Murine models of liposome reactions: technical challenges and mechanisms

Gábor Szénási

Biological therapies and vaccines: safety issues, physiological and immunological mechanisms

Summer School for PhD students and young researchers

15-16. 06. 2021.

Why is it reasonable to study infusion reactions (pseudoallergy/anaphylactoid reactions) in rodent models

if

rats and mice are 100/1000-fold less sensitive than humans

(and pigs are similarly sensitive)?

1. **Rats and mice are suitable for testing the (very complex) mechanisms of pseudoallergy – lower cost, TG mice, etc.**

   – Exploring the mechanisms of pseudoallergy is important to predict safety.

The problem:

pseudoallergy is observed in sensitive humans while, pseudoallergy is uniform in all animal models

The explanation:

humans are very heterogeneous genetically/epigenetically, while experimental animals are homogeneous

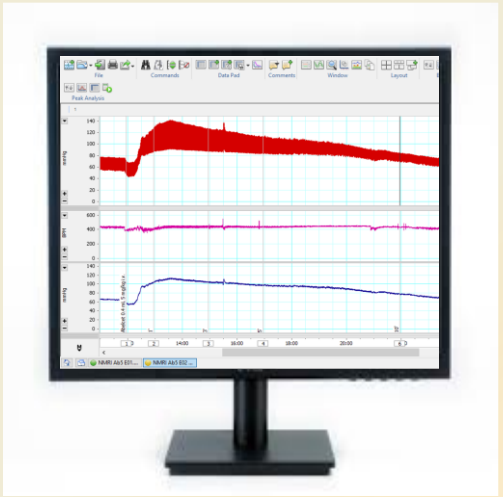
i.e.

the mechanisms of pseudoallergy is likely heterogeneous in humans

2. Exploring the mechanisms of pseudoallergy in rodents can help to better understand variations in human pseudoallergy

Methods of CARPA/CIPA testing in mice

1. Hemodynamic study



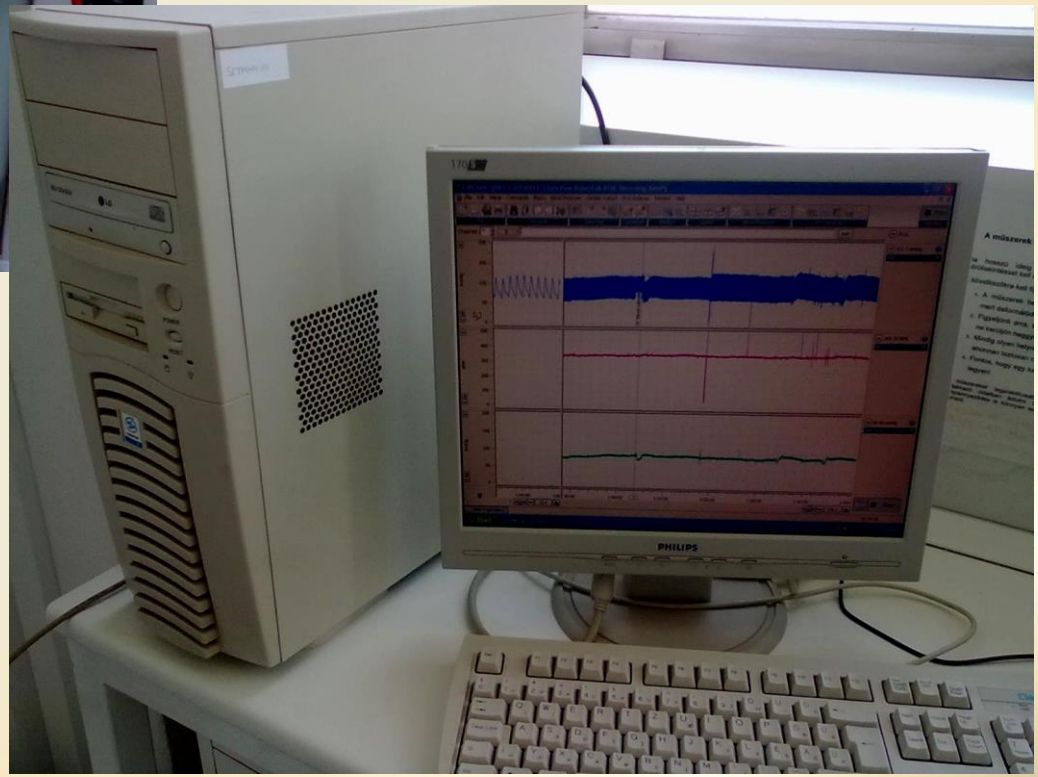
anesthesia and surgery

injection of test agents

BP and HR monitoring

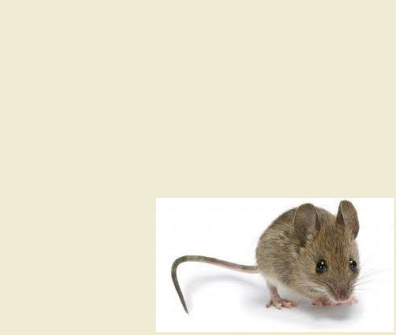
Equipment


PowerLab
BLOOD PRESSURE
BALANCE
CAL
SELECT
RANGE (mmHg)
GAIN
EXP
PW-01


PHILIPS

Methods of CARPA/CIPA testing in mice

2. Blood count and laboratory tests (ELISA)



anesthesia

injection of test agents

Anesthesia and sacrifice

Blood samples at 3-30 min

Blood cell counts

TXB2

C3a

CH50

PanC3

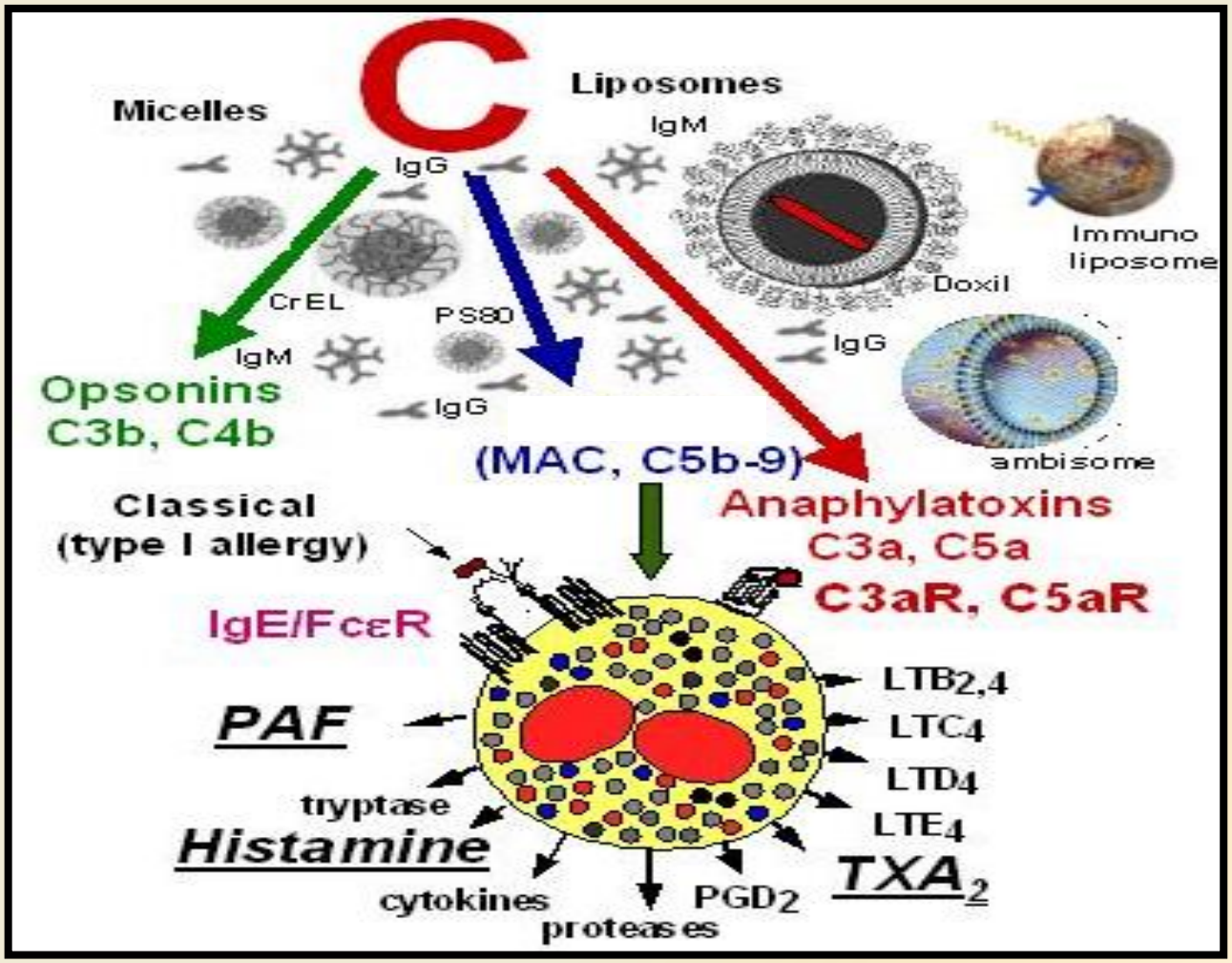
Hematology analyzer

Basic characteristics of liposomes and reference materials

Name	API	Lipids	The shape and nominal size	Origin
AmBisome	amphotericin B	HSPC, Chol, DSPG, αT	spherical SUV, <100 nm	Gilead, US
Abelcet	amphotericin B	DMPC, DMPG	Ribbon-like complexes >10 μm	Sigma-Tau Pharmaceuticals
Zymosan	Zymosan is a glucan with repeating d-glucose units connected by β-1,3-glycosidic linkages. It binds to TLR 2 and is a direct C activator. Zymosan is found on the surface of fungi, like yeast.			
cobra venom factor	CVF forms a CVFBbD complex, or convertase that is capable of activating 100 % of C3 in a wide variety of species via the alternative complement pathway.			

Abbreviations: API, active pharmaceutical ingredient; HSPC, hydrogenated soy phosphatidylcholine; Chol, cholesterol; DSPG, distearoyl-phosphatidylglycerol; αT, α-tocopherol. SUV, small unilamellar vesicles

Pseudoallergy/anaphylactoid reaction



Complement activation-related pseudoallergy - CARPA

International Journal of Nanomedicine
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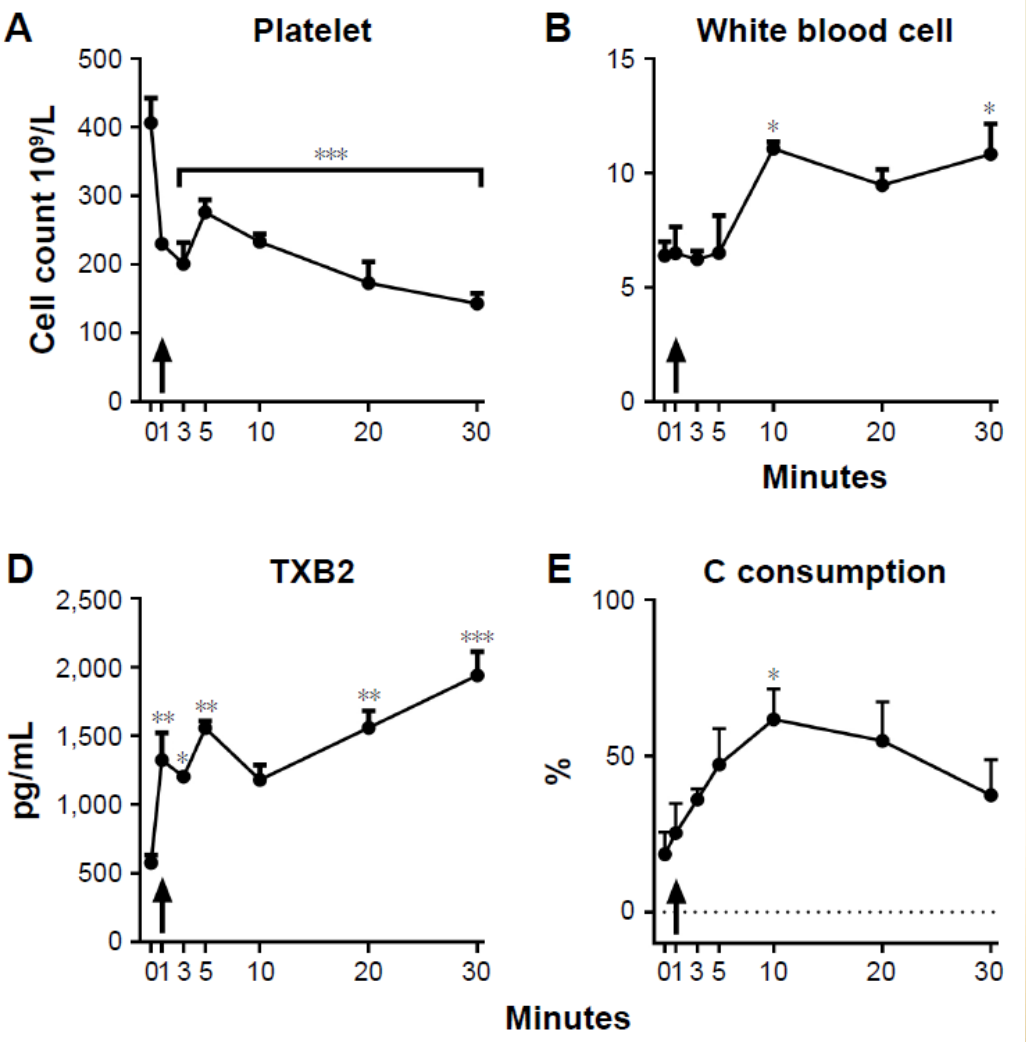
open access to scientific and medical research
2019 Feb 26;14:1563-1573. doi: 10.2147/IJN.S187139.
Open Access Full Text Article
ORIGINAL RESEARCH

