RE: RESUS Trial

Lawrence Landau, M.D.
Via Donald Jehn <donald.jehn@FDA.HHS.gov>

Group 1 A: Wilford Hall controlled hemorrhage model

i. Henderson CL, et al

1. The study does not directly address resuscitation. It addresses use of HBOC 201 in membrane oxygenators.
   1a. No, the model does not simulate RESUS protocol.
   1b. The data do not support use of resuscitation.
2. The study is complex, but fairly well designed. I have reservations, though.
3. Administration does not simulate the RESUS setting.
4. Monitoring does not simulate RESUS setting.
5. The experiment does identify appropriate end points, but minimal or no advantage is seen compared with blood, when used for pump priming.
6. All animals are accounted for.
7. The model is confusing in that the need for an oxygen carrying prime solution is not demonstrated to begin with. Suggestions that vasopressor effects due to hemoglobin solution may be advantageous in hypovolemic shock (page 12) are inappropriate.
8. The study documents are relative anticoagulation due to HBOC (page 13, paragraphs 4 – 5). These effects may be problematic in resuscitation.
9. This study provides no support for exemption from informed consent.

ii McNeil JD, et al. Hypotensive resuscitation…

1a. The model does simulate the RESUS trial setting.
1b. The preclinical data had some minimal support to HBOC use in the RESUS trial.

2. The study is well designed.
3. In general, yes.
4. Probably not, unless hypotensive resuscitation is part of the RESUS trial
5. The end points (survival and lactate production) are reasonable markers.
6. Yes.
7. Yes – hypotensive resuscitation is not generally performed in the United States, it may be in the future, however.
8. The study does provide support for HBOC use.

iii. Fitzpatrick CM, et al – Prolonged low volume resuscitation…

1a. No, this trial addresses a military scenario of field resuscitation with HBOC versus Hestend over eight hours.

1b. These pre-clinical data are equivocal, they demonstrate superior blood pressure response, but this is due to increased vascular resistance, a potential deleterious effect of hemoglobin solutions. There is, however, a small (non-statistically significant) increase in survival.

2. Yes.
3. No.
4. No, as expected, monitoring is more extensive than in the pre-hospital setting.
5. Yes.
6. Yes.
7. No, the model is straight forward.
8. No, the evolution of decreased mixed venous oxygen saturation (SV O_2), lower cardiac index and higher SVR are of significant concern.
9. The study does contribute some support to the concept of hemoglobin solution use in resuscitation.

iv. York GB, et al – Low-volume resuscitation…

1a. Yes, this is a reasonable model for clinical resuscitation.

1b. The data do provide some support, but there are problems as well.

2. Yes.
3. Yes.
4. No, the monitoring is more extensive.
5. Yes.
6. Yes.
7. No.
8. A significant degree of hepatocellular damage was seen in the pigs given HBOC and not in the other groups. This is worrisome.
9. Yes, evidence supports a potential benefit, but not without potential risks.

v. Fitzpatrick CM, et al. – Resuscitation with a blood substitute…

1. A)Yes. B) The data do not support use in humans.
2. yes
3. yes
4. No, monitoring was more extensive
5. The end point used probably will correlate with clinical outcome, but will not be able to be used in clinical settings.
6. Yes
7. The model addresses the potential for vasoconstriction due to NO scavenging. The use of "resuscitation volumes needed to achieve

equivalent BP" as an endpoint can easily confuse the effects of HBOC as a resuscitation fluid and of HBOC as a NO scavenger. In fact, it seems clear that in this model HBOC led to systemic vasoconstriction as well as to diminished responses to acetylcholine in the vascular response model. The finding that arterial NO is not increased means very little and is not reassuring.

8. The findings are not reassuring.
9. The findings do not support exemption from consent.

vi. Sampson et al. – A comparison of the hemoglobin based …

1. A) No. The protocol simulates low-volume resuscitation in the 'far-forward' military scenario. B) The data do not support use in humans.
2. Yes, but only as regards low-volume resuscitation in the 'far-forward' military environments.
3. Yes, dosing may occasionally fall within RESUS ranges.
4. No, monitoring is more extensive than that used in pre-hospital settings.
5. Yes
6. Yes
7. No, the model is straightforward.
8. Several adverse outcomes are evident within this trial. Although HBOC did support survival, cardiac output, SvO2 and UO were lower throughout resuscitation that they were with standard fluids. Since HBOC required less fluid volume to achieve the target BPs sought, we cannot rule out the possibility that the BP was supported by increased SVR, presumably du to NO scavenging. The authors' final conclusion that when HBOC is administered "common clinical markers for determining adequacy of resuscitation may not be useful" appears to me deceptive.
9. The findings do not support exemption from consent.

