A porcine model of complement-mediated infusion reactions to drug carrier nanosystems and other medicines

János Szebeni, Péter Bedőcs, Domokos Csukás, László Rosivali, Rolf Bünger, Rudolf Urbanics

Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary
Department of Anatomy, Physiology and Cell Biology, Uniformed Services University of Health Sciences, Bethesda, MD, USA
Department of Pathophysiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary
Institute of Pathophysiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary
Seroscience Ltd, Budapest, Hungary
Bay Zoltan Applied Research Nonprofit Ltd, Budapest, Hungary
Department of Nano-Biotechnology and Regenerative Medicine, Miskolc University, Miskolc, Hungary
Hungarian Academy of Sciences–Semmelweis University, Research Group for Pediatrics and Nephrology, Budapest, Hungary

ABSTRACT

Intravenous administration of low (milligram) doses of nanoparticulate materials in pigs can lead to acute cardiopulmonary, hemodynamic, hematological, biochemical and dermatological changes within minutes, mimicking the human infusion (or anaphylactoid) reactions to many state-of-the-art (nano)medicines and biologicals. Because of the causal role of complement (C) activation, the phenomenon was called C activation-related pseudoallergy (CARPA). This review summarizes the available information on porcine CARPA caused by different liposomes and polymers. It provides methodical details of the model and addresses the quantitation, sensitivity, specificity, reproducibility and variability of symptoms caused by different reactogenic drugs. We describe a unique feature of the model: the rise of tachyphylaxis (self-induced tolerance) as a function of structural properties of reactogenic agents. For drugs that cause tachyphylactic CARPA, such as liposomal doxorubicin (Doxil), the review recapitulates a recently reported method of desensitization, which may prevent this, as well as many similar hypersensitivity reactions. In explaining the underlying mechanism of tachyphylactic CARPA, a new theory on “double hit” is outlined, wherein the pulmonary intravascular macrophages (PIM cells) of pigs give exaggerated response to simultaneous stimulation of their anaphylatoxin and other surface receptors (e.g., toll-like, PAMP, DAMP or mannose) that recognize vesicle surface molecular patterns. The porcine CARPA model might provide unique advantages in studying the mechanism of severe hypersensitivity reactions in man to i.v. drugs, as well as in identifying drugs and drug carriers that may cause such reactions.

© 2012 Elsevier B.V. All rights reserved.
1. Introduction

Today's pharmacotherapy utilizes a wide range of drugs that contain carrier vehicles with complex multimolecular, multicompartment structures (micelles, liposomes), polymers (proteins, polysaccharides) or polymer-conjugates (PEGylated proteins) whose size is in the lower end of the nanometer range (8–100 nm), i.e., the dimension of nano-technology. Besides their unique therapeutic or diagnostic advantages, these "nanomedicines" seem to share the problem of being recognized by the immune system as foreign, which may lead to the rise of adverse reactions and/or loss of efficacy. Activation of the complement (C) system may play a key role in this immune response in the case of liposomal drugs, micellar solvents, radiocontrast media, PEGylated proteins, monoclonal antibodies and many other protein or polymer-based state-of-the-art nanomedicines [1–4]. Nevertheless, the prediction and prevention of C activation-related pseudoallergy (CARPA), a mechanistic term for infusion or anaphylactoid reactions, have not yet been solved.

As reviewed earlier, CARPA entails a wide variety of symptoms in almost all organ systems [1–5]. Among these, the most frequent symptoms are flushing, skin rash, dyspnea with chest or heart pain, increased or decreased blood pressure and arrhythmias, while the rare, life-threatening symptoms include major arrhythmias, circulatory shock, myocardial infarction and cardiac arrest. The latter events may lead to death in a small but not negligible percentage of patients (mainly people with a history of heart disease), making CARPA a special safety issue in the case of non-life-saving applications of many nanomedicines and biologicals. For this reason, recognition of the "CARPAgenic potential of drugs and diagnostic agents, and finding ways for the prediction and prevention of such reactions are important goals that are substantially aided by the porcine CARPA model reviewed in the present paper.