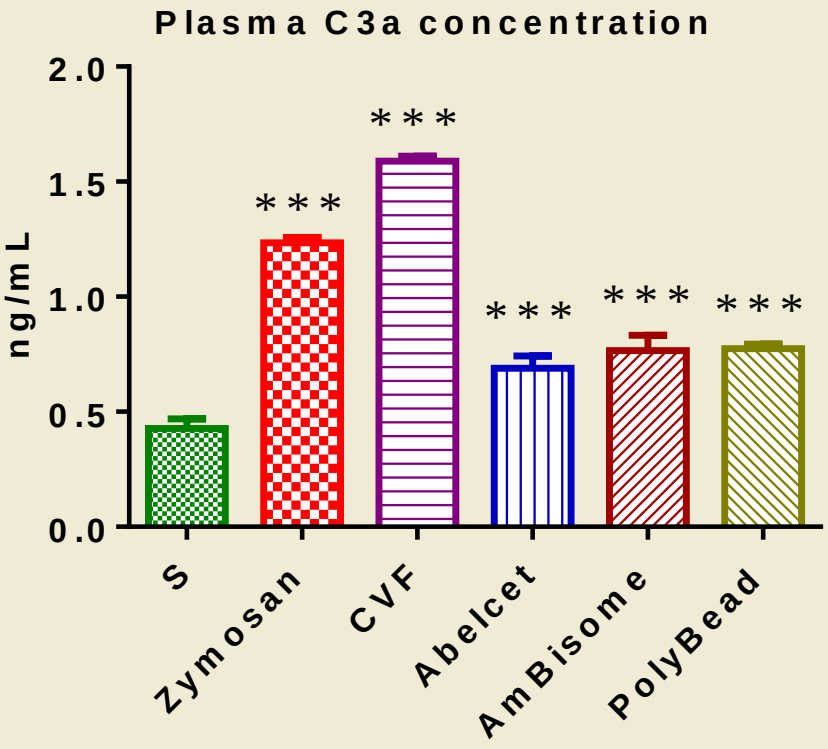
Acute physiological changes caused by complement activators and amphotericin B-containing liposomes in mice

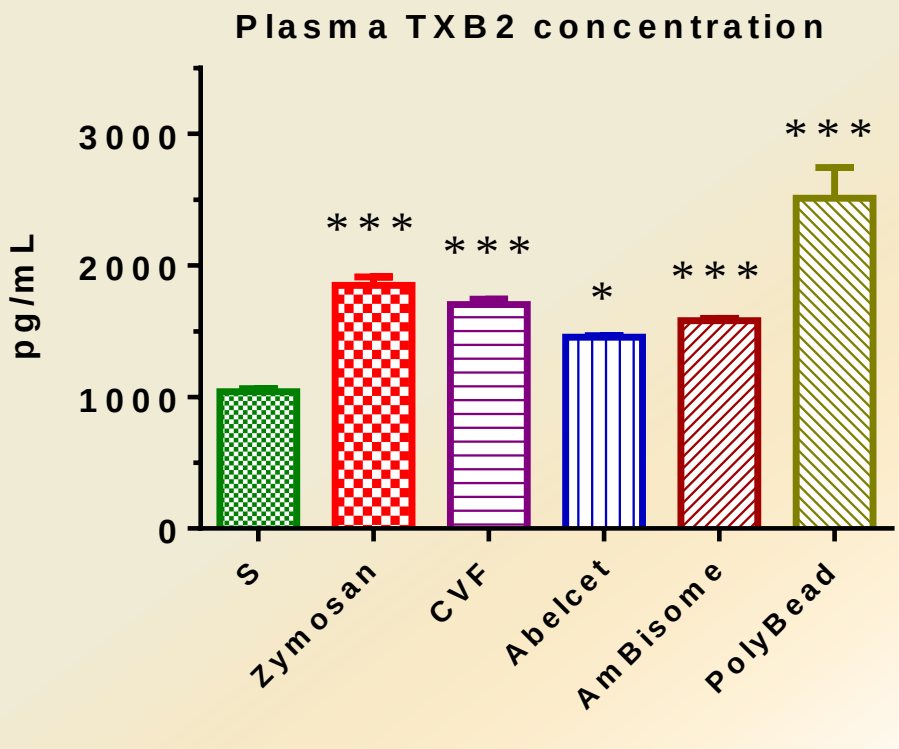
Erik Őrfi,[1,2] Tamás Mészáros,[1,2] Mark Hennies,[3] Tamás Fülöp,[1,2] László Dézsi,[1,2] Alexander Nardocci,[1] László Rosivall,[1,2] Péter Hamar,[4,5] Barry W Neun,[6] Marina A Dobrovolskaia,[6] János Szebeni,[1,2,7,*] Gábor Szénási[1,8,*]

Effects of zymosan at 30 mg/kg i.p.

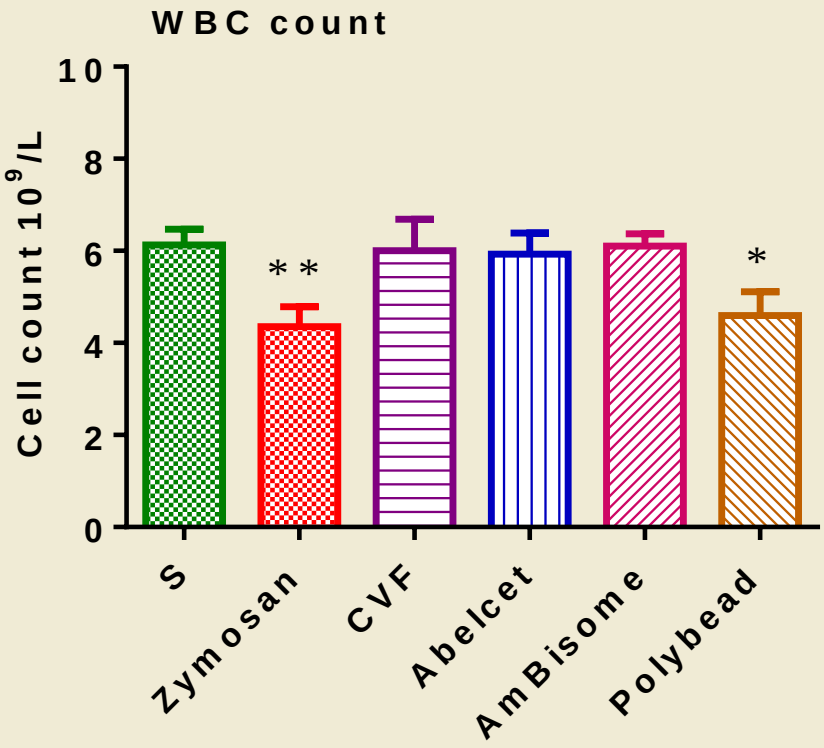


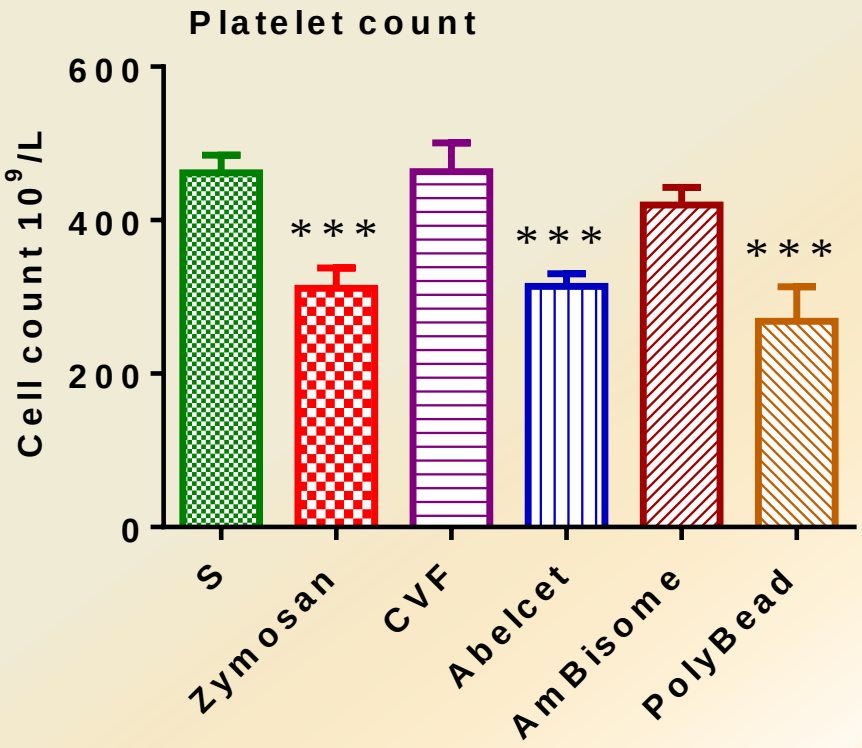
Effects of liposomes and complement activators on plasma C3a and thromboxane (TXB2) concentrations 3-5 min after treatments in mice



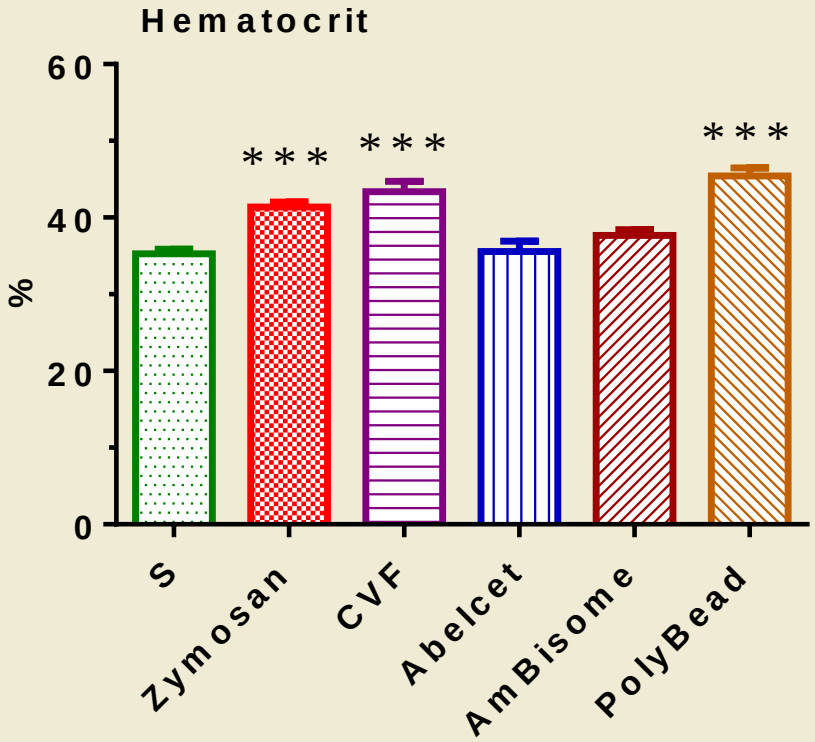


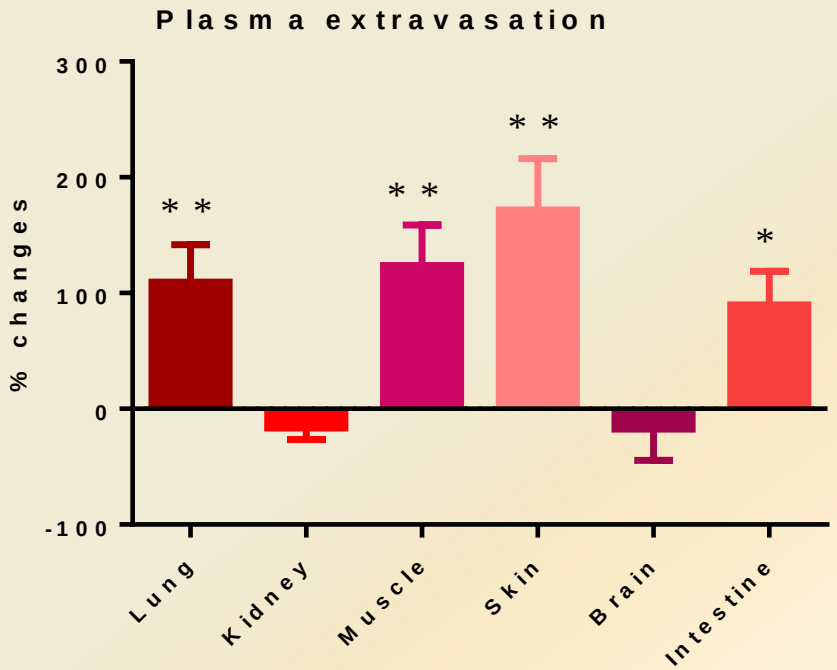
Effects of liposomes and complement activators on white blood cell (WBC) and platelet counts 3-5 min after treatments in mice





