Does Evans Blue Dye or Blood Sampling (Preconditioning) Improve Survival Time After Hemorrhage in Inbred Rat Strains?

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Introduction

Eight-fold differences in survival time were detected among 15 inbred rat strains after a severe controlled hemorrhage without resuscitation (Klemcke et al., *Shock* 29:748, 2008), suggesting an inherent genetic influence on the ability to survive hemorrhage. In a subsequent study, however, we showed that differences in survival time after hemorrhage might reflect strain-dependent differences in blood volumes (Klemcke et al., Exp Biol 2009). To clarify that observation, the current study was conducted wherein multiple inbred rat strains were consistently hemorrhaged ~47% of their blood volumes based on blood volumes measured 24 hrs earlier in each rat and their survival times measured.

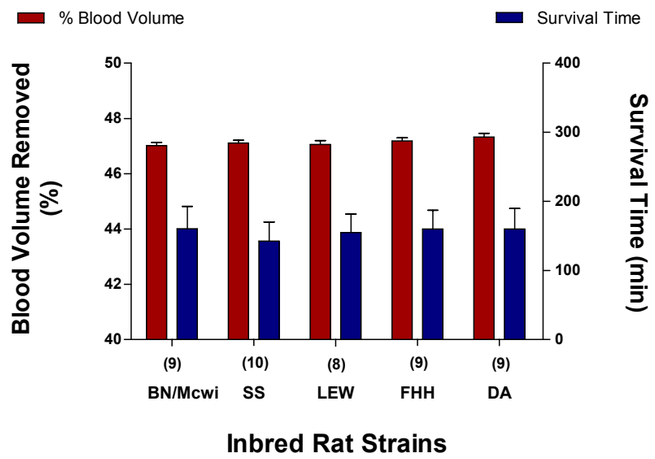
Methods

- Anesthetized male rats (~13 weeks of age) were chronically catheterized in the carotid artery.
- Blood volumes were measured in each rat using an Evans Blue procedure (Klemcke et al., Exp Biol 2009) and rats were recovered. Briefly, an initial ~400 µl of arterial blood sample was removed; a volume of 0.5% Evans Blue dye (100 µl/100g body weight) was injected into an exposed femoral vein; and after 5 min, a 2nd ~400ul of arterial blood was collected. Absorbancy was measured at 605 nm. Hematocrits were measured and used in the calculation of blood volumes (Wang, *Am J Physiol* 196: 188, 1959).
- 24 hrs later each rat was subjected to a conscious controlled hemorrhage by removing ~ 47% percent of their measured blood volume during a 26 min period.
- Rats were observed until death or euthanized after 4 hours.

Results

- The percent blood volume removal and survival times did not differ among strains (P > 0.38; Fig 1).
- Clear differences exist in survival times between this study and our initial study (Klemcke et al., *Shock* 29:748, 2008; Table 1).
- Previously, DA rats were bled only slightly more (based on blood volumes measured in subsequent studies), but survived 2 hr less than in our current study.
- Previously, FHH rats were bled less than currently, but survived 1.5 hr less.
- Previously, LEW rats were bled almost identical to the current study, but survived ~ 1 hr less than in the current study.
- Finally, for BN/Mcwi and SS, less blood was removed and survival times were longer previously than in the current study.

Figure 1



Each bar represents the Mean ± SEM of the number of rats shown below the bars. Inbred strains used in this study were: BN/McWi, Brown Norway from the Medical College of Wisconsin; SS, Dahl Salt Sensitive; LEW, Lewis; FHH, Fawn Hooded Hypertensive; and DA, Dark Agouti.

Table 1

Comparison of hemorrhage severity and survival times in the current study with our initial previously-reported study (Klemcke et al., *Shock* 29:748, 2008).

Rat Strain	Difference in % blood volume removed in initial *vs.* current study	Volume of blood equivalent to difference in % volume removed (ml)	Difference in survival times in initial *vs.* current study (min)
DA	1.58% more	(+) 0.264	120 less
FHH	1.29% less	(-) 0.321	96.5 less
LEW	0.24% more	(+) 0.049	56.9 less
SS	3.41% less	(-) 0.846	55 more
BN/Mcwi	4.81% less	(-) 1.023	47.9 more

For our previous initial study, the percent of total blood volume removed was calculated from the average blood volume of each inbred rat strain and from the known amount of blood removed from each rat. These percent of blood volume hemorrhages were then compared with those in the current study. These differences in percent blood volumes were also converted to an absolute volume of blood. The average difference in survival time between the initial and current study are presented for each inbred rat strain.

Discussion and Conclusions

- Under conditions of the current study, comparable hemorrhages among strains resulted in comparable survival times.
- Comparison of the previous with the current study denote differences in survival time despite similar hemorrhages.
- Such observations suggest some intriguing hypotheses: 1) The Evans Blue dye injected to measure blood volumes might influence survival time; and 2) The blood samples (~0.8ml) removed for blood volume determinations might constitute a preconditioning stimulus that improves survival time.

1. Evans Blue is not completely inert, but rather has multiple biological effects such as an ability to increase activity of calcium-dependent potassium channels associated with hyperpolarization and a decrease in cellular excitability (Hollywood et al., Eur J Physiol 435: 631, 1998; Wu et al., *Biochem Biophys Res Commun* 254, 666, 1999). It also blocks the action of purinoceptors (mediate the action of extracellular ATP) in endothelial and vascular smooth muscle cells (Eltze and Ullrich, Eur J Pharm 303: 139, 1996). Such actions could potentially influence survival to hemorrhage.
2. Preconditioning refers to a procedure wherein a noxious stimulus—in this case hemorrhage—near to but below the threshold of irreversible damage is applied to a tissue or organism. Shortly after preconditioning, the tissue or organism develops a certain resistance or tolerance to that or even a different noxious stimulus (Dirnagl et al., *Lancet neurology* 8, 398, 2009). Hence, preconditioning protects against subsequent injury. Thus, blood removal for measurement of blood volumes 24 h earlier might precondition the rats to survive better after a more severe hemorrhage. Both hypotheses will be tested in a subsequent study.