2. C activation-related hypersensitivity reactions in pigs: rise of the CARPA concept

To our knowledge, the first description of liposome-induced anaphylactoid reactions in miniature pigs was in a study by Wassel et al., who observed the reaction in search for in vivo evidence for liposome-induced C activation by autoantibodies to cholesterol, which these authors detected and purified from pig blood [6]. This discovery led to using pigs for studying liposome reactions, the first time those caused by hemoglobin-containing liposomes (LEH), tested as a blood substitute [7]. Later on, for the past 13 years, the model has been used and extended to develop the CARPA concept, explore the effects of different liposomes, polymers and other particulate agents, and delineate the conditions and factors influencing the reactions [8–11]. Most of these studies included, or focused on liposomal doxorubicin (Doxil®/Caelyx®) as CARPA inducer [8–11].

The train of thought leading to the CARPA concept started with the recognition that the porcine reaction to LEH and other liposomes closely mimicked the symptoms described in patients following i.v. injection of Doxil or other lipid-based drug formulations [12–24]. These symptoms included signs of cardiovascular distress (dyspnea, hypo- and hypertension, chest and back-pain, arrhythmias) and skin changes (flushing, rash), suggesting that these hypersensitivity reactions (HSRs) and the pigs' reaction to liposomes might have a common pathomechanism, i.e., C activation. That liposomes can activate the C system had been known since the late 1960s [25–27], and the fact that the symptoms of C activation in animals include all symptoms of CARPA had also been known for long, since the early 1980s [28–31]. In addition, the liposome reactions in man and pigs resembled very much the HSRs caused by paclitaxel (Taxol) and other taxanes administered i.v. in micellar solutions (Cremophor EL or polysorbate-80), which too were shown to activate the C system [32–34]. All these facts taken together led to the concept that CARPA may be the sole cause, or major contributor to many non-IgE-mediated HSRs observed with novel particulate drugs and other nanomedicines, and that CARPA may represent a new subclass of Type I HSRs [35].

As for the term, “C activation-related pseudoallergy”, it should be mentioned that it was used for the first time in 1999 [7] with the intent of pointing to the likely mechanism of a non-IgE-mediated (pseudoallergic) immune reaction. The official, World Allergy Organization-recommended name for these reactions is “non-allergic hypersensitivity” [36] which, in our opinion, is a questionable nomenclature in light of the convention of using allergy and hypersensitivity synonymously in the biomedical literature.

3. Current approaches for CARPA prediction

The standard immune toxicology tests applied to date, focusing on lymph organs and immune cell functions, do not predict the acute CARPAgenic potential of drugs, and the same is true for standard allergy (skin prick and patch test, intradermal test, blood IgE ELISA, RAST, ImmunoCAP, UniCAP) tests. In fact, at present it is impossible to predict with certainty the risk of severe CARPA in an individual. As it was recently pointed out [5], measurement of C activation in vitro, as a hemocompatibility test, is presently a regulatory requirement only for the commercialization of medical devices [37], not for nanomedicines for systemic use.

In vitro measurement of C activation by a drug is possible with numerous commercially available ELISA kits, however, the presence of C activation per se does not necessarily predict a full-blown CARPA episode; a significant correlation between in vivo C activation and clinical CARPA was shown only in the case of massive C activation with plasma SC5b-9 levels, taken as a biomarker of C activation, rising a minimum of 2–3-folds, often >10-folds over the baseline [38]. In addition, there is substantial individual variation in the in vitro C response to different C activators, necessitating the application of a large number of different sera to assess the C activating potency of the test agent in the population and frequency of strong responders. Complement activation was suggested to be an essential, but not rate-limiting step in the development of CARPA, indicating the need to explore further biomarkers and other laboratory predictive tests.
4. The porcine CARPA model

4.1. Experimental set-up

4.1.1. Animals, instrumentation

For the studies quoted in this review pigs of both sexes were used, weighing 15–40 kg. They were purchased from approved experimental animal vendors and were rested 2–3 days before the experiments. The experiments started with sedation of animals with intramuscular ketamine, followed by intubation and anesthesia with isoflurane or xylazine/ketamine mixture and pentobarbital. Fluid supply was provided via a venous catheter to maintain circulatory stability.