Group B

i) King DR et al. – Resuscitation with a hemoglobin based …

1. A) Yes. The study simulates the combination of traumatic brain injury (TBI) with shock. So to the extent that the RESUS trial contains such patients, the study may be reflective. B) Yes, the data support the use of HBOC in humans with TBI and shock.
2. Yes.
3. In general, yes.
4. No, monitoring is more extensive.
5. Yes (assuming the trial includes TBI patients)
6. Yes
7. The study refers to HBOC-301. I'm not sure if this is a 'typo' or a different product.
8. In general, the effects on TBI appear quite favorable and would support BBOC use in this population. As with the other studies, SvO2 was diminished, suggesting a peripheral NO scavenging effect.
9. Yes - the findings support in a general way, exemption from consent.

ii) Malhotra et al. – Resuscitation with a novel hemoglobin-based …

1. A) Yes. The study simulates rapid, uncontrolled 'peri-operative' hemorrhage. Such situations can be fairly similar to pre-hospital uncontrolled hemorrhage. B) Yes, the data support the use of HBOC in humans with TBI and shock.
2. Yes.
3. In general, yes.
4. No, monitoring is more extensive.
5. Yes (assuming the trial includes TBI patients)
6. Yes
7. The study refers to HBOC-301. I'm not sure if this is a 'typo' or a different product.
8. In general, the effects on TBI appear quite favorable and would support BBOC use in this population. As with the other studies, SvO2 was diminished, suggesting a peripheral NO scavenging effect.
9. Yes - the findings support in a general way, exemption from consent.

iii) Malhotra et al. – Cerebral perfusion pressure elevation…

1. A) Like King DR et al, the study simulates the combination of traumatic brain injury (TBI) with shock but it uses diaspirin-linked

hemoglobin. B) No, the data do not support the use of HBOC in humans with TBI and shock.

2. Yes.
3. No – it uses a different product.
4. No, monitoring is more extensive.
5. Yes (assuming the trial includes TBI patients)
6. Yes
7. The study involves use of DCL-Hb. This is known to have greater effect on SVR than HBOC-201. The study concept was to see whether this enhanced hypertensive response was cerebral-protective in the setting of TBI. The DCL-Hb did appear to have some salutary effect.
8. There were systemic adverse effects here such as cardiac failure associated with the higher doses of DCL-Hb, but the extent to they will reflects upon HBOC use is unknown.
9. No - the findings do not support exemption from consent.

iv) Maxwell et al. – Resuscitation of severe chest trauma…

1. A) The study attempts to study the 'toxicity' of Hgb solutions that scavenge NO. It does so by comparing four different types of hemoglobin (none of which to my knowledge is under consideration) in a model that simulates the combination of blunt chest injury with hemorrhagic shock. B) No, the data do not support the use of HBOC in humans.
2. Yes.
3. Yes, generally.
4. No, monitoring is more extensive.
5. Yes – the primary endpoint is survival time.
6. Yes
7. Yes. The study involves use of a variety of different hemoglobin solutions. All were vasoconstrictors and lowered cardiac index. Some of these hemoglobin solutions led to significantly decreased survival time compared to saline. Other Hgb's led to significantly increased survival, ***but only in comparison with the Hgb's that caused early death***. No comparison is made to saline. I find this a deliberate obfuscation of the lack of significant benefit observed vs saline.
8. All the Hgb solutions showed some association with adverse effects, but the extent to which it reflects upon HBOC is unknown.
9. No - the findings do not support exemption from consent.

GROUP C – Frielich model

i) Gurney et al – A hemoglobin based oxygen carrier….