Effects of liposomes and complement activators on hematocrit and plasma extravasation (zymosan, 30 mg/kg, i.v.) at 3-5/30 min after treatments in mice

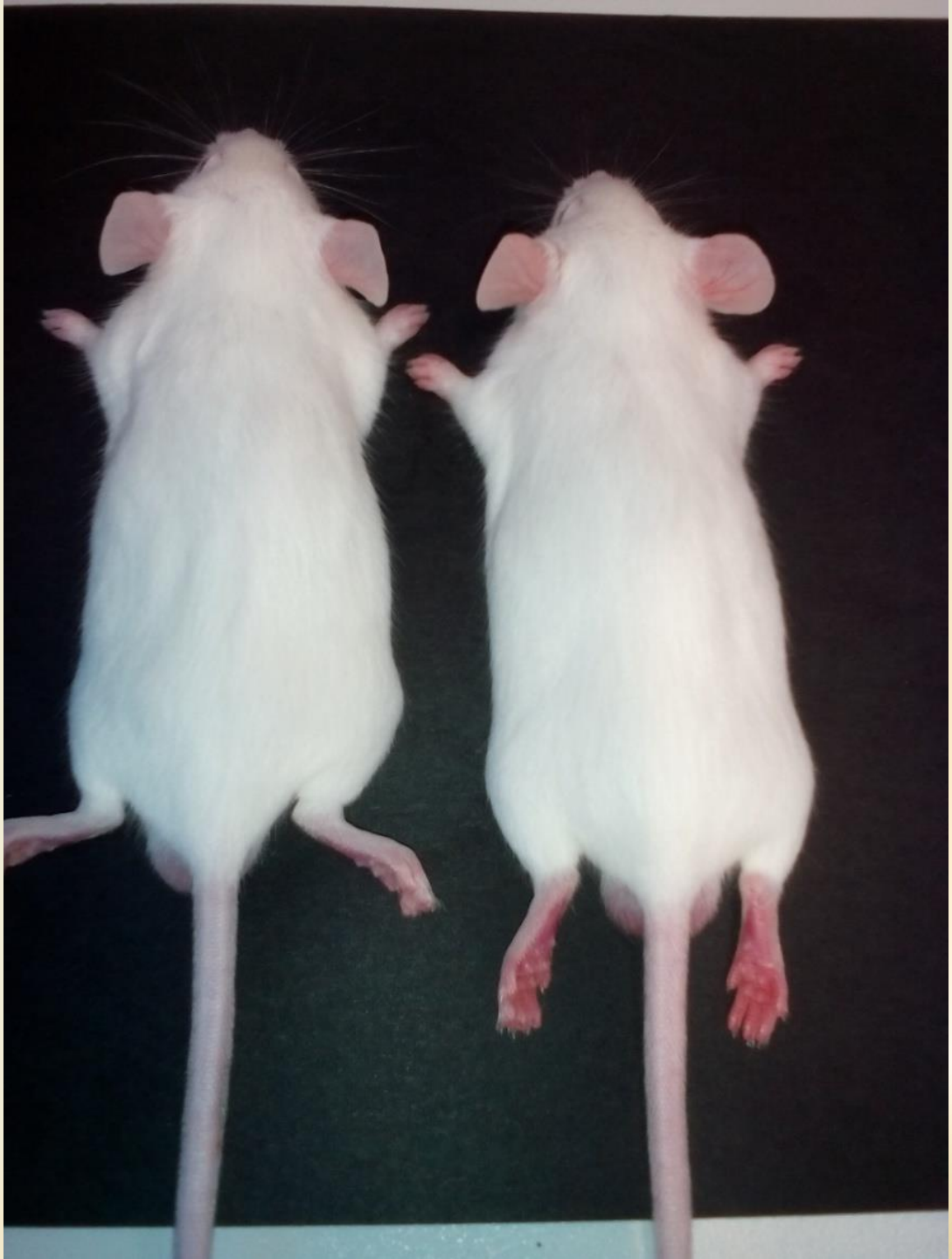




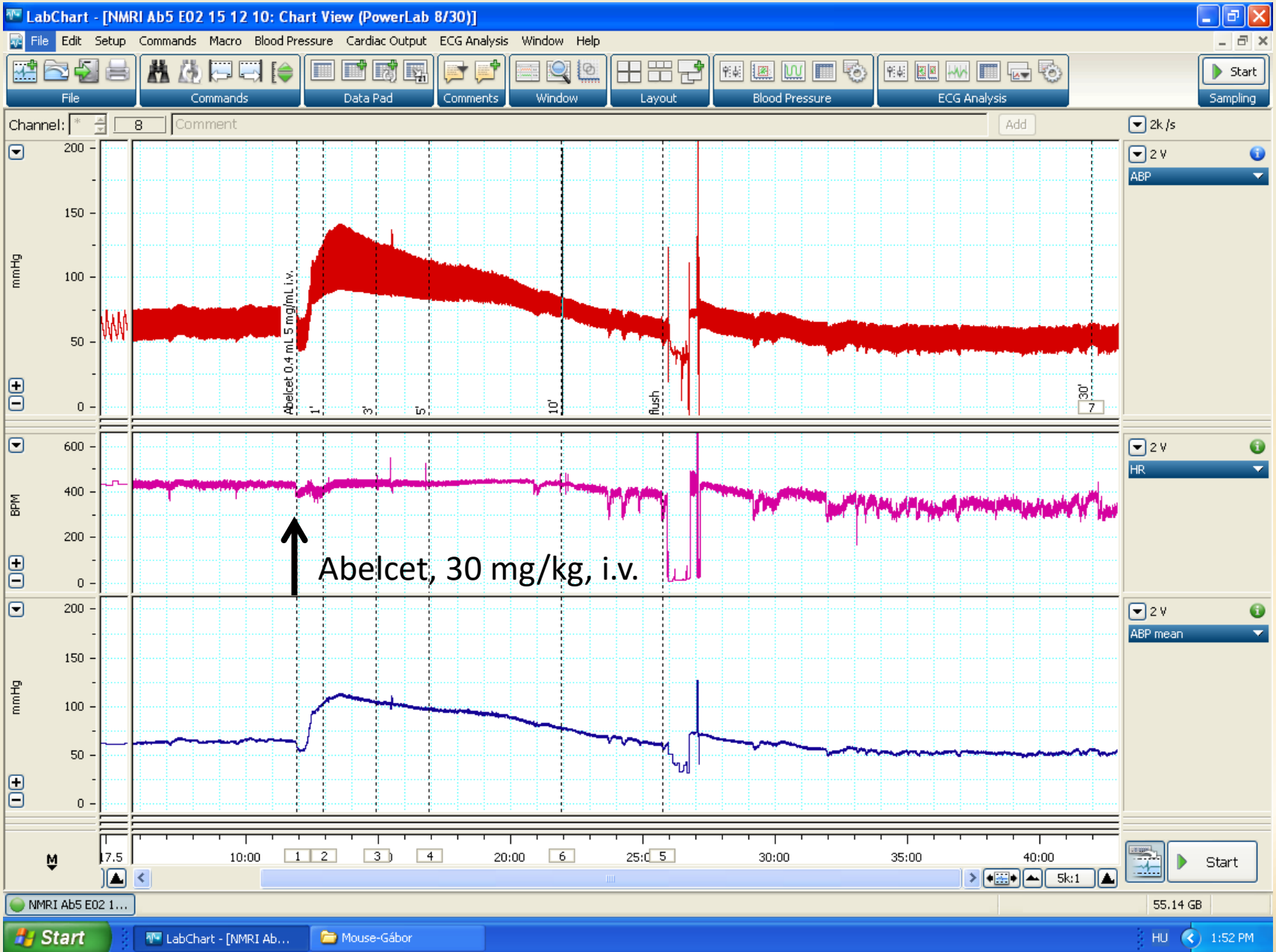
Plasma extravasation (zymosan, 30 mg/kg, i.v.) in mice

Rare CARPA symptom: urticaria

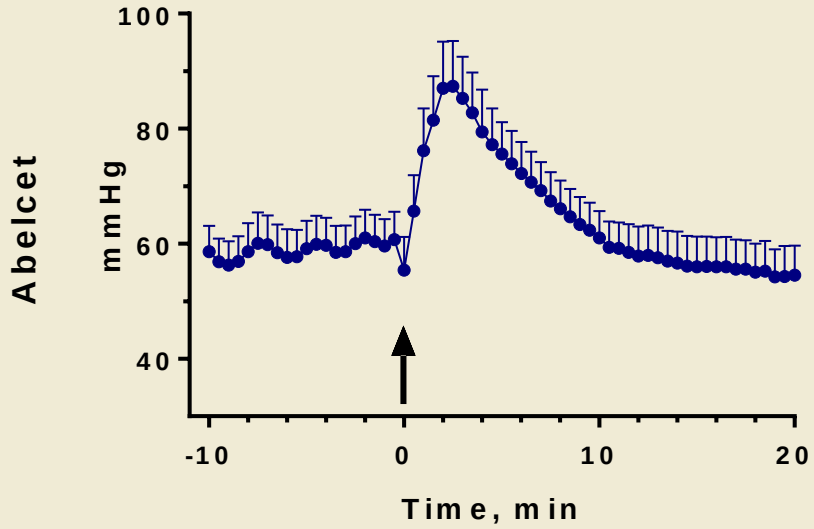
Effects of mannan (a linear polymer of the sugar mannose).