Surgery was performed to cannulate the right external jugular vein for introducing the Swan–Ganz catheter in the pulmonary artery to measure pulmonary arterial pressure (PAP). The right femoral artery was also cannulated for sampling for blood cell counts and TXB₂ levels, and to measure systemic arterial pressure (SAP). The ECG was monitored at the standard Einthoven’s 3-lead detection points. Hemodynamic parameters (PAP, SAP), heart rate and ECG were continuously monitored and recorded using state-of-the-art data acquisition systems. Other details of surgery, instrumentation and hemodynamic analysis were described in the original studies [7–11].

4.1.2. Testing procedure

Baseline blood samples were taken and recording of physiological parameters of the animals started 5–10 min before the first injection of test materials. Liposomes and/or other test agents were diluted in saline or PBS, and their administration into the left external jugular vein at time 0 started either as bolus or as slow infusion. Boluses were flushed into the circulation with 5 mL of PBS or physiological saline solution. The complete CARPA test involved quantitation of the cardiopulmonary, hemodynamic, skin, hematological and blood chemical changes alone or combined. Hemodynamic changes were expressed either in absolute terms (mm Hg, l/min, etc.) or in relative increase or decrease (%) compared to the baseline reading. The ECG complexes and intervals and heart rate were analyzed digitally. Skin changes were assessed visually and/or by photography. For blood cell (white blood cell, WBC, and platelet) and biomarker (e.g., TXB₂, CH₅₀, etc.) analyses, samples were collected before and after the injection of the test substances at different times (for example 1, 3, 5, 10, 15, 20, 30, min). WBC and platelet counting was done in a Coulter Counter and the measurement of plasma TXB₂, a stable metabolite of TXA₂, was performed with ELISA.

4.1.3. Symptoms

Fig. 1 provides a graphic summary of the different manifestations of porcine CARPA caused by different drugs and agents. The picture insets within the pig’s sketch remind the main organs afflicted: lung, heart, circulation, skin and blood, with specific symptoms listed for each organ (system). The changes depended on each other in a complex cause–effect relationship funneling a vicious cycle of lethal anaphylactic shock (Fig. 2).

4.1.4. Quantification of physiological changes

Because the spectrum and severity of individual symptoms of CARPA caused by different liposomes or other agents varied substantially in pigs, it was important to develop a universal, comprehensive approach to quantifying the severity of reactions that allowed comparison of the effects of different drugs. Therefore we developed a scoring system that took into consideration all adverse physiological changes measured during CARPA in order to differentiate among combinations of symptoms with quantitatively distinguishable level of severity. The proposed method established a so-called cardiopulmonary abnormality score (CAS) for each reaction, on a scale of 0 to 5. The arbitrary definition of different CAS values, based on combinations of symptoms, and the corresponding clinical qualifying descriptors (i.e., minimal, mild, moderate, severe and lethal reactions) [9] are listed in Table 1.

5. Unique features of porcine CARPA

5.1. Sensitivity, specificity and reproducibility

It can be stated in general that the porcine CARPA model is highly sensitive to nanoparticles, and that it is remarkably reproducible and variable at the same time. The model is highly sensitive, because certain nanoparticles at a dose as low as 5–10 μg/kg can cause major hemodynamic and other physiological derangements. As a reminder, the LD₅₀ of the most toxic inorganic compounds in nature, such as cyanide or arsenic salts, are in the 1–10 mg/kg range, implying up to three orders of...
magnitude more toxicity of nanoparticles compared to the most reputable poisons.

The changes characteristic of CARPA are remarkably reproducible insomuch as the individual variation of pulmonary vascular changes to strong reactogenic particles is very small, and different nanoparticles cause essentially the same, or very similar reactions. For example, in our studies bridging some 13 years by now, we observed quantitatively reproducible pulmonary hypertension in practically 100% of pigs in response to zymosan, MLV liposomes and, most recently, AmBisome [7–11,39]. On the other hand, the reactions to Doxil showed substantial inter-experimental variation depending on drug batches and other, yet unidentified experimental conditions [10].

As for CARPA caused by different nanoparticles, polymers or other particulate supramolecular structures, all can cause the same, characteristic hemodynamic, cardiopulmonary, skin and blood alterations (Fig. 1), although in different combinations. Reactive nanoparticles that have been analyzed in detail include PEGylated and non-PEGylated liposomes [7–11,39], polyethyleneimine and polyethyleneimine-graft-ethylene glycol) block copolymers [40], carbon nanotubes (CNTs) as well as phospholipid microbubbles, dendrimers, gold nanoparticles and lipid emulsions (unpublished data). They all act in about the same (0.01 to 1.0 mg/kg) dose range; thus, the high sensitivity of pigs to nanoparticle-induced CARPA is paralleled by low specificity.