1. A) In general yes. B) Yes, the data do tend to support the use of HBOC in humans.
2. Yes.
3. Yes, generally.
4. No, monitoring is more extensive.
5. Yes – the primary endpoint is survival time.
6. Yes
7. No, the study was rather clear.
8. Yes, the results are reassuring.
9. Yes - the findings support exemption from consent.

ii) Arnaud et al – Effects of bovine polymerized ....

1. A) In general, yes. B) Yes, the data do tend to support the use of HBOC in humans.
2. Yes.
3. Yes.
4. No, monitoring is more extensive.
5. Yes, in part. The primary endpoints (some of which, like PFA-CT are quite sophisticated) look at the effects of HBOC on coagulation. Early changes will reflect the likelihood that patients will continue to bleed, which will affect outcome. The late outcome points are irrelevant, and some (like late microthrombus formation in the lung) reflect an outdated understanding of the pathogenesis of shock lung injury.
6. Yes
7. No, the study was rather clear.
8. Yes, the results are reassuring.
9. Yes - the findings support exemption from consent.

iii) Dong et al – Immune effects of resuscitation....

1. A) In general, yes. B) Yes, the data do tend to support the use of HBOC in humans.
2. Yes.
3. Yes, though the shock insult is rather moderate.
4. No, monitoring is more extensive.
5. Yes.
6. Yes
7. No.
8. Yes, the results are reassuring.
9. Yes - the findings support exemption from consent.

iv) Philbin et al – A hemoglobin based oxygen carrier....

1. A) In general, yes. B) The data lend some support the use of HBOC in humans.
2. Yes.
3. Yes.
4. No, monitoring is more extensive.
5. No, not really. There was no significant difference in death rate, and late parameters like the amount of blood used in the 'hospital phase' are not interesting.
6. Yes
7. No.
8. Yes, the results are somewhat reassuring.
9. Yes - the findings support exemption from consent.

v) Johnson et al – Bovine polymerized hemoglobin.... (CCM in press)

1. A) Yes. B) The data lend some support the use of HBOC in humans.
2. Yes.
3. Yes.
4. No, monitoring is more extensive.
5. Yes.
6. Yes
7. No.
8. Yes, the results are generally reassuring.
9. Yes - the findings support exemption from consent.

Group 1 d. Geoffrey Manley hemorrhage model

i. Knudson MM et al. Tissue oxygen monitoring during hemorrhagic shock...

1a. No
1b. These data supply very modest support
2. The study has both good and bad aspects to its design. The assessment of the tissue oxygen tension is state of the art. The shock protocols are unequal and tend to favor the HBOC. Specifically, the lactated Ringer's group is not resuscitated to the same extent and neither is the hypertonic saline dextran group. The hemodynamic data reported in Figure III only extend through shock and not into the resuscitation phase, which would have made the difference obvious. It annoys me that the original Journal of Trauma reviewers didn't pick up that disparity.
3. Possibly
4. No, the monitoring is much more intensive.

5. Yes: direct monitoring of tissue oxygenation is probably the best single marker for resuscitation.
6. Yes.
7. Yes, the model incorporates unequal resuscitation in the various treatment groups, moreover, in the "Conclusions" the authors tend to discount their own findings when it suits their purposes – note the conclusions, in paragraph 1. Although HBOC exerted a clear pressor effect, the authors discounted this in the last paragraph of the conclusions. Also, they claim tissue hypoxia was not seen when in fact it ***was*** seen in the liver which is exactly the tissue most at risk and most expected to manifest hypoxia.
8. The presence of a pressor effect and the related hepatic hypoxia suggests a clear mechanism for the hepatic enzyme elevations and alterations in liver histology seen in other animal studies. This may or may not be significant in terms of the ability of HBOC to support patients in shock.
9. Some support with respect to subparagraph ii is produced, however, with respect to subparagraph iii there is some suggestion of the possibility of excess risk.

