Complement Activation Related Pseudoallergy and Its Animal Models

PhD theses

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Introduction

Complement activation related pseudoallergy (CARPA) is a new class of hypersensitivity reaction. Unlike IgE-mediated allergy, these reactions arise without prior sensitization and symptoms often lessen or disappear on later encounters with the provoking agent. Activation of the complement system through the various pathways or their combination leads to the generation of anaphylatoxins, opsonization, and the formation of the membrane attack complex. The outcomes include activation of mast cells, polymorphonuclear cells and platelets, the release of vasoactive mediators, such as thromboxane and histamine, with cardiovascular and other effects.

Several dugs and chemicals were shown to trigger CARPA, including particulate radio contrast media, drug delivery systems, carbon nanotubes, liposomes (Doxil, Ambisome) and micellar solvents, such as Cremophor EL in Taxol. The monitoring of CARPA became an important aspect in the development of these pharmaceuticals. Underlining the importance of this new type of hypersensitivity, in vitro and in vivo testing of

complement activation became a recommended toxicology test by the US Food and Drug Administration. Utilizing a combination of in vitro and in vivo models for detailed understanding of the interaction between nanomaterials/nanoparticles and the complement system is essential. There is a wide range of in vitro assays currently used for biocompatibility testing and to study the activation of the various complement pathways in human sera. For in vivo tests animal models are the closest experimental alternatives to the human conditions. The interaction of the complex homeostatic systems is kept intact, allowing the analysis of physiological and pathophysiologic processes as they occur in a living organism.

Objectives

The new generation of micro- and nano-sized drug delivery systems may bring an increased risk of recognition by the immune system as foreign. It can be predicted that CARPA will be a returning safety problem in the upcoming age of nanomedicines. Hence, its understanding and prevention may become a critical step

in the R&D of these agents.

In our attempt to study complement activation by various nanoparticles and improve the safety and utility of these compounds, we had the following specific aims:

- Establish an in vivo animal model for the testing of complement activation related pseudo-anaphylaxis by intravenously administered substances.

- Identify the benefits of the in vivo model compared to in vitro complement testing.

- Examination of the hemodynamic effects of serial intravenous injections of Doxil, a clinically used liposomal drug.

- Based on the observations during in vivo testing of Doxil, develop a method for the prevention of complement activation related pseudo-anaphylaxis to the drug.

- Evaluate the differences between in vitro and in vivo methods for the prediction of complement activation related pseudo-anaphylaxis provoked by polyethylene

imine polymer nanoparticles.

- Test utility of alternative animal species for studying complement activation by nanomedicines.

Methods

In vivo tests of complement activation related hemodynamic reactions

Experiments using pigs and dogs were performed at the Semmelweis Medical University in Hungary and at the Uniformed Services University of the Health Sciences (USUHS) in the USA, and were approved by the local Animal Subject Review Committees and followed their guidelines, treating the animals humanely. Swine were sedated with i.m. ketamine and anesthetized with either i.v. pentobarbital or isoflurane through an endotracheal tube. After instrumentation the following parameters were continuously monitored and recorded: pulmonary arterial pressure (PAP), systemic arterial pressure (SAP), central vnous pressure (CVP), electrocardiogram (EGG), heart rate (HR), end-tidal carbon dioxide ($ETCO_2$). Test materials were injected through the Swan-Ganz catheter

into the pulmonary circulation and flushed with 5 ml saline solution. Between injections of test and/or reference material, a resting period of at least 20 minutes was maintained. Experiments with dogs followed the same protocol.

In vitro complement assay

Human serum samples from healthy volunteer donors, obtained through an institutionally approved phlebotomy protocol, were stored at -70°C until use. An ELISA-based method for quantification of serum S-protein-bound C terminal complex (SC5b-9) and levels of the catalytic subunit of Complement factor B (Bb) was performed. The test polymers, at the concentration determined to be the IC50 value, and control compounds were incubated with different human sera for 45 min at 37°C in a shaking water bath (shaking rate of 80 rpm). After terminating the reaction by adding chilled specimen diluent (provided in the ELISA kits) at a 20-fold volume, samples were tested for SC5b-9 and Bb levels using the respective ELISA kits (TCC and Bb kits, Quidel Co, San Diego, CA), following

the manufacturer's instructions. All reactions were tested in duplicates.