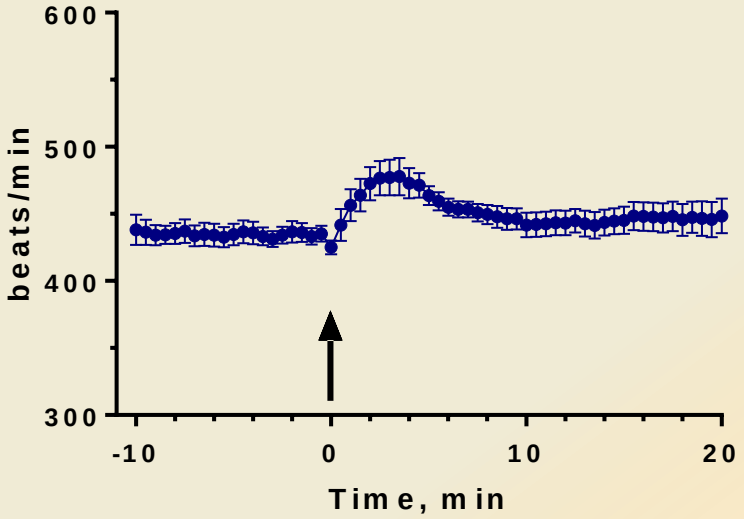


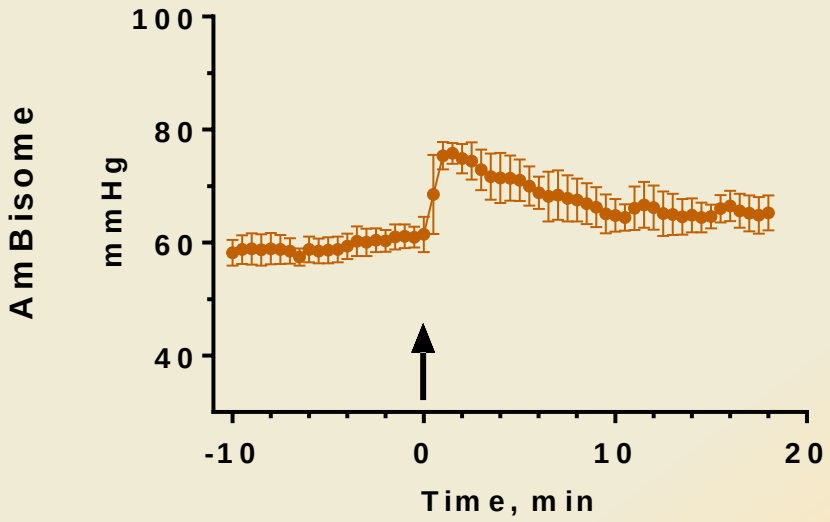
Original recording in a mouse

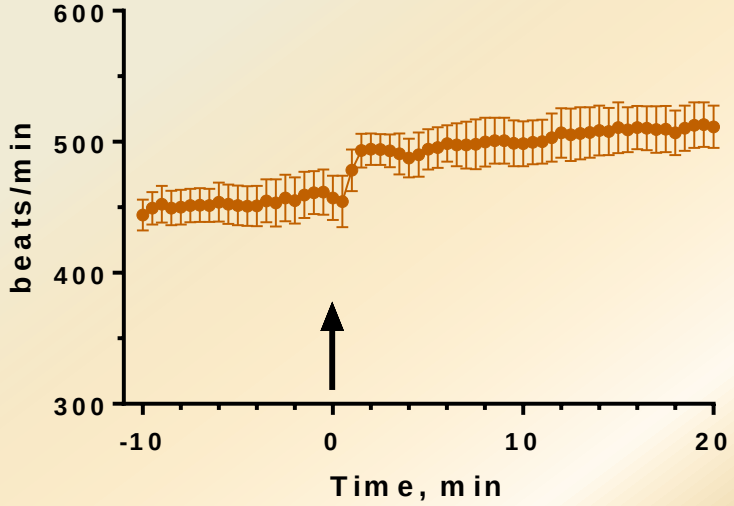


Effects of liposomes (Abelcet, 30 mg/kg; Ambisome, 300 mg/kg, i.v.) on blood pressure (left) and heart rate (right) in mice

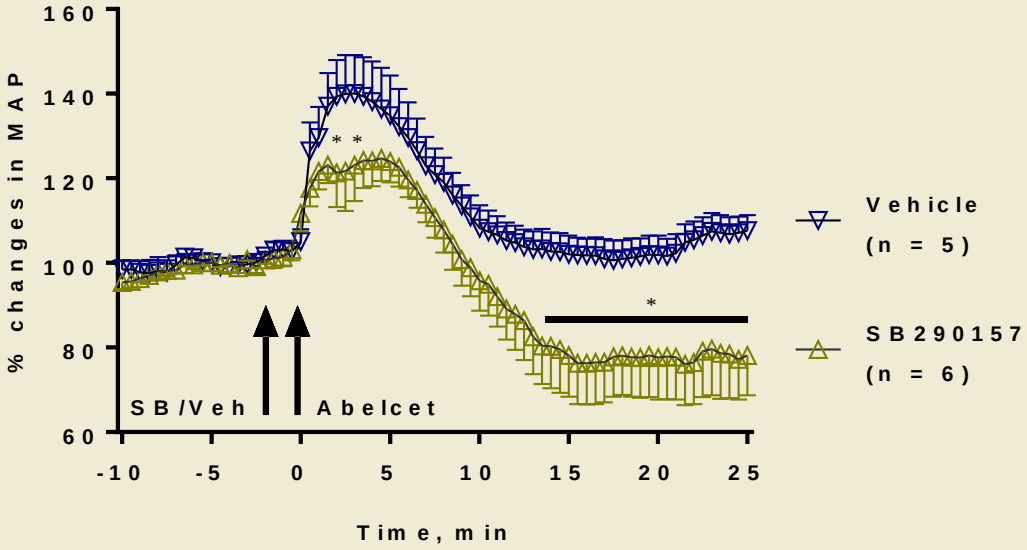


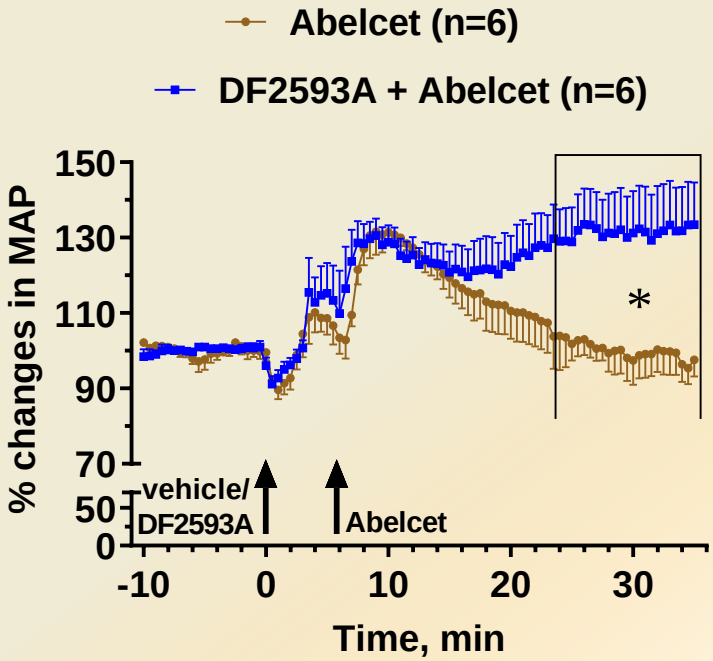






Mechanism of blood pressure changes: the roles of complement fragments C3a and C5a in mice

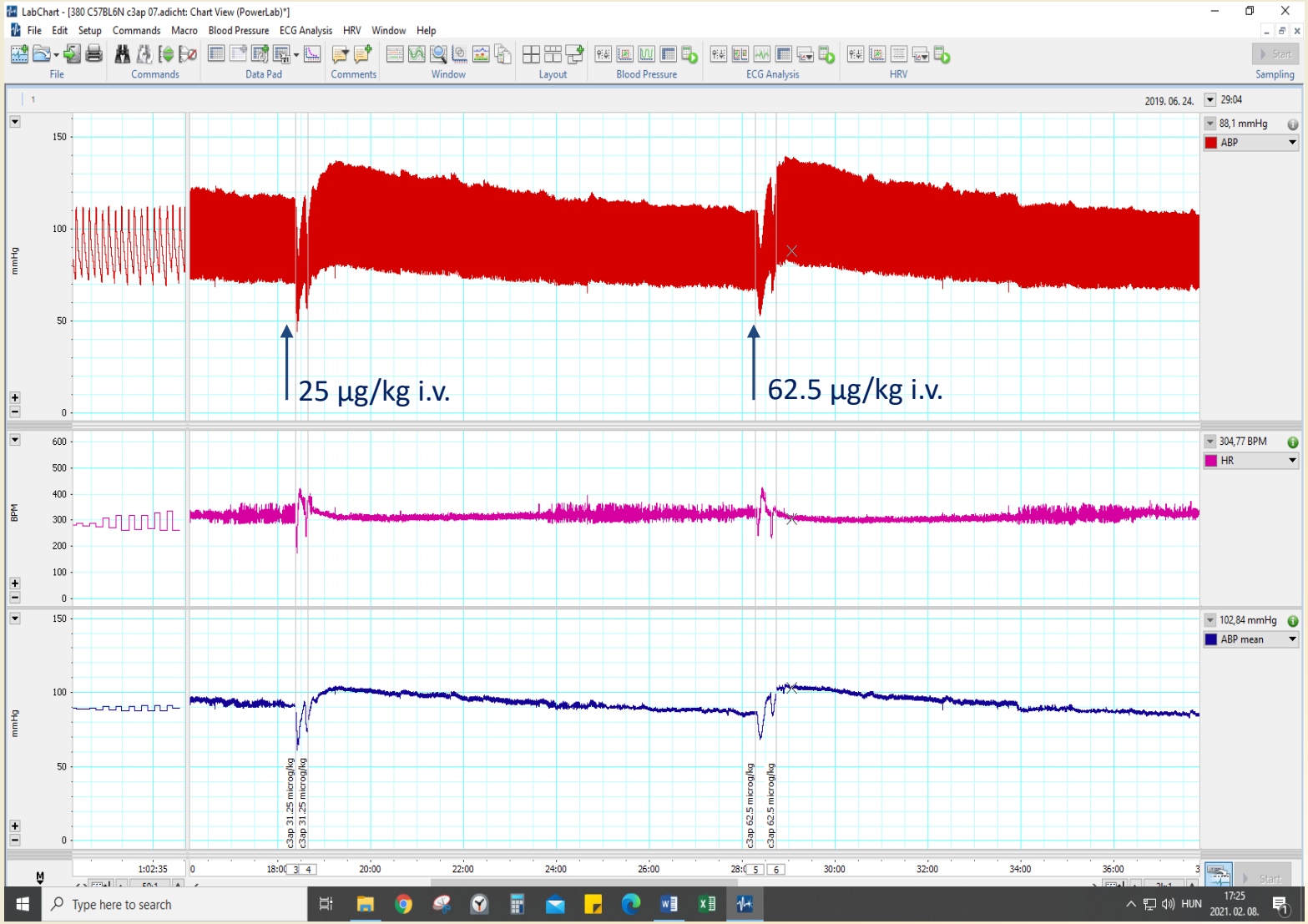




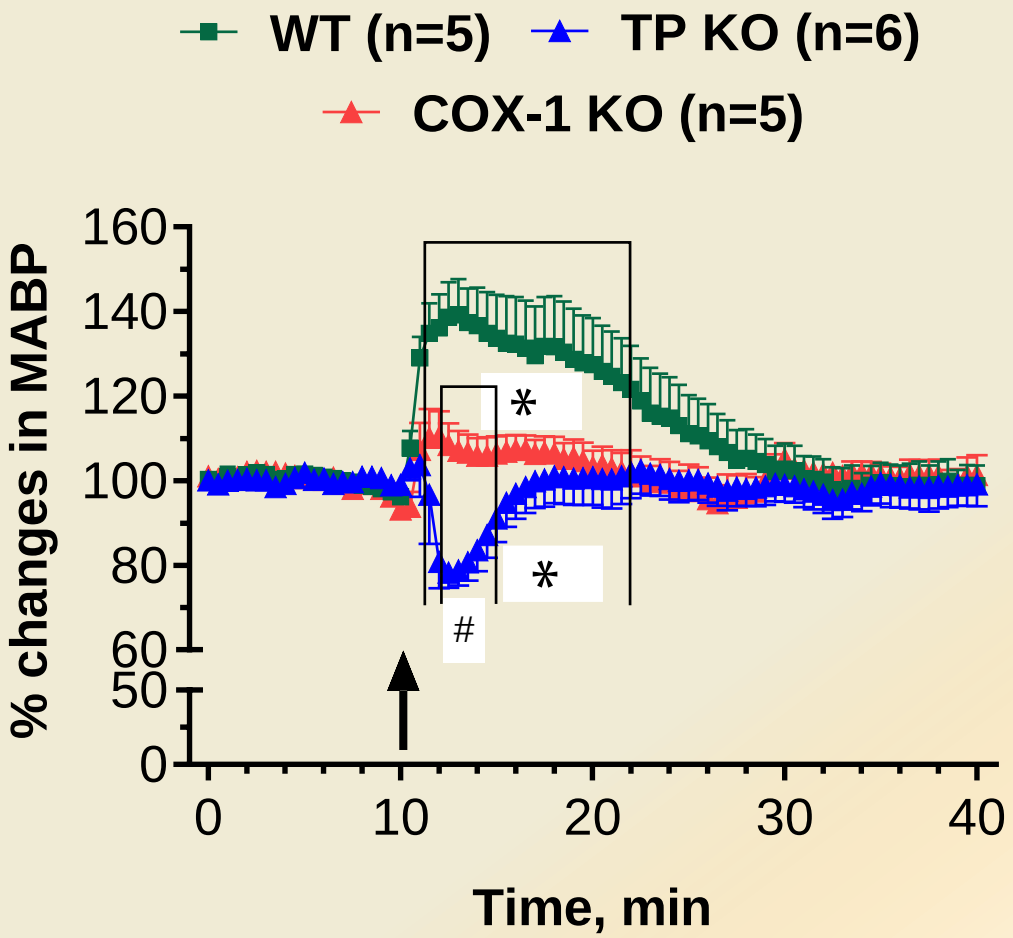
SB290157 – C3a receptor antagonist

DF2593A – C5a receptor antagonist

Mechanism of blood pressure changes: role of complement C3a (63-77 AA) peptide fragment in mice

