 0.008 for HES after hemodilution. §: p = 0.005 for GEL and *: p = 0.004 for HES after hemodilution.

Figure 5. Resistance index for blood flow in the central retinal artery before and after hemodilution. prae HD: before hemodilution, post HD: after hemodilution. $: p = 0.007 between GEL and HES after hemodilution.

47.3 cm · s^{-1} (p=0.014) (figure 3), while there were no significant differences in or between the groups for the diastolic retinal blood flow (figure 4).

Resistance index significantly increased from 0.56 to 0.67 only for HES after hemodilution (p=0.007) (figure 5), while there was no difference between HES and GEL before or after hemodilution.
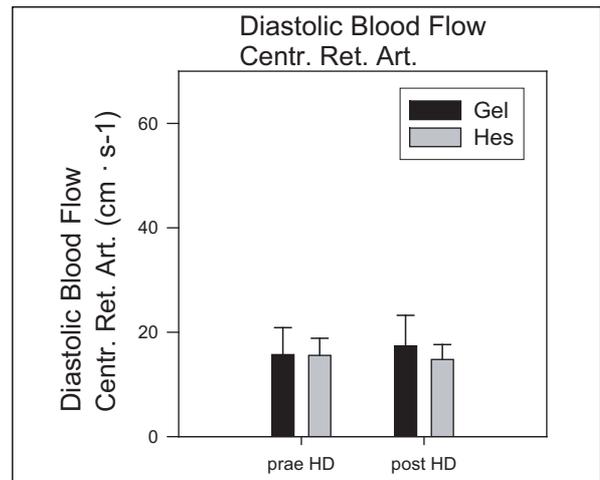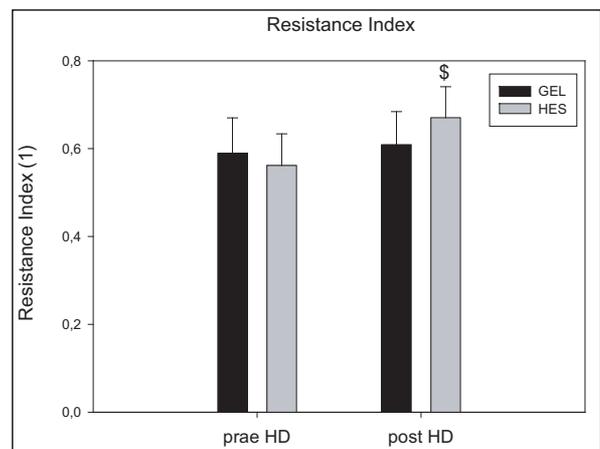
Discussion

The present study was designed to compare the effects of two colloids on rheological and hemodynamic parameters in an acute experimental setting with 3 months old pigs. The described setup allowed us to compare the effects of a hypervolemic dilution with

two different colloids (gelatin or hydroxyl ethyl starch) on hematocrit, plasmaviscosity and systolic and diastolic blood flow in the central retinal artery.

The hematocrit was primarily affected by the hemodilution but decreased significantly as an effect of the single hypervolemic hemodilution in both groups. The low hematocrit of 26.3 % before hemodilution is due to the chosen animal model of the 3-month-old pigs, as each species may have its own hematological and rheological profile [16].

In our study the infusion of high amounts of colloids with a single application (30 ml · kg^{-1}) within 30 min for hemodilution reduced the hematocrit by 38 % in GEL and by 33 % in HES.

Higher hematocrits (44 and 47 %) were observed in clinical studies in humans between and before hemodilution [8,17]. In these clinical studies smaller single volumes of colloids were repeatedly infused for the treatment of retinal perfusion disturbances as an isovolemic hemodilution while the same volume of blood was drawn simultaneously. For up to 10 days 250 - 500 ml of a colloid were infused corresponding to 3-4 ml · kg^{-1} body weight in a 70 kg subject.

Using hypervolemic and isovolemic hemodilution techniques hematocrits were reduced to 39 % by one hemodilution each day within a period of 10 days or to 35 % by 8 infusions during a period of 6 weeks. This corresponds to a decrease of hematocrit by 17% and 20 %, respectively.

Therefore, the higher volume applied as a single hemodilution of 30 ml · kg^{-1} colloid in our study decreases the hematocrit more than the smaller but repeatedly given infusions that are used in daily routine so far.

The plasma viscosity increased in both groups after hemodilution and led to a significant difference between the two groups. The plasma viscosities for GEL and for HES themselves were higher than the original plasma viscosities in both groups before dilution. The hypervolemic diluted blood showed a higher plasma viscosity in both groups due to the mixture of blood and colloid.

Plasma viscosity is one method of describing the rheological properties of the blood.

The plasma viscosity is determined by the temperature controlled capillary tube plasma viscometer, which could be used as a bed-side technique close to the experimental setting. Further advantages are the low cost and the immediate generation of results.

In our chosen animal model with 3-month-old pigs a lower plasma viscosity was shown than those known

from other clinical studies. As the plasma viscosity of pigs is lower than the viscosity of the applied colloid, hemodilution leads to an increased plasmaviscosity in this animal model.

In hitherto existing clinical studies the plasma viscosity decreased under an isovolemic hemodilution infusion with 10 % HES from 1.34 to 1.30 mPas reaching significancy [17], from 1.34 to 1.25 mPas [18], 1,32 to 1,23 mPas [19], 1.34 to 1.29 mPas [20] or 1.33 to 1.29 mPas [11]. Another clinical study measured a change of plasma viscosity from 1.13 to 1.12 without any difference [9]. This range of results may be a consequence of different methods and apparatus for the determination of plasma viscosity. All studies were performed with a temperature-controlled viscometer. In the studies cited first the same capillary viscometer was used as that in our research, while in study [9] the sefam aggregameter was used.