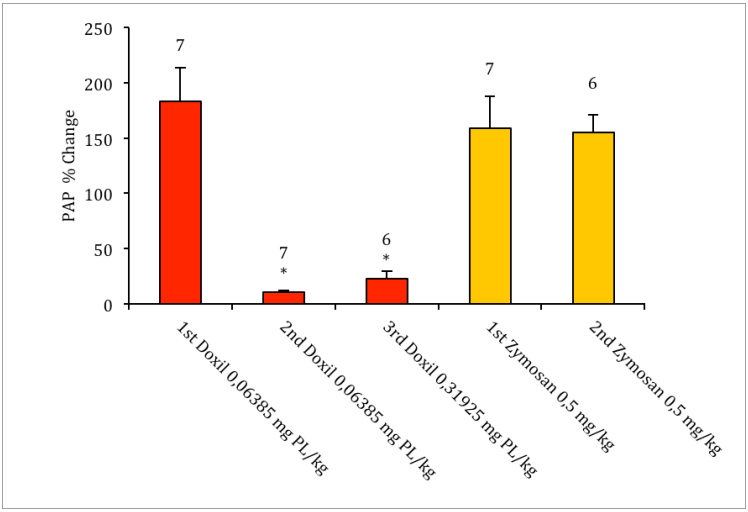
Results

To test complement-activating properties of Doxil, 12 pigs were administered 0.01 mg/kg Doxil, the equivalent of 0.06385 mg phospholipid per kg bodyweight (0.06385 mg PL/kg), as first dose. After the injection of Doxil, we observed acute pulmonary hypertension in all animals, within a few minutes following the injection. The mean pulmonary arterial pressure (PAP) increased significantly ($p=0.0005$, $n=12$) from a median of 16.5 (15-20.25) mmHg to 42 (34.5-48) mmHg.

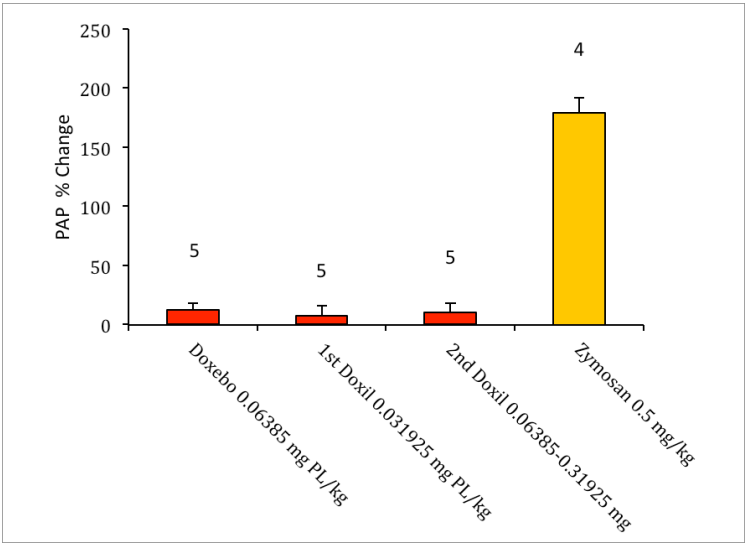
To test whether the first reaction can be repeated with subsequent doses, in seven animals the first Doxil injection was followed by a second identical dose of Doxil injection (0.06385 mg PL/kg). Although the change in PAP from a median of 17 (14-19) mmHg to 19 (16-20) mmHg was statistically significant ($p=0.0177$, $n=7$), such a slight increase is clinically irrelevant, and hence we don't consider it to be a serious anaphylactic reaction.

To test whether tolerance can be breached with a higher dose, six animals received an additional five-fold bolus dose of Doxil (0.31925 mg PL/kg) as a third injection. No statistical difference could be shown between pre- and post-injection pressures either in the pulmonary arterial pressure (17 (12.75-19.5) and 20 (17.75-22.25) mmHg, respectively, p=0.625, n=5), or in the systemic arterial pressures (89.5 (77.75-97.75) and 86 (74.5-99.75) mmHg, respectively, p=0.7865, n=5).

To test whether tolerance is specific to Doxil, we administered two sequential doses of the complement activator Zymosan (0.5 mg/kg) subsequent to the Doxil injections, which both triggered increase in PAP from 17 to 46 mmHg (p=0.0156, n=7) and from 21 to 50.5 mmHg (p=0.0355, n=6) respectively.



To study whether the tachyphylaxis phenomenon can be used to prevent severe CARPA reaction provoked by Doxil injections, we treated five animals with a relatively slow infusion of Doxebo, which is a liposome preparation with the same chemical composition as Doxil, with the sole difference that it does not encapsulate doxorubicin. Netiher the Doxebo infusion ($p=0.25$), nor the subsequent low and high dose Doxil injections ($p=4164$ and $p=0.625$, respectively, $n=5$) triggered CARPA.