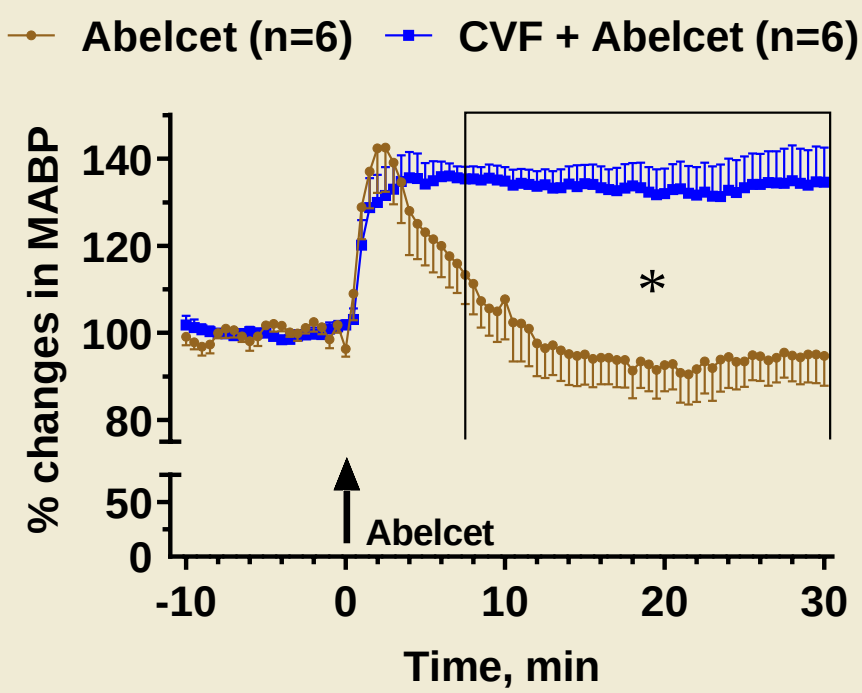


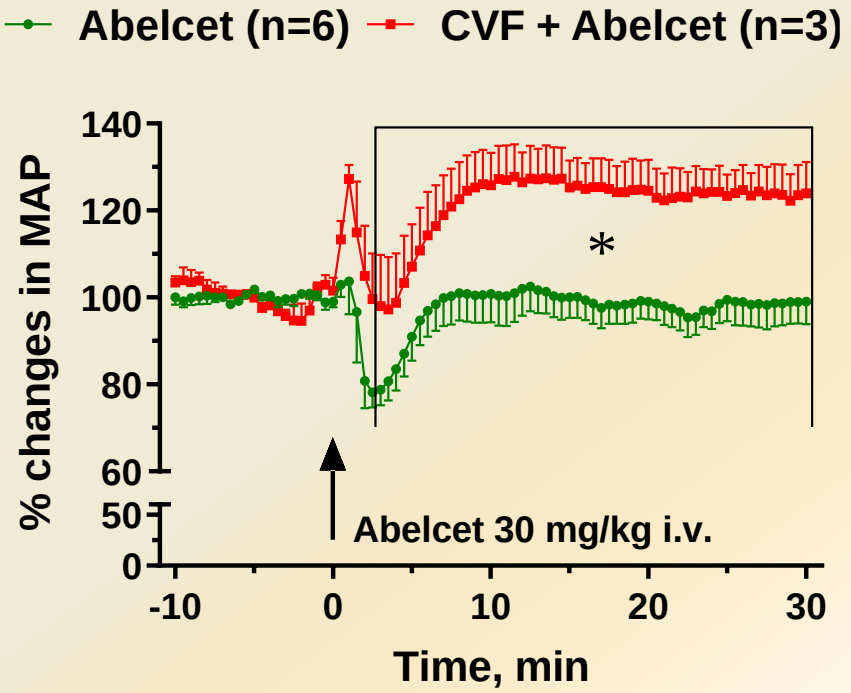
Mechanism of blood pressure changes: role of cyclooxigenase-1 (prostaglandins) and thromboxane A2 in mice



Mechanism of blood pressure changes: the role of complement and thromboxane A2 in mice

TP KO mice ↓

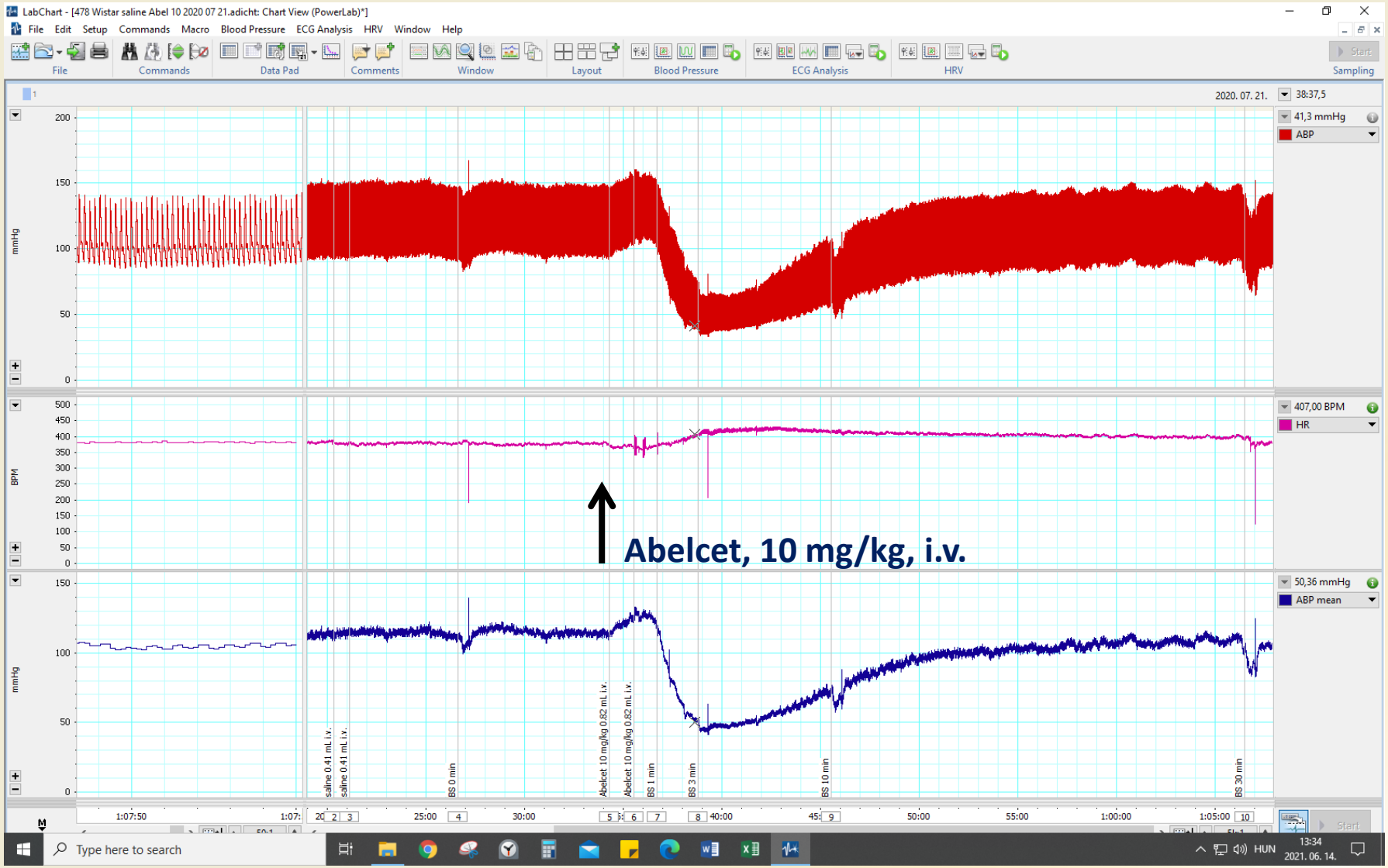




In summary

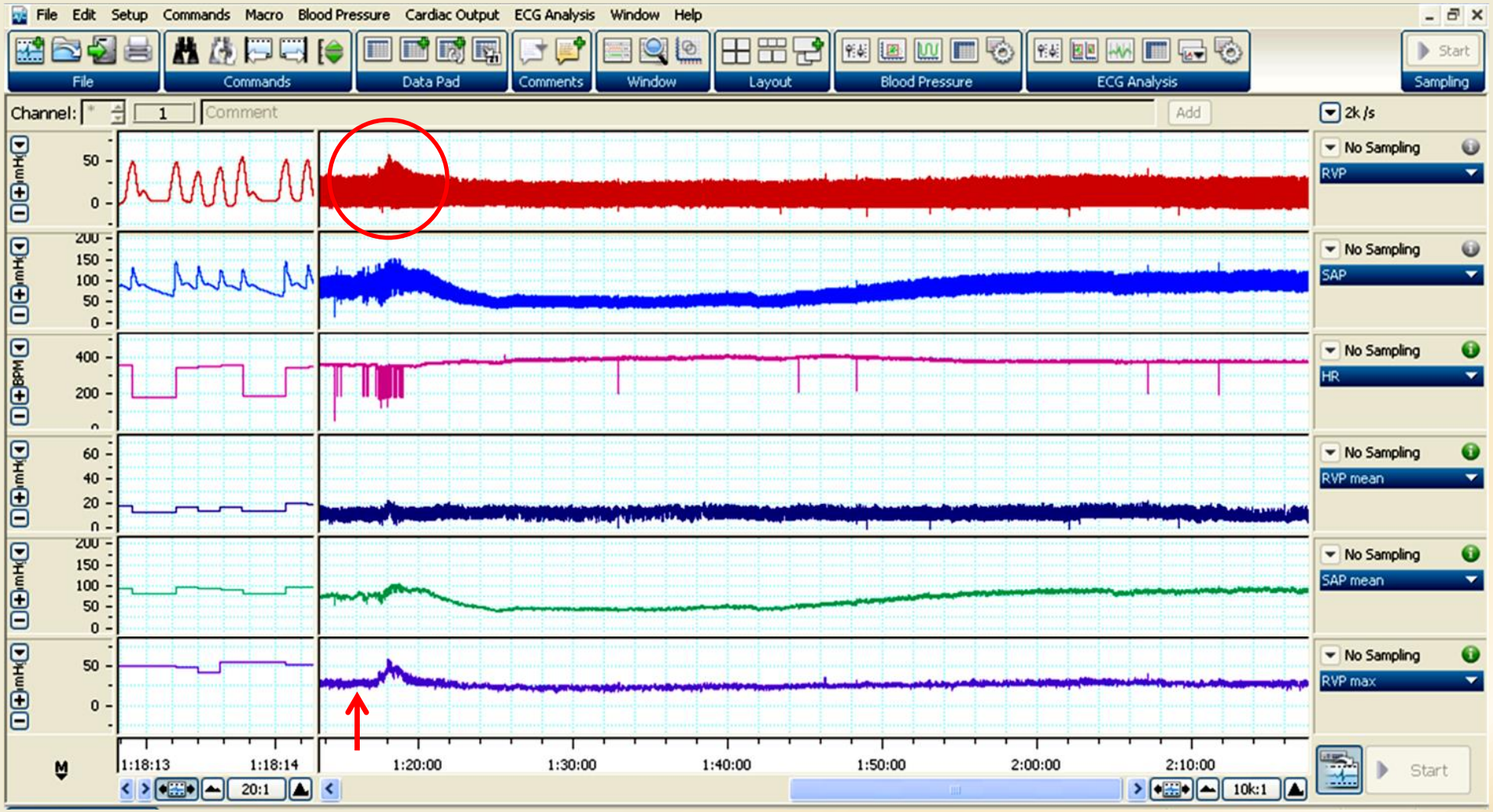
1. (Patho)physiological consequences of mouse pseudoallergy are acute hypertension, changes in blood count, plasma concentrations of complement fragments and vasoactive mediators in mice.
2. Liposomes and nanoparticles do cause complement activation in mice.
3. Complement activation contributed to changes in BP (CR3a ↑, CR5a ↓).
4. BP increase is mainly mediated by TXB2 via COX-1 activation.
5. In mice, complement activation suppressed a mechanism responsible for a late phase of hypertension, which mechanism needs to be explored.
6. All symptoms strongly depend on the characteristics of inducers.