The conclusion that the porcine CARPA model is highly variable is based on the observations that depending on numerous factors, pulmonary hypertension can be associated with both systemic hypotensive and hypertensive, tachycardia or bradycardia, decreased or unaltered cardiac output, decreased or increased etCO₂, and presence or absence of skin rash and ECG alterations. Also, within one animal, the reactions to certain particles can be repeated several times, or they occur only at the first administration, with the subsequent doses causing less or no reaction at all. The latter, self-limiting reactions are referred to as being tachyphylactic [11].

To illustrate the similarity and, at the same time variability of porcine CARPA to different nanoparticles, Fig. 3 shows typical reactions to liposomal doxorubicin (Doxil), liposomal amphotericin-B (AmBisome) and a 25 kDa polymer, polyethyleneimine (25K-PEI) polymers. All these materials caused typical CARPA with cardiopulmonary distress; nevertheless, the pattern and kinetics of blood pressure changes were slightly different. Doxil, for example, caused closely paralleling pulmonary hypertension and systemic hypotension with transient narrowing of pulse pressure (Fig. 3A); AmBisome led to complex, biphasic rises and falls of PAP and SAP initially with systemic hypertension, and biphasic variation of pulmonary pulse pressure (Fig. 3B); while 25K-PEI caused slowly rising pulmonary hypertension with expanding pulmonary pressure but no changes in SAP (Fig. 3C).

The reproducibility of PAP and SAP changes upon repeated injection of reactogenic drugs varied between two extremes: full or none. Fig. 4A and B shows examples of fully reproducible reactions.
caused by multilamellar DMPC/DMPG/Chol (45:5:50 mole ratio) liposomes (MLV, A) and AmBisome (B) [10]. These liposomes greatly differ from each other in size (MLV: 2–10 μm, AmBisome: 100 nm) while they share the feature of being strongly negative due to the presence of phosphatidylglycerol. Thus, surface charge, more than size, may be a critical factor in intra-animal reproducibility of liposome reactions.

The non-reproducible types of cardiopulmonary responses to repetitive injection of the same or increasing doses of reactogenic inocula are illustrated in Fig. 5A and B. Fig. 5A shows an entire lack of reproducibility, referred to as full tachyphylaxis, while Fig. 5B demonstrates a case of semi-reproducibility, or partial tachyphylaxis. In particular, in Fig. 5A, after a major initial reaction to the first injection of Doxil, no more changes in PAP (or SAP) could be induced by further injections of this drug despite the fact that the animal did not lose responsiveness to another C activator, zymosan. In Fig. 5B the SAP response to low dose zymosan showed a switch from severe to mild reactions after the third dosing. A further variation of the phenomenon was seen with 25K-PEI, which induced gradual tachyphylaxis with cross-tolerance induction to a PEGylated derivative [40]. This type of reaction could be overridden by increasing the bolus dose (Fig. 5C).

Fig. 3. Variation of pulmonary and systemic blood pressure responses to different inducers of CARPA. Screen shots of real time recordings of the initial (4–6 min) period of pulmonary (upper panels) and systemic (lower panels) changes following i.v. injection of 0.01 mg/kg Doxil (A), 0.1 mg/kg AmBisome (B) and 0.05 mg/kg 25K-PEI (C). Data taken or modified from Ref. [10].