ii. Lee SK et al. Small-volume resuscitation with HBOC…

1a. Yes, at least initially.
1b. The data do lend some support to human use especially in the setting of combined head injury and shock.
2. The study is small and lacks controls using another fluid, however, the data are interpretable.
3. The schedules of administration tend to simulate early resuscitation in the pre-hospital phase.
4. No, the monitoring is far more intensive.
5. Yes, again the study uses tissue oxygenation, in this case in the brain, as an end point of study which is excellent. It also identifies appropriate hemodynamic parameters, but does not report them all. This downplaying of systemic vascular resistance (SVR) is seen in several studies where it was clearly available, and would have directly assessed pressor effect.
6. Probably not, at least one animal suffered a cardiac arrest during "preliminary studies" due to rapid infusion of HBOC through a Swan-Ganz catheter. I suspect the data from this animal were omitted from the study which therefore has an "n" of seven instead of eight.
7. First, as noted above, this study included no control animals using other fluids. It did however look at the effects of HBOC in a linear fashion fairly well and the data obtained as to cerebral oxygenation are outstanding. The problem I have with the data and its interpretation is again that systemic vascular resistance was not reported and I suspect on the basis of the hemodynamic data that it was indeed increased. This was probably most apparent at the last time point which the authors did not discuss.

8. As noted above, there was a death due to rapid infusion of HBOC into the pulmonary artery, but on the other hand the data suggests that cerebral oxygenation was improved rather than simply maintained. This may lend support to HBOC use in cerebral injury.
9. The data do support exemption from informed consent, especially in the head injured group.

iii. Manley GT et al. Small-volume resuscitation…

This paper is an invited chapter in a non-peer reviewed publication. It essentially duplicates the prior paper (Lee SK et. al, Academic Emergency Medicine) using an 'n' of 6 rather than seven; I suspect this is a subset of the animals reported in the other paper and this paper should not be considered separately.

Group 1 e. Carolina Resuscitation Research Group

i. Katz LM et al. HBOC-201 improves survival in a swine model…

1a. Yes, in extreme cases.
1b. These results supply significant support for the use of HBOC in humans.

2. The study design is rather innovative in that it creates a situation where animals not resuscitated with the hemoglobin containing solution become profoundly anemic as well as hypovolemic during the hepatic hemorrhage phase and all the rest. Thus, this model does simulate the ***extreme*** case of hemodilution during resuscitation from shock. In another sense, however, these comparisons are unfair because ***the standard of resuscitation at this late point would be blood rather than a plasma expander***, and therefore blood resuscitation would be the appropriate comparator for HBOC.
3. No, the monitoring is more intensive.
4. The primary end point of this study is death and ***the model is constructed such that all animals that do not get HBOC die*** in the acute phase. In comparison, seven of eight animals given HBOC survived. With respect to the hemodynamic parameters the surviving HBOC animals appeared relatively well, but SVR was not reported. Interesting also, transaminases were not reported, reportedly because HBOC interfered with testing for them. This has not been the case in other studies I've reviewed. The remainders of the laboratory evaluations were essentially normal.
5. Yes.
6. As noted, these studies essentially referred to an end stage of resuscitation where progressive hemodilution causes the hemoglobin concentration to go to zero in animals that are not resuscitated with HBOC (or presumably in humans not resuscitated with blood). Thus,

although this study makes an excellent case for the use of HBOC in conditions where transport is prolonged and blood is not available, the results can not be realistically applied to conditions where transport times were shorter or blood is available in a reasonable time frame.

7. The absence of adverse events is reassuring however the study was not powered to look for them. Moreover, there is no comparative group available using this preparation.
8. This work does support exemption from informed consent in the situation where transports are prolonged and blood is unavailable.

ii Manning JE et al. Bovine hemoglobin based…

1a. Yes, very well.
1b. These data provide excellent support for the RESUS trial.
2. The study is well designed.
3. In general, yes.
4. No, the monitoring is more intensive.
5. A major outcome is survival which was markedly improved with HBOC. The secondary endpoints such as Ph base balance, etc., not surprisingly demonstrated superiority of HBOC. No effort was made to look at adverse events such as organ dysfunction.
6. Yes.
7. There are some minor issues with the speed and volume of resuscitation as seen in Figure III especially since resuscitation was supposed to be to equivalent blood pressures.
8. There is no evidence of excess adverse events in the HBOC group; the severity of shock here is such that the one mortality in the HBOC group should be expected.
9. These data do support exemption from informed consent in the setting of rapid hemorrhage especially with unavailability of blood.