Systolic blood flow in the central retinal artery significantly increased for both colloids. Systolic and diastolic blood flow can be measured in the central retinal artery by the described Doppler technique. In both groups the hemodilution led to a significant increase in systolic blood flow, while the effect on diastolic blood flow was minor and not significant. As the plasma viscosity increased in our study the acute volume expansion by hypervolemic hemodilution may alone be responsible for this increased systolic blood flow. So far, there are no results concerning systolic blood flow from clinical studies. The longer lasting effect of HES compared to GEL was found for the mean blood flow velocity of the middle cerebral artery in healthy volunteers undergoing isovolemic hemodilution [21].

The increase of the resistance index as a calculated parameter is an expresssion of the more pronounced increase of systolic in comparison with diastolic blood flow, which showed only minor and not significant increases. As a consequence the resistance index could significantly increase after hemodilution for HES. For GEL both systolic and diastolic blood flow increased and did not lead to a significant change, therefore.

The impact of acute single hemodilution by an apheresis technique on arterial and venous velocity was confirmed by Doppler analysis in a clinical study [13]. The resistance index such as the relation between systolic and diastolic flow in the central retinal artery was diminished by isovolemic hemodilution in ischemic central retinal and branch retinal vein occlusion, while there was no change in the non-ischemic central retinal venous occlusion.

In further clinical studies other parameters were used to reveal the accelerating effect of isovolemic hemodilution by hydroxyl ethyl starch on blood flow. The capillary blood cell velocity increased after isovolemic hemodilution [17] while the retinal arterial time and arterio-venous transit time were reduced [9].

To date, the preferred therapy in the acute stage of an impairment of optic nerve head or retinal perfusion is hemodilution. In a few cases surgical intervention such as pars plana vitrectomy with sheathing of the arterio-venous branching at the site of occlusion or radial optic neurotomy with consecutive decompression may be an alternative [22]. In arterial occlusions selective catheterization of the A. ophthalmica has been investigated, results are still contradictory and the procedure is difficult and dangerous [23].

Summarizing, we established an acute animal model to compare the effect of a single hypervolemic hemodilution by gelatine and hydroxyethyl starch, respectively, on haematological, rheological and haemodynamic parameters and blood velocity. This acute single bolus model is able to describe the decrease of hematocrit and the increase of systolic blood velocity by gelatine and hydroxyethyl starch better than in chronic clinical studies with repeated infusions.

Perspectives

Besides monitoring the effects of therapeutical interventions aimed to improve retinal perfusion during different disease states, monitoring central arterial blood flow may be an attractive alternative for monitoring cerebral perfusion. This may be rather interesting for the monitoring of patients during stages of compromised perfusion especially for cardiac surgery and cardiopulmonary bypass. Besides comparing the effects of different colloids, this model may also be useful to investigate the effect of inotropic agents and anaesthetics on retinal perfusion.

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Second Biennial Meeting

Cardiac Surgery Care 2008

"Neuroprotection during cardiac surgery"

3. - 4.10.2008 Lübeck, Germany
MARITIM Seehotel - Timmendorfer Strand

Friday, 3.10.2008
16:15 -18:00
Opening Session
(Sievers, Schmucker)
– Characteristics, incidence, and pathomechanisms of neurological damage during cardiac surgery
  H. P. Grocott, Winnipeg, Canada
– Neurocognitive deficits after cardiac surgery: the neurologists perspective
  W. Müllges, Würzburg, Germany

Saturday 4.10.2008
08:30 - 9:45
Pathophysiology and pharmacology
(Werner, Grocott)
– Pharmacological prevention and modulation of stroke
  T. Unger, Berlin, Germany
– CPB and neurological dysfunction - results from animal models
  B. Jungwirth, München, Germany
– Pharmacological prevention and modulation of cerebral ischemia and neurological dysfunction during cardiac surgery
  H. P. Grocott, Winnipeg, Canada

10:30 - 12:00
Clinical anesthesia and monitoring
(Schirmer, Gehring)
– The effects of general anesthesia and variations in hemodynamics on cerebral perfusion
  K. Engelhardt, Mainz, Germany
– Transcranial doppler, EEG-and SEP Monitoring during cardiac surgery
  H. Gehring, Lübeck, Germany
– Cerebral oxygenation monitoring
  J. M. Murkin, London, Canada

13:30 - 15:00
Surgical and perfusion techniques
(Feindt, Müllges)
– Surgical concepts to decrease neurological complications during Off-pump surgery
  J. Babin-Ebell Neustadt (Saale), Germany
– The optimal hematocrit during CPB and circulatory arrest
  L. Dübener, Lübeck, Germany
– Perfusion concepts to decrease neurological complications - "Best practice bypass"
  J. M. Murkin, London, Canada

15:30 - 16:30
Specific surgical situations
(Sievers, Weigang)
– Anatomical aspects of the blood supply to the CNS
  J. Hendrikse, Utrecht, Netherlands
– Neuroprotection during ascending aortic and aortic arch surgery
  M. Bechtel, Lübeck, Germany
– Neuroprotection during descending aortic surgery and stenting
  E. Weigang, Mainz, Germany

16:30 - 17:00
Closing session and poster award
(Bechtel, Heringlake)
– Anything new to change my clinical practice. Future perspectives
  P. Feindt, Düsseldorf, Germany
  U. Schirmer, Ulm, Germany

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