A NOVEL PROTEIN-BINDING RNA APTAMER, APTA-1, EMERGES AS A NEW THERAPEUTIC TOOL TO COMBAT SEPSIS

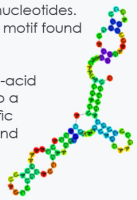
L Jedlina[1]; O Olin[1]; A Bylock[1]; V Casslén[1]; M Eriksson[2]; M Brewinska-Olchowik[3]; K Piwocka[3]; M Lindstam[1]

[1]Aptahem AB, Malmo, Sweden; [2]Section of Anesthesiology and Intensive Care, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden; [3]Laboratory of Cytometry, Nencki Institute of Experimental biology PAS, Warsaw, Poland.

INTRODUCTION

Aptamers are single oligonucleotide chains, which constitute a novel class of chemical compounds. The chains are long enough to allow formation of unique secondary and tertiary structures. The 3D structures are crucial for their capability to recognize specific functional motifs on their tailored targets, for binding affinity and specificity.

Apta-1 is an aptamer consisting of a single RNA chain with 90 nucleotides. Apta-1 binds with high affinity and specificity to an amino-acid motif found in a cell membrane receptor protein DBL1. However, the same amino-acid motif is also present on other extracellular proteins. This ability, to specifically recognize a target as subtle as amino-acid motif rather than a target as large as a protein, holds the key to a very unique feature of Apta-1. By specifically targeting a specific amino-acid motif, Apta-1 can act on multiple protein targets and thus display multiple *in vivo* effects at the same time.



In this study we have investigated *in vivo* **effects of Apta-1 treatment on hemostasis, inflammation and tissue repair.** These three arms of immune response are commonly dysregulated in sepsis and in the Systemic Inflammatory Response Syndrome (SIRS). We present results from studies on non-human primates, rats and mice using *in vivo* models for thrombosis and endotoxemia.

METHODS

In order to mimic the human Systemic Inflammatory Response Syndrome, we used an *in vivo* model for Endotoxemia where **non-human primates (NHP)** or **mice** were challenged with endotoxin lipopolysaccharides (LPS endotoxin). To explore the effects of Apta-1 on blood coagulation we used an Arteriovenous shunt model for Thrombosis in **rats.**

In all experiments, the **untreated** group received the vehicle (saline) i.v. while the second group received **Apta-1** i.v. A third group of animals treated with **standard of care** drug used in the clinic (e.g. the anti-coagulant Rivaroxaban or the anti-inflammatory drug Dexamethasone) was also included in some experiments.

Untreated (LPS + Saline) | **Standard** (LPS + Standard treatment) | **Apta-1** (LPS + Apta-1 treatment)

The doses used across the animal species varied in concentration (mg/kg). However, all dosing was converted based on an equivalent surface area dose for other species and thus **each species received an equivalent dosing.**

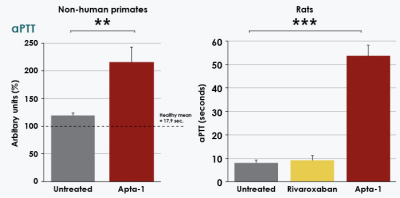
Blood samples were collected **2-3 hours** after vehicle/treatment administration regardless of animal species or *in vivo* models used. For parameters such as D-dimers and indicators of complement activation we also present results from samples taken at the **24 hours** time point.

RESULTS

HEMOSTASIS

Coagulation

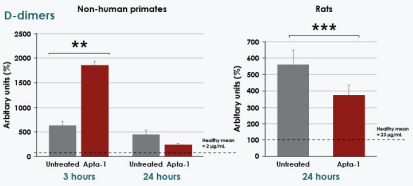
Apta-1 significantly prolongs blood clotting times



Blood coagulation times were assessed by aPTT in plasma of non-human primates and rats. Apta-1 significantly prolongs blood coagulation in both animal species and in two *in vivo* models, endotoxemic as well as thrombotic. In contrast to Rivaroxaban, Apta-1 does not affect extrinsic coagulation pathway in Rat Arteriovenous Shunt Model for Thrombosis.

Fibrinolysis

A brief induction of D-dimers by Apta-1

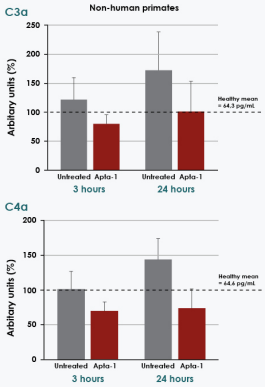


D-dimers in plasma of non-human primates and mice were quantified by ELISA. D-dimers are 3-fold increased in Apta-1 treated animals at 3 hours post LPS-administration. This pro-fibrinolytic effect of Apta-1 is short-lived and D-dimers fall below the level observed in untreated groups already at 24 hours.