An original recording in a rat

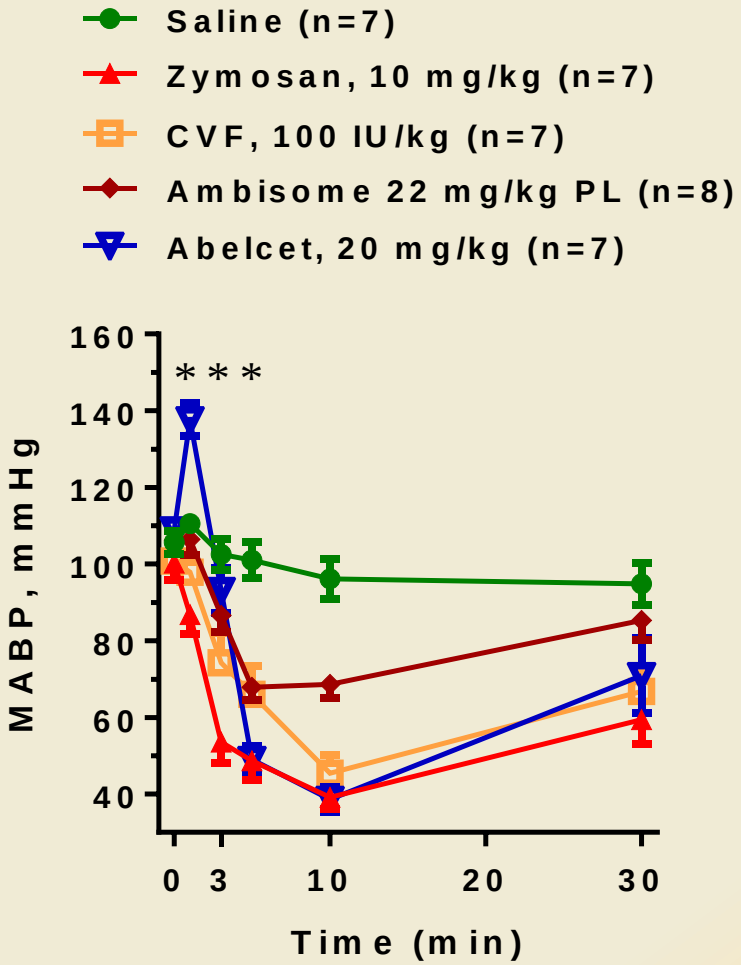


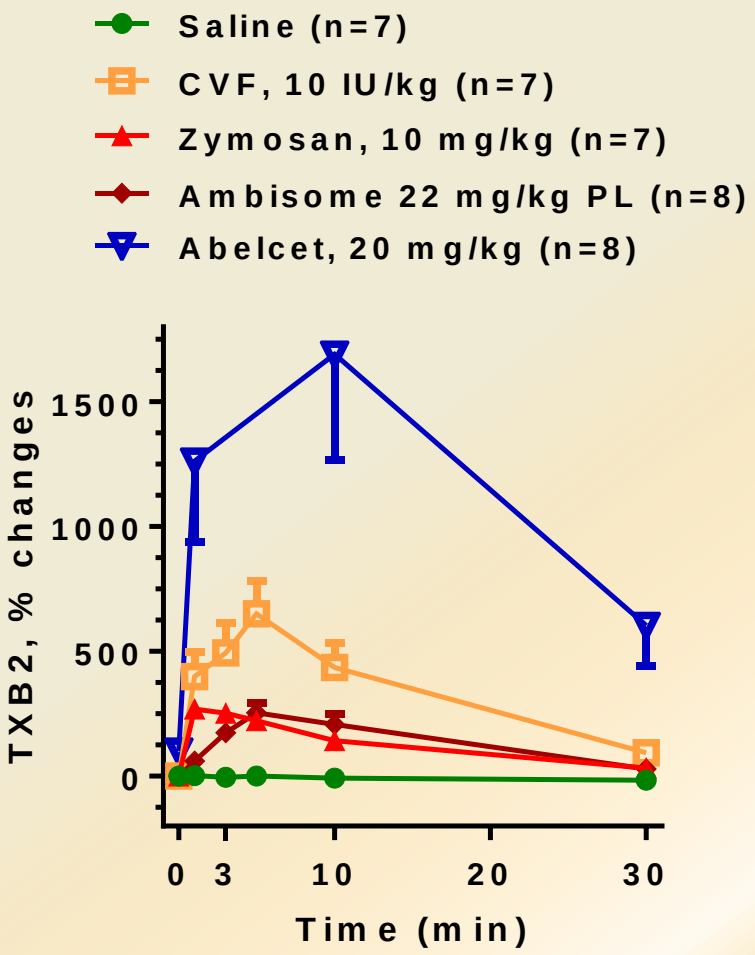
The recommended daily dosage of ABELCET® for adults and children is <u>5 mg/kg</u> given as an intravenous infusion at a rate of 2.5 mg/kg/h.

Hemodynamic effects of zymosan in rats

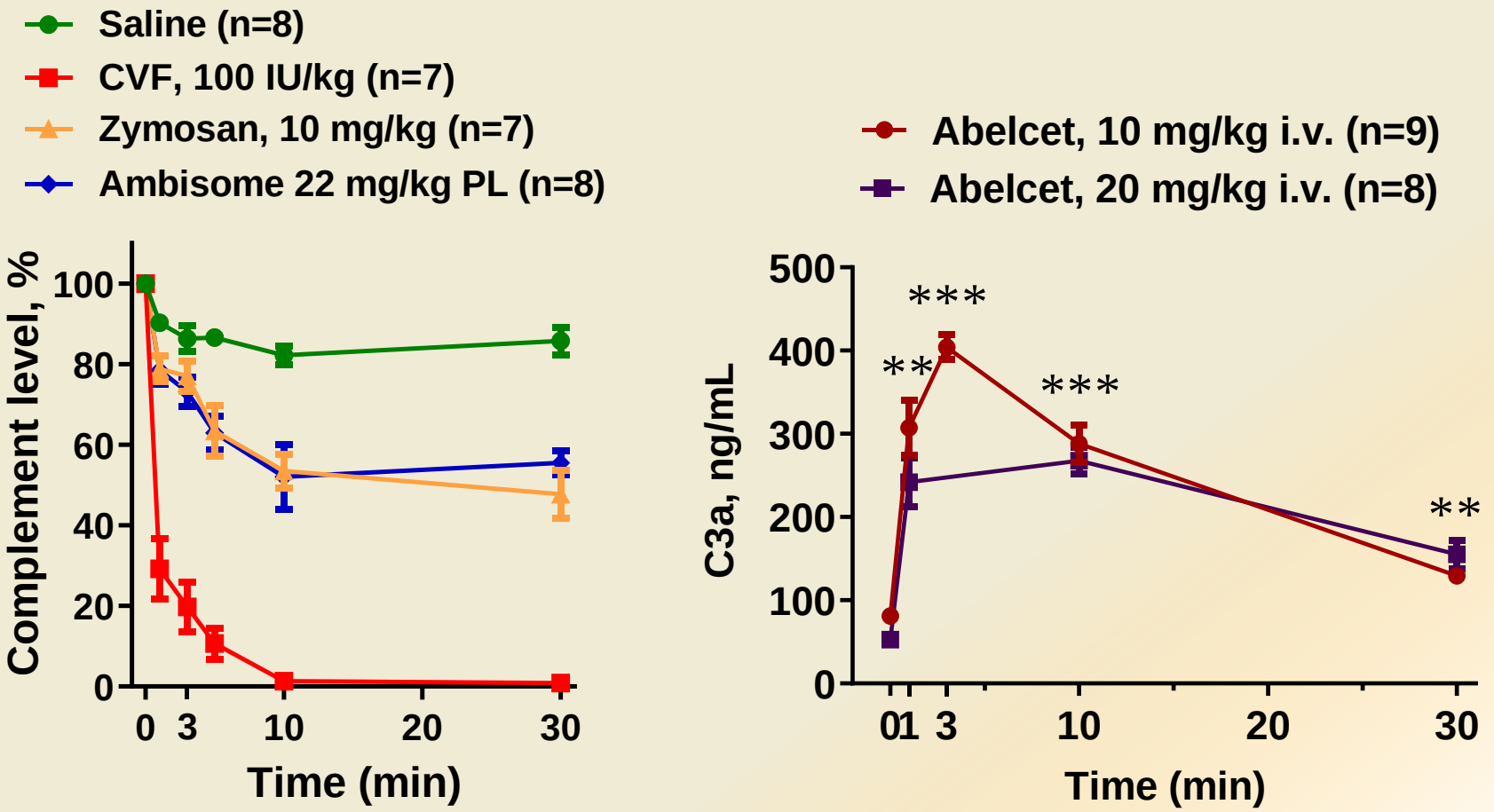


Effects of liposomes and direct complement activators on blood pressure (MABP) and plasma thromboxane (TXB2) concentrations in rats