Group 1 e: Carolina Resuscitation Research Group uncontrolled hemorrhage model (pig)

iii. Manning JE et al. Selective aortic arch perfusion…

1. This is an interesting model which evaluates the compared use of oxygenated Ringers lactate and oxygenated HBOC-201 in swine hemorrhaged to cardiac arrest and then resuscitated after two minutes with intra-aortic infusion of the study fluid. Shock was achieved by resection of four lobes of the liver with spontaneous hemorrhage. The fluids were given at identical rates i.e. 70 ml per kilogram per minute or the equivalent of approximately two units of blood per minute in a 70kg man.

The study makes several assumptions as to resuscitation which are futuristic to say the least, especially that an aortic cannula could be placed within two minutes of cardiac arrest from hemorrhage. There are also very real issues as to the

relative volumes of fluid used (as in multiple other studies). A 3-1 or 4-1 ratio of LR to HBOC (which is colloidal) must be followed The 2-1 ratio is inadequate. In addition, oxygenating the LR is of no interest since it won't carry the oxygen that is bubbled through it. In other ways this is a very good study. It is well done and there are no "untoward" adverse effects seen. Obviously the animals treated with non-oxygen carrying solution required epinephrine and eventually died, whereas the others survived for the course of this acute experiment. Of note also, lactic acidosis was seen in this paper like as with other uses of HBOC-201. This suggests an element of tissue hypoxemia, not unexpected in this circumstance, but worrisome for HBOC sumping of NO by HBOC-201. In general, therefore, this study does not mimic the study conditions of the RESUS protocol and does not give confidence for the waiver of informed consent in humans.

Group1 f: Rat models of shock

i. Handrigan MT et al. Choice of fluid influences…

These data are derived from a rat model that uses hypotensive resuscitation of the type suggested by Bickell and Mattox in a rat model. I have several concerns about this study. It compared either normotensive resuscitation with LR, hypotensive resuscitation with LR with hydroxyethyl starch and compared it to hypotensive resuscitation with HBOC. The HBOC however, appears to have been ***Polyheme*** and I'm not sure whether this is the same HBOC formulation that is proposed for use in the RESUS trial. In this study, the polyheme animals actually did less well than hypotensive resuscitated animals that were treated with ***either*** lactated Ringers or HEX. The model, therefore, does not suggest any benefit to resuscitation with HBOC and therefore does not support waiver of consent. These are, however, of course rat studies. Of interest also; there was a distinct increase in the base deficit seen after treatment with HBOC as compared to all the other interventions. This suggests once more that there was increased tissue hypoxia and does not support waiver of consent. Also of interest, although these results were quite striking they were not mentioned at all in the Discussion, suggesting possible bias in the reporting of the data.

ii. Hayward R. and Lefer AM. Administration of polymerized bovine hemoglobin…

This model used HBOC-201 in rats submitted to "Noble-Collip drum shock". This is a shock model rarely used anymore because of animal use considerations. The rats typically develop a diffuse capillary leak syndrome and hypotension due to loss of fluids into soft tissues, approximating a severe crush injury. In this set of experiments rats were resuscitated from their drum shock with a variety of fluids. HBOC animals had longer survival time, demonstrated improved arterial blood pressure and had lesser Ach-initiated vasoconstriction of SMA vascular rings. The authors also looked at infiltration of neutrophils into the bowel and found no differences with HBOC. All animals here were appropriately disposed of, these studies were well performed. But since these

studies do not address hemorrhagic shock *per se* they cannot give confidence as to the use of HBOC without informed consent.

Group 2. Reports of preclinical studies conducted and /or funded by the sponsor. Summary report and raw data for Protocol KO04-02.

a. Summary report and raw data for Protocol KO04-02.

i. HBOC-201 versus Hetastarch resuscitation after 40% blood volume loss

This group of studies by the Naval Medical Research Center, in Silver Springs, resulted in several publications that were previously reviewed and are noted in section 9.2 ("Manuscripts").

Comments about methods:

1. The instrumentation used is standard.
2. The tissue injury and controlled hemorrhage is a standard preparation.
3. The use of a Kocher clamp to crush the rectus muscle does not, in my judgment, constitute a significant soft tissue injury of the type which would activate systemic inflammatory responses. I believe it is marginally better than the laparotomy alone.
4. The resuscitation protocols used are fairly standard. The liver injury and uncontrolled hemorrhage protocol is relatively standard.
5. The resuscitation protocol routinely used 6% hetstarch in lactated Ringer's (LR) as a comparator. The post-op clinical observations and the euthanasia and tissue collection were all standard. Among the various assays used, the nitrotyrosine tissue analysis is probably the most interesting and relevant to the generation of peroxynitrate by nitric oxide. The use of transcutaneous PO_2 was also important to measure tissue oxygenation directly.