To examine the in vitro complement activation by various polyethileneimine (PEI) polymers and their PEGylated derivatives, nineteen series of SC5b-9 ELISA measurements were performed in 6 different human sera. Only PEI-25kDa caused significant ($p=0.0273$), dose dependent elevation of SC5b-9 in 3 out of the 9 sera, while the other substances did not exhibit C activation.

To explore the in vivo complement-activating properties of the polymers, 13 pigs were injected with the preparations and hemodynamic changes were analyzed.

All of the tested polymers were active in terms of causing significant hemodynamic changes, including a rise of PAP, rise or decline of SAP, change of heart rate, and ECG alterations. In some cases the reaction was practically lethal and required active resuscitation efforts to save the animal. However, because of the low number of animals per group, it is not possible to perform a meaningful statistical analysis of the hemodynamic changes provoked by each of the different polymers separately. Taking all the animals into account, the pulmonary arterial pressure significantly increased after the first injection from a median of 14 (12-15.75) to 34 (19.75-44.75) mmHg ($p=0.0039$, $n=10$). The increase after the second injections was also significant from 16 (11.5-21.25) to 28.5 (16.25-43.75) mmHg, ($p=0.0059$, $n=10$) and there was no difference between the changes of PAP after the 1st and 2nd injection (148.8% (31.73-233.6) vs. 82% (28.27-195.6) increase, respectively; $p=0.2324$, $n=10$).

To assess the utility of dogs as an in vivo animal model for testing complement activation by nanomedicines, we studied their hemodynamic reactions to the intravenously

administered liposomal drug Ambisome. Acute systemic hypotension developed after the first injections, SAP decreased from a median of 146.5 mmHg to 81 mmHg ($p=0.0313$, $n=6$). Just like in the pig model, we observed tachyphylaxis during repeated administration of the liposomes and neither low, nor high doses of Ambisom caused change in PAP ($p=0.0625$ and $p=0.0938$, respectively, $n=6$) or SAP ($p=0.8438$ and $p=1$, respectively, $n=6$).

Conclusions

1. First injection of Doxil causes clinically significant acute pulmonary arterial hypertension. The porcine model is extremely sensitive in terms of immune-reactivity to this liposomal formulation. The hemodynamic changes observed in the animals also correspond closely with the signs and symptoms presented by sensitive humans during clinical adverse events after administration of Doxil

2. There is tachyphylaxis for subsequent Doxil injections and they do not cause clinically significant

acute pulmonary arterial hypertension or changes in systemic blood pressure. The tolerance is maintained even at a 5-fold dose.

3. Tolerization with Doxebo can be used to prevent severe CARPA reactions to Doxil. This raises the possibility of taking advantage of the phenomenon in the clinical setting. Prevention of the adverse, often severe hypersensitivity reactions may enable its application even in those susceptible individuals who otherwise would not be able to benefit from the drug.

4. Tolerance is specific to Doxebo and Doxil. The rapid development of tachyphylaxis suggests that it is not related to active buildup of immune memory via specific cell activation. Consumption or depletion of complement factors can also be ruled out as the reactivity to other strong C activators as Zymosan is maintained in the liposome-tolerized animals.

5. PEI-25kDa causes dose-dependent complement activation in vitro, while all PEI formulations induce CARPA in the pig model. PEI 25kD showed the highest reactogenic potential in the porcine model as

well, and during the limited number of experiments with the polymers we have not observed complete tachyphylaxis

6. Dogs exhibit signs of CARPA when administered Ambisome, but these differ from the hemodynamic reactions in pigs after Doxil injections. The typical hemodynamic reaction in dogs to Ambisome is a drop in systemic arterial pressure without pulmonary arterial pressure changes, while to Zymosan the response is acute pulmonary hypertension without systemic arterial pressure change.

Publications

On the subject of the dissertation:

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5: Szebeni J, Alving CR, Rosivall L, Bünger R, Baranyi L, Bedöcs P, Tóth M, Barenholz Y. Animal models of complement-mediated hypersensitivity reactions to liposomes and other lipid-based nanoparticles. J Liposome Res. 2007;17(2):107-17. Review.

On other subjects:

6: Hood MN, Song T, Bedocs P, Capacchione JF, Kasper CE, Haigney MC, Ho VB. Multiacquisition t1-mapping MRI during tidal respiration for quantification of myocardial t1 in Swine with heart failure. AJR Am J Roentgenol. 2013 Oct;201(4):W563-70.

7: Buckenmaier CC 3rd, Capacchione J, Mielke AR, Bina S, Shields C, Kwon KH, McKnight G, Fish DA, Bedocs P. The effect of lipid emulsion infusion on postmortem ropivacaine concentrations in swine: endeavoring to comprehend a soldier's death. Anesth Analg. 2012 Apr;114(4):894-900.