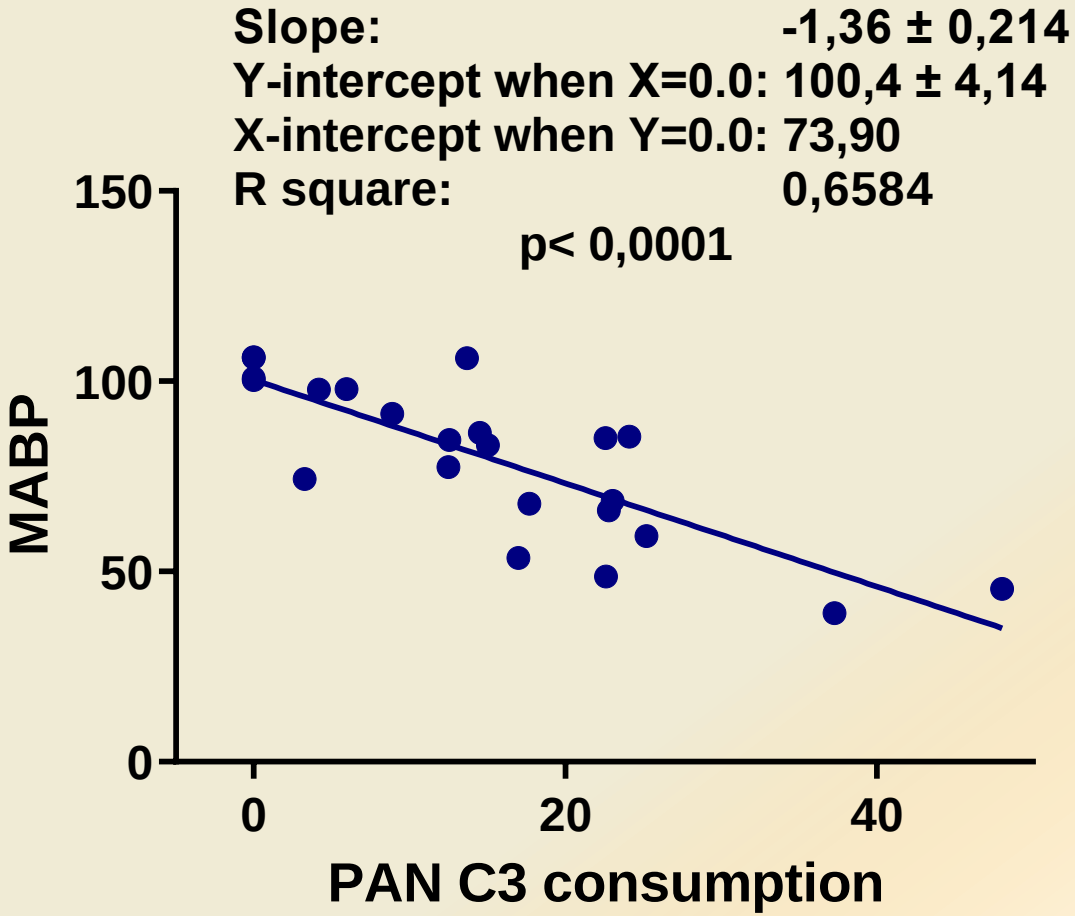




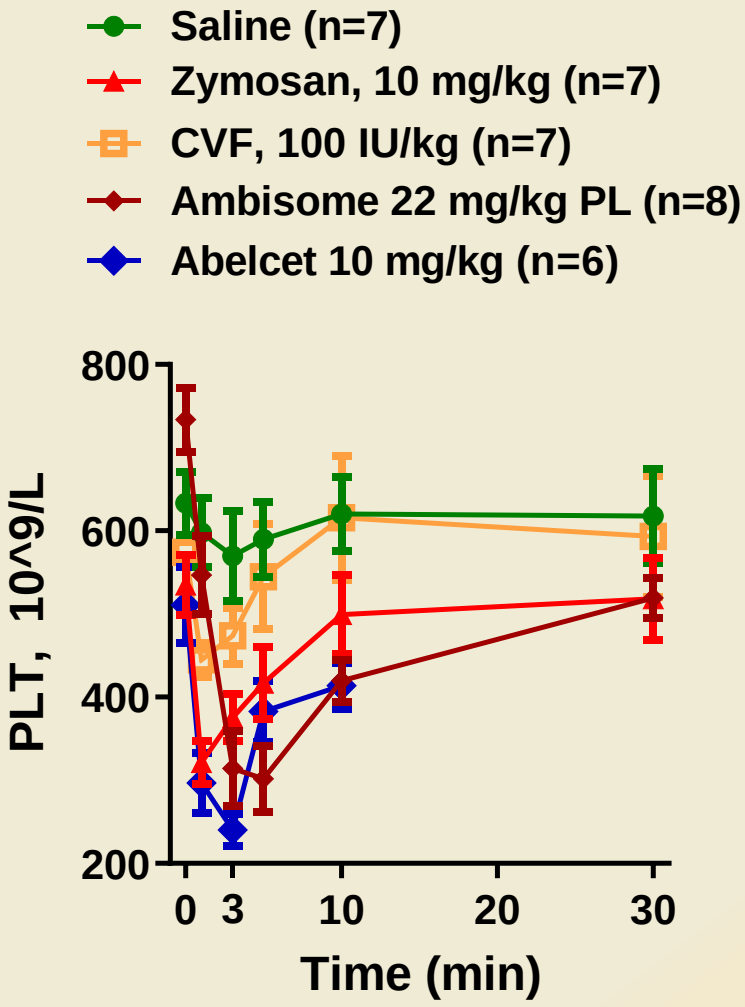
Effects of liposomes and direct complement activators on blood complement level in rats

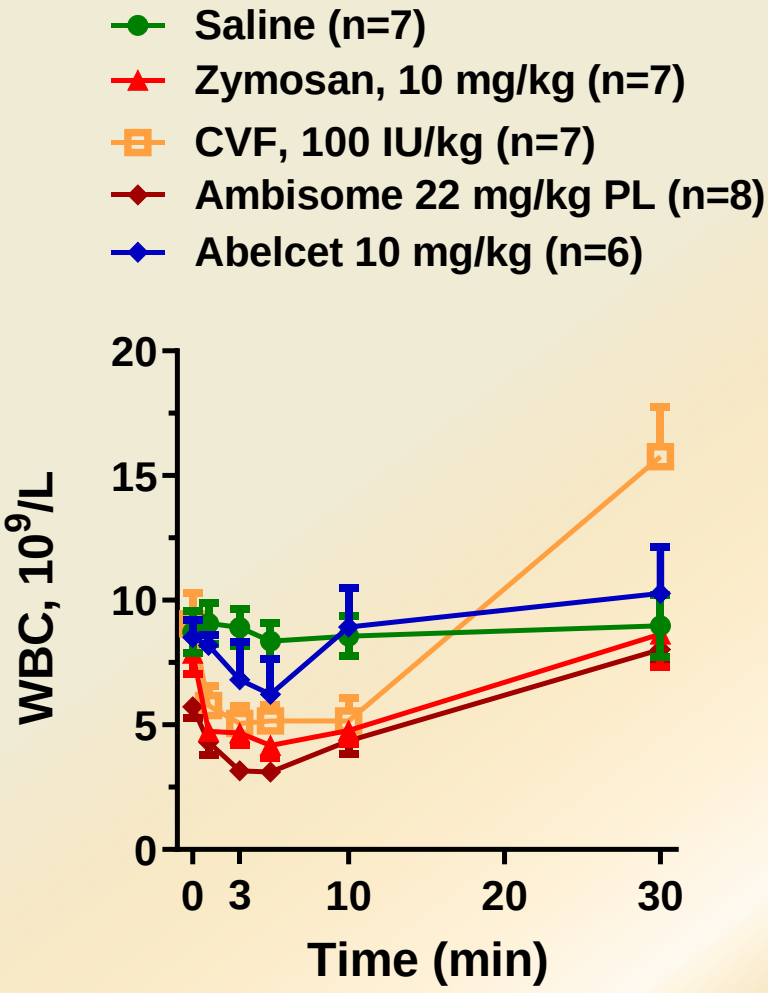


Correlation between the peak blood pressure effect (MABP) and complement consumption in rats



Effects of liposomes and direct complement activators on platelet and white blood cell counts in rats





Complement factors C3a and C5a have distinct hemodynamic effects in the rat

Lavinia M. Proctor [a], Tyson A. Moore [a], Peter N. Monk [b], Sam D. Sanderson [c], Stephen M. Taylor [a], Trent M. Woodruff [a,*]

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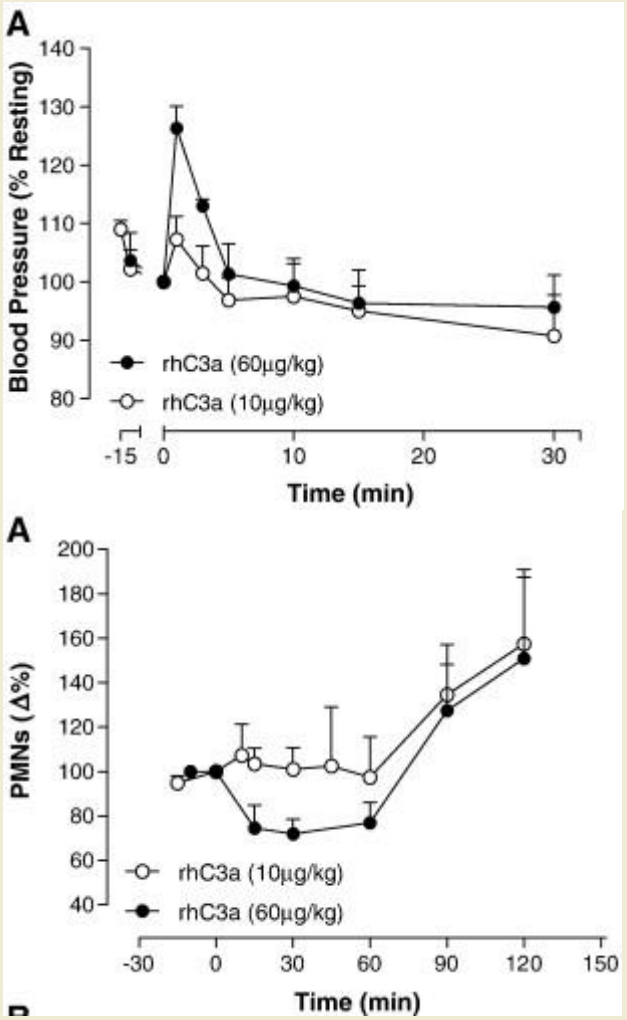
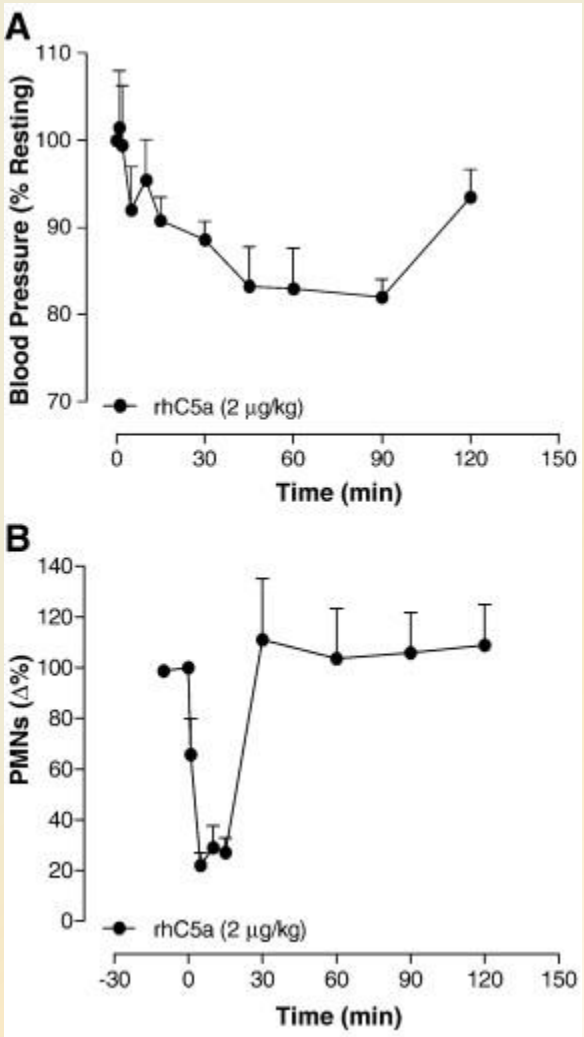
[b] Academic Neurology Unit and Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2RX, United Kingdom
[c] Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha NE 68198, USA

International Immunopharmacology 9 (2009) 800–806



C3a – BP ↑
WBC ↓ ↑



C5a – BP ↓
WBC ↓

ELSEVIER

Nanomedicine: Nanotechnology, Biology, and Medicine
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Complement-mediated hypersensitivity reactions to an amphotericin B-containing lipid complex (Abelcet) in pediatric patients and anesthetized rats: Benefits of slow infusion

Gergely Milosevits, MD[a,b], Tamás Mészáros, PhD[b,c], Erik Őrfi, PharmD[b], Tamás Bakos, MsC[b], Miklós Garami, PhD[a], Gábor Kovács, MD, PhD, DsC[a], László Dézsi, PhD[b], Péter Hamar, MD, PhD, DsC[b], Balázs Győrffy, MD, PhD, DsC[a,d], András Szabó, MD, PhD, DsC[a], Gábor Szénási, PhD[b,1], János Szebeni, MD, PhD, DsC[b,c,d,e,*,1]

[a]*Second Department of Paediatrics, Semmelweis University, Budapest, Hungary*
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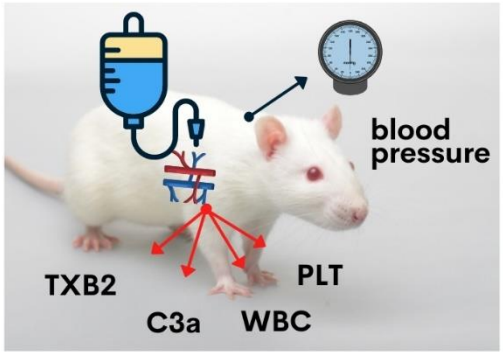
[c]*SeroScience LCC, Budapest, Hungary*
[d]*Cancer Biomarker Research Group, Institute of Enzymology, Research Center for Natural Sciences, Budapest, Hungary*
[e]*Department of Nanobiotechnology and Regenerative Medicine, Faculty of Health, Miskolc University, Miskolc, Hungary*

Revised 6 January 2021

INFUSION OF ABELCET

Hypersensitivity reactions in pediatric patients

Physiological and laboratory changes in rats



BENEFITS OF SLOWER INFUSION

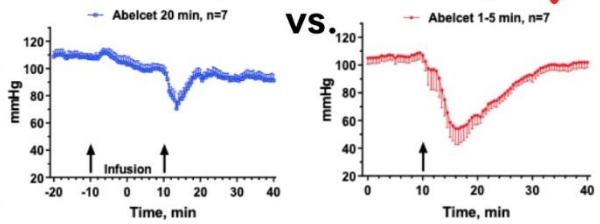
Number of infusion reactions ↓

>4 h infusion

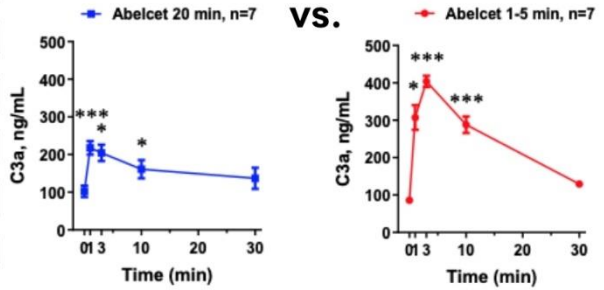
vs.