INFLAMMATION

Complement

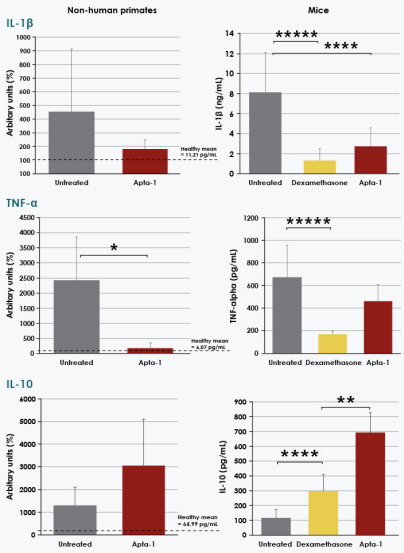
Apta-1 prevents over-activation of complement cascade



The amount of complement fragments C3a and C4a in non-human primates treated with Apta-1 does not exceed the amounts observed in healthy animals during the first 24 hours after LPS administration. Apta-1 prevents over-activation of complement cascade atearly stages and thus exhibits anti-inflammatory effect in NHP animal model for endotoxemia. Both complement fragmentswere quantified by Cytometric Bead Array human anaphy-latoxin kit for flow cytometry.

Cytokines

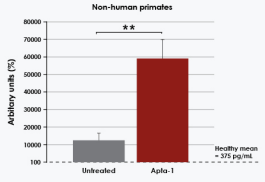
Apta-1 inhibits hyperinflammatory response in two animal models for endotoxemia



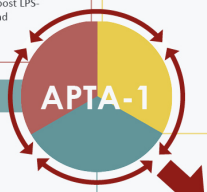
Apta-1 decreases circulating levels of clinically monitored pro-inflammatory cytokines IL-1β (2-fold) and TNF-α (25-fold fold, p=0.03) at 3 hours after LPS administration in non-human primates. Also, treatment with Apta-1 results in an increase of the anti-inflammatory cytokine IL-10 in blood circulation. Furthermore, Apta-1 increases IL-10 levels significantly higher than corresponding increase in Dexamethasone treated mice (p=0.006). Cytokines were quantified by LEGENDplex kit for flow cytometry.

TISSUE REPAIR

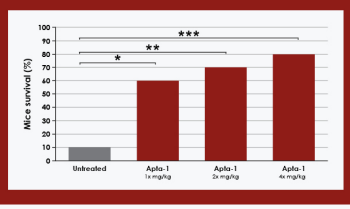
Pentraxin 3, a key player in tissue repair, is significantly increased by Apta-1



Apta-1 induces tissue repair by up-regulating circulating levels of Pentraxin 3. Pentraxin 3 was quantified by LEGENDplex for flow cytometry.



APTA-1 DRAMATICALLY IMPROVES SURVIVAL OF ENDOTOXEMIC MICE



Mice challenged with LPS endotoxin were monitored for 72 hours and survival was recorded. Survival in untreated group (n=10) is only 10%. Survival in groups treated with relative 1x mg/kg, 2x mg/kg, and 4x mg/kg of Apta-1 (n=10 in each group) is dose-dependent and ranges from 60 to 80%. Apta-1 significantly improves the outcome in animals with endotoxemia.

CONCLUSIONS

This study identifies Apta-1 as an anti-coagulant, an inhibitor of hyperinflammatory response and an inducer of tissue repair. Such broad spectrum of *in vivo* effects can only be explained by Apta-1 acting simultaneously on multiple proteins, which share an identical amino-acid motif – a motif specifically targeted by Apta-1.

A survival rate of endotoxemic mice was dependent on the treatment dose of Apta-1, with up to 80% survival in mice given the highest dose. Since survival in the untreated group was only 10%, Apta-1 dramatically improves the outcome in animals with endotoxemia.

Apta-1 has a potential to act therapeutically in pathophysiological conditions characterized by an exaggerated response of the immune system, as seen in SIRS and sepsis.

APTAHEM

CONTACT INFORMATION
info@aptahem.com
www.aptahem.com

References
1. Barfod A. et al. *Parasitology Research (2009) 105:1557–1566*
2. Fletcher B et al. *Journal of Cellular and Molecular Medicine (2012) 16, 4: 672-682*
3. Doni A. et al. *Frontiers in immunology (2019) 10: 712*