Fig. 4. Intra-animal reproducibility of CARPA. A) PAP curves from a pig injected 8 times with the same dose (0.2 mg lipid/kg) of MLV. The experiment showed essentially identical rises of PAP after each injection, over 8 h [7]. B) PAP (red), SAP (blue) and heart rate (black) changes following consecutive injections of AmBisome (two times), Doxil and zymosan, at doses (mg/kg) shown under the curves. The experiment showed identical changes following AmBisome injections, which did not interfere with subsequent responses to Doxil and zymosan [10].
Fig. 5. Different forms of tachyphylactic responses to i.v. nanoparticles in pigs. A) Full tachyphylaxis, i.e., tolerance induction by 0.1 mg/kg Doxil bolus in a pig, as evidenced by the lack of PAP response to the 2nd and 3rd similar boluses. Nevertheless the animal remains responsive to the C activator zymosan. B) Real-time SAP readings in a pig repetitively injected with zymosan, showing a “switch” after the 3rd hypotensive bolus, i.e., significant reduction of the severity of blood pressure response to zymosan. C) PAP, SAP and heart rate readings in 2 pigs (upper and lower panels) injected with the indicated doses of PEI polymers and zymosan.
In summary, the intra-animal reproducibility of CARPA responses to sequential injections of nanoparticles shows a substantial particle dependent variation between near full to almost no reproducibility of the first reaction. The presence of tachyphylaxis in the case of Doxil treatment has led to the development of a desensitization protocol for the prevention of Doxil reactions \[11\], and may also help in understanding the pathomechanism of CARPA, as detailed below in Sections 6 and 7.

5.2. Thromboxane and blood cell changes, infusion rate dependence and inhibitors of CARPA

Fig. 6A provides evidence that a rise of thromboxane \(A_2\) (TXA\(_2\)) in blood may play a rate limiting, maybe sole causal role in inducing pulmonary hypertension in pigs. In the experiment shown (Fig. 6A) there was a remarkable quantitative correlation between the rises of PAP and levels of TXB\(_2\) in the arterial plasma of pigs on the time scale of minutes.

Fig. 6B shows that stepwise increases of the infusion rate of MLV led to gradual increases of PAP until a new plateau was reached, implying a very close, real-time dose–effect relationship between MLV and PAP. Fig. 6C shows relatively minor changes in blood platelet and WBC counts during MLV-induced CARPA, which is in sharp contrast to the very sensitive blood cell response of other animal species to i.v. liposomes (rats, dogs) \[42\]. In any way, the issue of blood cell changes in pigs needs to be revisited as other, unpublished observations do show major changes after injection of various liposomes and other nanoparticles. Finally, Fig. 6D presents evidence that indomethacin and soluble C receptor type 1 (sCR1), a known C inhibitor \[7\] can almost completely block, while an anti-C5 antibody partially inhibits the pulmonary response of pigs to MLV.

6. Desensitization for liposome reactions based on tachyphylaxis

The tachyphylaxis to Doxil raised the possibility of taking advantage of the phenomenon for the prevention of adverse, often severe HSRs to Doxil that may preclude the use of this drug in hypersensitive individuals, and may even cause death in rare cases. The beneficial effect of slow infusion is well known in the clinical practice \[2,3\] and in fact, it had been known from our porcine studies shown in Fig. 6B, that the infusion rate is a key determinant of HSRs to liposomes. Thus, in an earlier study \[11\] we tested the hypothesis that slow, non-reactogenic infusion of Doxil-like but doxorubin-free liposomes, called “Doxebo”, may prevent the reaction to bolus doses of original Doxil. As shown in Fig. 7A, bolus injection of Doxebo significantly reduced, although did not entirely prevent a major reaction caused by Doxil as the first injection under similar conditions \[11\]. However, when Doxebo was given in slow infusion, the Doxil-induced rise of PAP was entirely abolished (Fig. 7B and C). These findings suggest that tolerization with Doxebo might represent a possible approach to prevent the HSRs to Doxil.

7. Additional features of porcine CARPA: skin changes

Fig. 8 shows the typical flushing and rash reactions seen in pigs during CARPA. The changes start and persist in parallel with the hemodynamic alterations, although return to the baseline usually takes longer than the normalization of blood pressure. Importantly, skin changes are not present in all animals that develop cardiopulmonary reactions, and the only factor that seems to correlate with their presence is the overall strength of the reaction, inasmuch as skin changes were observed solely as manifestation of severe (CAS 4–5) reactions. Until
now no study has been devoted to explore the exact conditions and mechanism of skin changes during CARPA in pigs.

8. Mechanism of porcine CARPA

As illustrated in Fig. 2 and discussed in previous studies and reviews [7,8,10,11,35,41–43], the molecular and cellular mechanisms of CARPA are extremely complex. Major contributors to complexity are the multiple, redundant couplings of C activation to stimulation of mast cells, basophils and macrophages via their anaphylatoxin (C3a, C5a) receptors, and the multiple synergistic and antagonistic effects of these cells' secretory products at the level of smooth muscle and endothelial cells.