≤4 h infusion

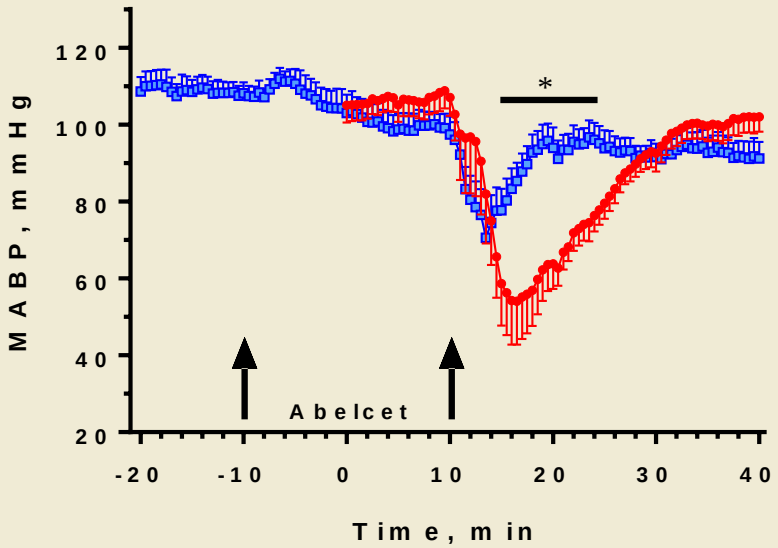
Blood pressure change ↓

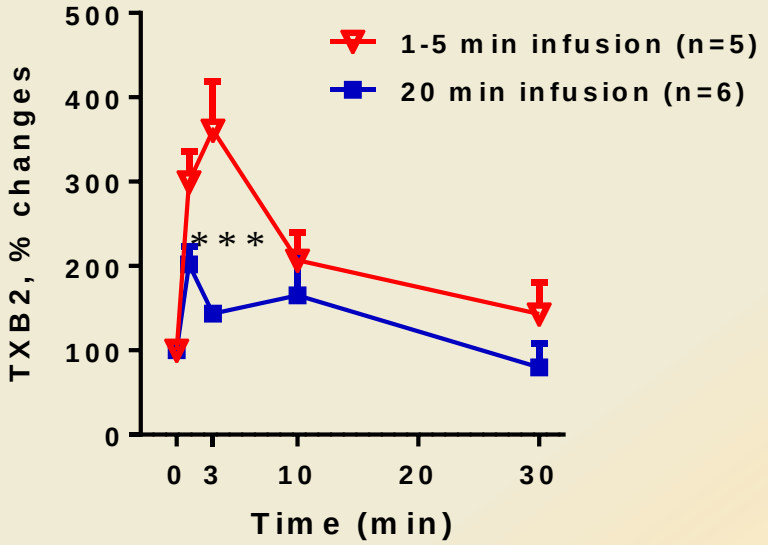


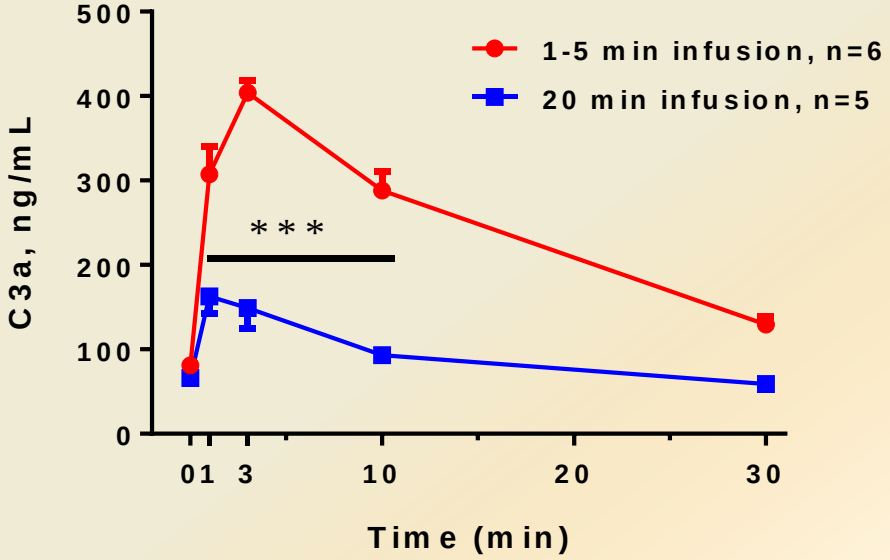
Complement activation ↓



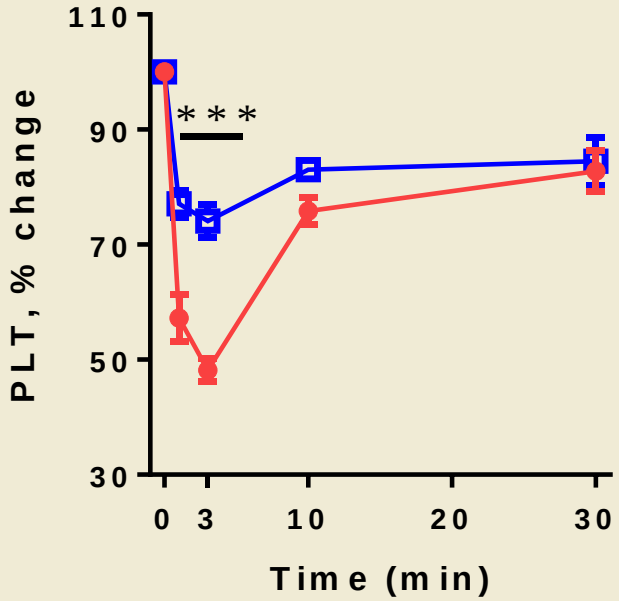
Mechanism of blood pressure changes: the role of infusion rate in rats. Effects on blood pressure (MABP) plasma thromboxane (TXB2) and C3a concentrations

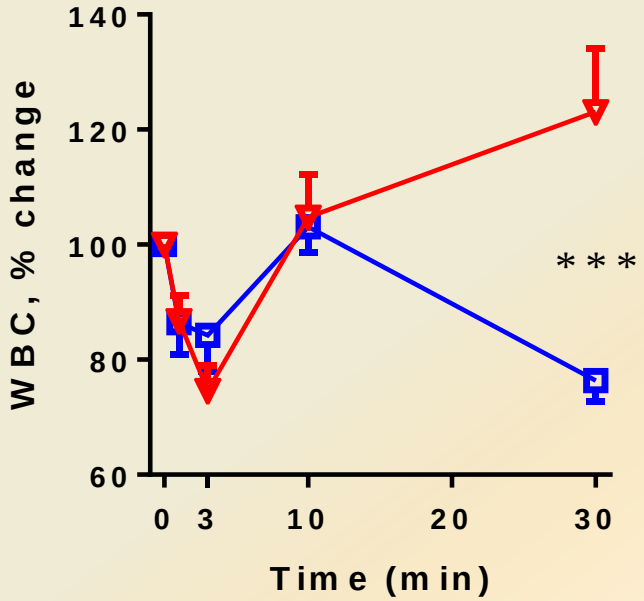




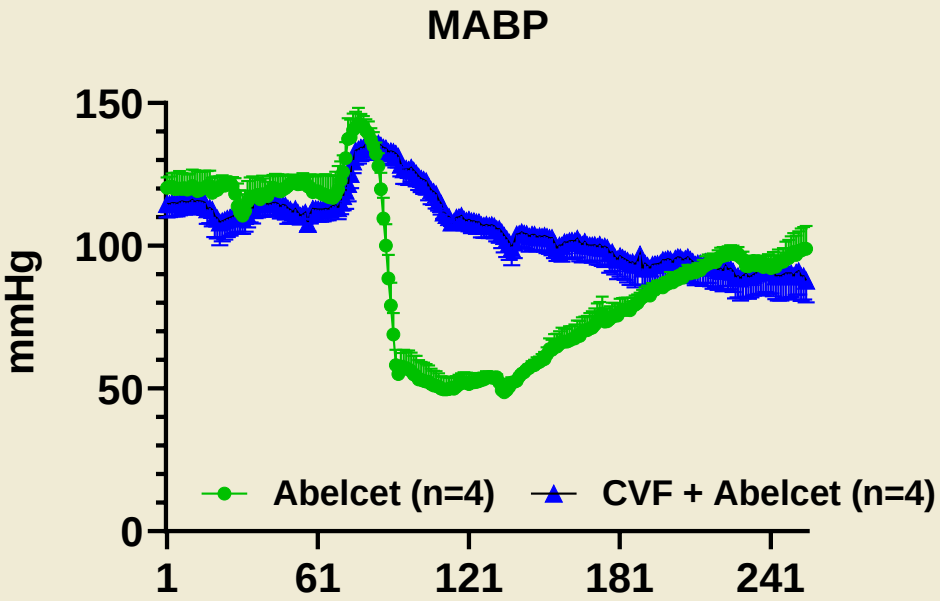


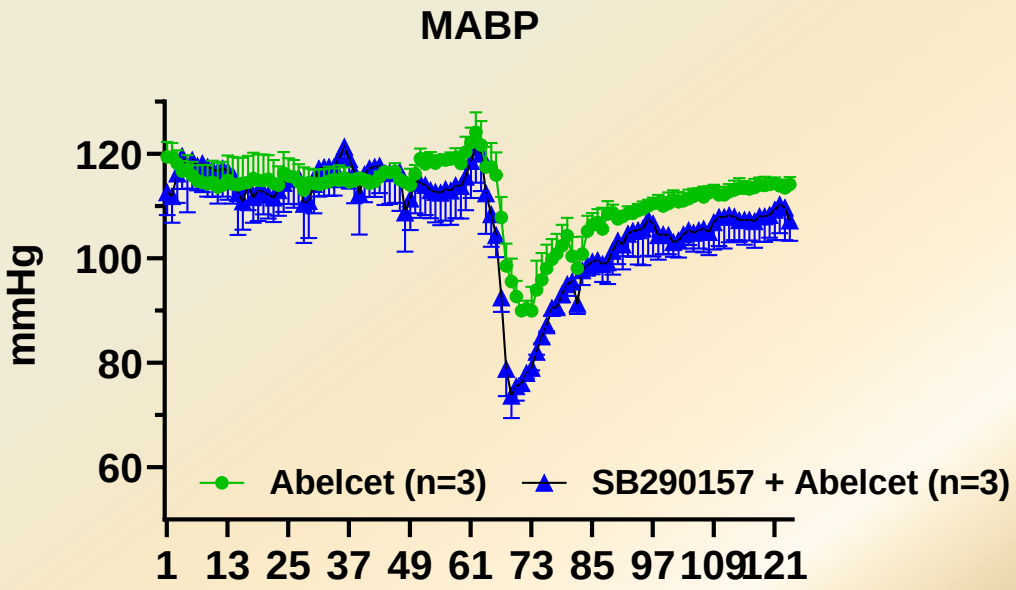
Mechanism of blood count changes: the role of infusion rate in rats. Effects on white blood cell and platelet counts.





Mechanism of blood pressure changes: role of complement depletion and complement fragment C3a in rats





In summary
(mice vs rats)

1. (Patho)physiological consequences of pseudoallergy are similar in mice and rats except the direction of blood pressure changes.
2. Complement depletion lengthened the hypertensive effect of Abelcet, but abolished the Abelcet-induced hypotension in rats.
3. The BP effect of Abelcet is mainly mediated by TXB2 in mice, but TXB2 hardly attenuated the Abelcet-induced hypotension in rats.
4. All symptoms strongly depend on the characteristics of inducers.

Conclusion

1. Mice and rats are suitable for safety testing of potential infusion reactions.
2. Mice and rats are rather sensitive to some (few) liposomal products and nanoparticles.
3. Despite, mice and rats studies can be used primarily for exploring the mechanisms of pseudoallergy.

Conclusion

1. Pseudoallergy is induced by two basic mechanisms:
    1. Complement activation (CARPA)
    2. Complement independent (CIPA) mechanisms (phagocytosis).
2. Complement activation contributed to changes in BP (CR3a ↑, CR5a ↓) and WBC count (CR3a ↑, CR5a ↓).
3. Both mechanisms trigger the production of secondary and tertiary mediators, i.e. a sequence of events is induced leading to a complex array of symptoms that varies a lot depending on the characteristics of the drug and the sensitivity of the recipient.

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Thank you for your attention!