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# Complement Activation-related Pseudoallergy (CARPA)

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Article by Phil Barrington

## What keeps me awake at night?

There are several things: Did I lock the back door? Should the £2.16 have been put in Box 3.6 or 3.7 on my tax form?

Despite more than 30 years of experience of administering drugs to man, on the night before the administration of the first dose of a new study drug, I still struggle to sleep.

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Thankfully, in the vast majority of cases, everything is fine but as we know from the TeGenero incident and the more recent Bial trial, things can, very occasionally, go badly wrong.

With increasing numbers of nanotechnology enhanced (liposomal, micellar, polymer-conjugated) and protein-based (antibodies, enzymes) drugs being developed, the potential for hypersensitivity reactions increases.

The molecular mechanism of mild to severe allergy symptoms may differ from case to case and is mostly not known, however, in many cases a major cause or contributing factor is activation of the complement (C) system. The clinical relevance of complement activation-related pseudoallergy (CARPA), a non-immunoglobulin E (IgE)-mediated pseudoallergy, lies in its unpredictability and occasional fatal outcome.

## What is different between CARPA and IgE mediated (Type I allergy)?

In order to develop an IgE mediated hypersensitivity, the experimental drug needs to bind to an antigen i.e., anti-drug antibodies. These would not normally be present at the time of administration of the first doses of a new agent. However, in the case of cetuximab, it has been shown that hypersensitivity reactions that occur with the first administration are due to cross-reactivity with environmental allergens.

In the case of CARPA, the reactions occurred mostly at the first exposure of the drug. In some cases, this is believed to be due to previous exposure to polyethylene glycol (PEG) which is present in many medications and ‘everyday’ products such as toothpaste.

CARPA can also be induced by medical devices such as endovascular grafts, heart valves, and dialysis membranes.

## What are the symptoms of CARPA?

There are numerous symptoms of CARPA relating to cardiovascular, bronchopulmonary, haematological, mucocutaneous, gastrointestinal systems along with neuro-psycho-somatic and systemic effects (Szebeni 2005). Symptoms of flushing due to histamine release may be part of CARPA but could be also mediated via the MrgprX2 receptor present on peripheral blood basophils and eosinophils, another mechanism which bypasses the antibody-mediated pathway and directly triggers mast cell degranulation by activating the mast cell-specific receptor called Mas-related G protein-coupled receptor X2 (MrgprX2). Urticaria might also be confused with IgE mediated mast cell activation. Thus, CARPA can be difficult to diagnose. In the one case of CARPA I was involved with, the patient reported the unusual symptom of a metallic taste that occurred within seconds of the infusion starting.

## Complement activation

There are three pathways of complement activation: the classical pathway; the mannan-binding lectin (MBL) pathway; and the alternative pathway.

The classical pathway of complement activation is the pathway activated by C1, binding either directly to bacterial surfaces or to antibody, that serves as a means of flagging the bacteria as foreign.

The MBL, also called mannose-binding protein, is an acute-phase protein that binds to mannose residues. It can opsonise pathogens bearing mannose on their surfaces and can activate the complement system via this pathway, an important part of innate immunity.

The alternative pathway of complement activation is not triggered by antibody, but by the binding of complement protein C3b to the surface of a pathogen; it is therefore a feature of innate immunity. The alternative pathway also amplifies the classical pathway of complement activation.

Irrespective of the pathway, complement activation always leads to the cleavage of C3, forming C3a and C3b. Increased levels of C3b result in the generation of the C5-convertase, cleaving C5 in C5a, a powerful anaphylatoxin and chemoattractant, and C5b. Finally, C5b binds and interacts with C6–C9, forming the membrane attack complex (MAC/C5b-9) (Poppelaars et al. 2018).

CARPA can be particularly dangerous as there is amplification of the initial complement activation trigger that transforms the initial drug effect into a massive vicious cycle of abnormal physiological

changes that include pulmonary hypertension, systemic hypotension, myocardial hypoxia, and bronchospasm.

## How can you avoid / mitigate the risks of CARPA?

In essence, you first need to consider that CARPA could occur and then make sure that all the appropriate preclinical tests are performed and the medication is administered appropriately (should you decide to continue to develop the drug).

It should be noted that premedication such as acetaminophen, antihistamines and corticosteroids, may not prevent CARPA.

## Preclinical testing

The test compound should be screened for complement activation by incubation with normal human serum (samples taken from around 10 subjects) and measurement of sC5b-9 concentrations after 20-30 minutes of incubation. A 5-to-10-fold rise in sC5b-9 may be a realistic predictor of a clinical reaction. Increases in sC5b-9 of this magnitude were shown to correlate with clinical symptoms in patients treated with Doxil (PEGylated liposomal doxorubicin) which is known to induce this kind of reaction. If this test is negative, then it is recommended that a much larger number of samples of normal human serum samples (10 to 100) are tested (Szebeni 2005). In addition, seek advice on appropriate preclinical tests as susceptibility to CARPA varies considerably across different animal species.

Another point to consider is if the disease target is associated with the presence of autoantibodies or pre-sensitisation to PEG for PEGylated compounds e.g., PEGylated liposomal doxorubicin. It is possible that the candidate drug may only exhibit complement activation in the presence of the autoantibodies/antibodies to PEG. Therefore, testing with normal human serum may lead to a “reassuring” but totally erroneous result.

## Drug administration

Based on the Tegenero experience and investigations for how the potential for serious infusion reactions could be avoided, sentinel dosing is mandatory. Initial infusions should be given slowly e.g., over 4-8 hours and investigators should have a very low threshold for discontinuing the infusion should the subject develop any relevant symptoms. If the drug has been shown to induce CARPA and development of the compound is to continue, then sponsors should determine what

is a ‘safe infusion protocol’ in pigs and use this to determine the dosages and infusion rates to be used in patients.

Perhaps the best industrial and regulatory proof of the model’s utility is the fact that the safe human administration protocol for double stranded small interfering RNA (siRNA) delivering solid lipid nanoparticles was developed in the pig model. Patisiran (Onpattro R) was the first federal drug administration (FDA) approved gene therapy using (phospho) lipid-based nano-delivery vehicles (Bedőcs and Szebeni 2020).

## Samples to be taken in the event of an infusion reaction

As previously mentioned, it can be very difficult clinically to determine the cause of the infusion reaction. Therefore, several blood samples should be taken to help elucidate the mechanism. Blood samples include mast cell tryptase (elevated in IgE mediated reactions), histamine, IgE, panel of cytokines including IL-6, IL-8, TNF, complement (C3, C3a, C5a, and sC5b-9). It is important to obtain a sample at the peak of the reaction which is often around 30 minutes after the initial onset. Histamine can also be measured using a urine collection. In addition, a ‘reference’ sample should be taken, 48–72 hours after the anaphylaxis episode, to allow a comparison between the peak and the ’reference’ values (Doessegger and Banholzer 2015).

Ensure the samples are taken and processed correctly as histamine, for example, is particularly unstable.

Finally, I always like to take an extra sample so when an expert says, ‘Did you measure serum rhubarb concentrations?’, I can reply, ‘No but there is a sample in the freezer, so we can measure this if you think it would be useful’.

## References


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