Comments about results

Using these methodologies survival was generally superior in the HBOC groups except where hemorrhage was limited. My overall impression from the histopathology was that there were no differences in treatment groups with the exception that HBOC-201 seemed to yield hepatic injury (cholestasis) consistently. With respect to the various parameters used to look for evidence of differential immune activation using the different fluids, I must say that none of the fluids appeared to be particularly different from any of the others in any consistent way. The only consistent result I could see was the finding that apoptosis was decreased in neutrophils subjected to severe shock when HBOC was used in the resuscitation. This might be of concern if those neutrophils persisted in the circulation and caused systemic inflammation. This was not, however, particularly apparent in the lung histology, which would be expected to be the major early target organ.

The use of nitrotyrosine staining was a thoughtful addition to this group of experiments. These results were not particularly interesting except in the severe shock group. The data suggests animals getting HBOC did not sustain greater nitrosylation in the liver and lung than animals getting HEX or LR. Nitrotyrosine plasma levels, however, were markedly higher in animals getting HBOC than any other treatment. These data suggest that nitric oxide dependent nitrosylation does indeed take place in both plasma and tissues in shock, and that both HBOC and other fluids have effects upon them either by ameliorating shock or by direct attenuation of NO in the case of HBOC. These effects, however, are complex and not yet well worked out.

The effects on bleeding time and other hematologic parameters do appear to be a problem with HBOC. In all cases there appeared to be mild platelet deficits related to HBOC administration. Whether these will be more significant than the clear tendency to better tissue oxygenation in the human circumstance remains to be seen.

a. Summary report and raw data for protocol SUR-02-336 (USUHS)

i. Bowyer M. Resuscitative strategies for delayed evacuation…

These studies also used HBOC versus hetastarch versus normal saline in the setting of vascular injuries with delayed evacuation in pigs. They also looked at normotensive versus hypotensive approaches to resuscitation. No publications were produced by this study. I note many of the personnel involved were also involved in the performance of the KO04-02 study at the Naval Medical Research Center.

Global comments about methods:

1) The instrumentation used is standard.
2) They used normotensive as well as 'hypotensive' resuscitation, allowing application to realistic civilian settings.
3) Both the normotensive and hypotensive resuscitation strategies used 2x LR as equivalent to the colloids HEX and HBOC. This is incorrect and makes the crystalloids 'look bad'.
4) Anesthesia and instrumentation were standard
5) The femoral artery and vein stab-wound injury model was excellent and well calibrated.
6) The use of repeated phlebotomy (P3179) diminished the differences between animals due to continuing bleeding. This may be important if one fluid preferentially causes coagulopathy.

7) Exclusion of animals due to 'low initial hemorrhage' was probably wrong since such events can reflect hemodilution or coagulopathy due to the resuscitation fluids.

Comments about results

1) Some of the hemodynamic parameters were significantly different at baseline (P3181).
2) Cardiac Index was lower and SVR was higher with HBOC than HEX resuscitation.
3) Re-bleeding was more frequent using HBOC than HEX.
4) Both HBOC than HEX showed more tendency to re-bleed than saline. This probably relates to 1) their greater ability to maintain blood volume expansion at the resuscitation volumes used and 2) a likely mild coagulopathy (P3184).
5) The flow parameters and O2 extraction parameters are confusing due to the unequal volumes of resuscitation. ***The tissue oxygenation parameters however are instructive. See especially the bottom right graph on page 3189. There is a period (60-120 min) during which the BP is kept constant by fluid infusion. During this time, a clear improvement of tissue oxygenation due to HBOC is seen. After surgical repair and the re-infusion of shed blood these relative differences are lost. Nonetheless, it is clear that tissue metabolism can be preferentially maintained by HBOC.***
6) There were no mortality differences found between resuscitation fluids.

Final reports by Manning JE et al:

These data have already been reviewed in their published form and the 'complete data' reveal no new insights.