# *In vitro* and *in vivo* determination of antiTNFα activity in canine plasma from donors subject to preconditioning with endotoxin

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## BACKGROUND

Septic shock is characterized by cardiovascular and vasomotor failure that is induced by an uncontrolled cascade of inflammatory mediators such as TNFα, IL1β and IL6. In dogs, systemic bacterial infections, haemorrhage, trauma, gastric dilatation/volvulus and pancreatitis are the major causes of septic shock. Whilst endotoxin is a recognized effector molecule that can initiate an inflammatory cascade, it has been reported that preconditioning with endotoxin can down-regulate inflammatory cytokine responses to subsequent endotoxin challenge. This study reports the effect of endotoxin preconditioning on antiTNFα activity present in plasma from canine donors.

## MATERIALS

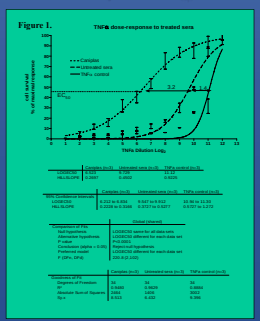
Plasma from preconditioned (Caniplas®) and normal dogs (FFP) was provided blind to the study by a commercial supplier (Plasvacc Pty Ltd).

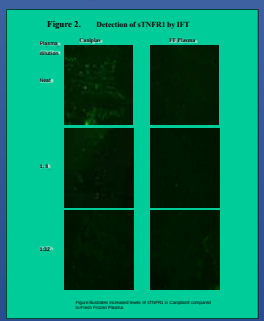
## METHODS

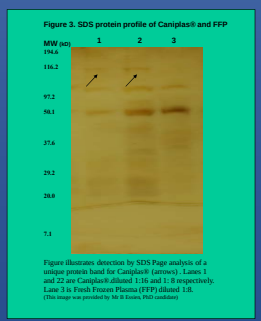
*In vitro* antiTNFα activity in canine donor plasma was determined by a L929 murine cell TNFα inhibition bioassay using recombinant murine TNFα. *In vivo* effects were tested by a rat subcutaneous skin pouch model. Rats were pre-treated for 3 days with either Caniplas®, FFP, saline (2mL/day, s.c) or carprofen (5mg/kg, s.c) and inflammation induced by injecting monosodium urate crystals into the pouch (5mg/ml in 5ml saline). Fluid was taken from pouches at specified intervals for cell count. TNFα and Il-6 levels were determined by Elisa. Protein profiles of Caniplas® and FFP were determined by standard SDS PAGE analysis. Examination of serum for soluble TNFα receptor 1 (sTNFR1) was performed by an immunofluorescence assay using a rabbit polyclonal anti sTNFR1 antibody and a FITC conjugated goat anti rabbit antibody as the detection fluorochrome. Data analysis: Normalized data was fitted to a Four-Parameter Logistic curve. Fitted midpoints were compared statistically for data sets using an F-test and calculated fitted hill slopes.

## RESULTS

In the rat skin pouch model, both Caniplas® and FFP reduced TNFα levels and Caniplas® was a more potent antagonist (data not shown). The heightened anti TNFα activity of Caniplas® compared to FFP was confirmed in the *in vitro* cell bioassay (Figure 1). Neither Caniplas® nor FFP reduced inflammatory cell infiltration or levels of IL6. There was also possible evidence that the effector mechanism in Caniplas® may be increased levels of soluble TNFα receptor 1 (Figure 2). A difference in the protein profile between Caniplas® and FFP by SDS Page analysis (Figure 3) was detected , although the nature and significance of this difference remains to be determined.




## CONCLUSION

Whilst we remain to confirm the mechanism, we report that preconditioning with endotoxin does illicit specific anti TNFα activity and that this observation has been confirmed in both in *in vitro* testing and *in vivo* animal models. It is plausible that preconditioning animals with endotoxin induces an increase in the concentration of soluble TNFα receptors I and II in donor plasma and that this is the likely source of TNFα antagonism. This report suggests that preconditioned plasma may be a beneficial treatment where inflammation causes increased expression of TNFα .