Focusing on some of the cardiovascular symptoms, the rise or fall of SAP, for example, depends on the net impact of changes in cardiac output, pulmonary circulation and peripheral vascular permeability and resistance, just to mention the main factors. The decrease of cardiac output also has several independent contributing factors, such as impaired coronary circulation as a consequence of myocardial ischemia, bradycardia and reduced left atrial (or left ventricular) filling due to pulmonary vasoconstriction. Pulmonary vasoconstriction, in turn, can arise as a consequence of arterial vasoconstriction due to TXA$_2$ liberation and capillary blockade caused by aggregated and/or sequestered platelets and leukocytes, i.e., pulmonary microembolism.

As a further aggravating factor, we provided pharmacological evidence for the liberation of adenosine in the coronary circulation, which likely contributes to bradycardia and systemic hypotension [9]. Actually, adenosine formation and release may explain a unique symptom of CARPA, paradoxical bradycardia, i.e. bradycardia despite severe arterial hypotension [9].

Yet another unique feature of CARPA is the decrease of exhaled CO$_2$ (etCO$_2$), which is an unexpected observation in light of the known elevation of this parameter in asthma. The finding is most easily rationalized with reduced elimination of CO$_2$ from blood at the alveolocapillary membrane due to decreased cardiac output [44], combined with pulmonary vasoconstriction and capillary blockage by microemboli. In asthma, on the other hand, CO$_2$ passage at the alveolar level is unimpaired but CO$_2$ builds up in the exhaled air as a consequence of bronchoconstriction that restricts exhalation.

8.1. Tachyphylaxis and its explanations

Perhaps the most puzzling feature of CARPA is its proneness to self-induced tolerance, i.e., tachyphylaxis with certain reaction inducers, while near perfect repeatability of the reaction in case of other reactogenic
molecules. In explaining the phenomenon, the rapid (minutes) development of tolerance argues against active buildup of immune memory via specific cell (lymphocyte) activation [11]. Complement consumption, or depletion of a component in the effector arm of CARPA can also be ruled out on the basis that reactogenicity to the strong C activator zymosan is maintained in liposome-tolerized animals [11]. One of our present hypotheses regarding the mechanism of tachyphylaxis to PEGylated liposomes is that the first dose depletes one or more early mediator(s) of CARPA, such as preexisting (natural) anti-PEG antibodies. The existence of such antibodies, as well as their induced form, has been known and studied for a long time both in man [45,46] and in animals [47–51]. Such natural antibodies usually occur at low concentrations, particularly IgM, thus it seems reasonable to assume that they could be depleted by the vast number of liposomes (in the order of $10^{10}$–$10^{11}$ [52]) that reach the blood with the first injection. However, this hypothesis needs to be tested in the future, just as our other hypothetical explanation, the down-regulation of a signaling pathway in allergy mediator secretory cells. Such downregulation could occur upon the binding of liposomes to surface receptors that specifically recognize PEGylated liposomes. The polymer on the surface of such liposomes could serve as a binding ligand, and pattern recognition receptors (PRRs) on secretory cells as innate binding sites, in analogy to the binding of mannose binding lectin (MBL) and/or ficolin to PEG on the surface of carbon nanotubes during the course of C activation via the lectin pathway [45,54]. The PRRs on allergy mediating (mast cell and myeloid line) cells that may serve as binding sites for PEGylated liposomes include pathogen- and/or danger-associated molecular pattern receptors (PAMP-R, DAMP-R), Toll-like receptors (2 and 4) (TLR-2,4) and mannose receptors [55,56]. A critical observation in pointing to PEG as a key player in tachyphylaxis caused by Doxil is that PEG-free AmBisome, which is similar to Doxil in size, and which causes similar first reactions in pigs as Doxil, does not lose reactogenicity upon repeated injections (its effect is non-tachyphylactic) [10].

In addition to repetitive PEG units, another type of surface-associated molecules on liposomes that may potentially act as a binding ligand to allergy-mediating myeloid or mast cells is the glycosylated or mannose-rich blood proteins, certain apolipoproteins that may rapidly bind to the vesicles.

The above discussed facts and considerations on the mechanism of Doxil-induced tachyphylaxis raise the possibility that the reactions to Doxil, other PEGylated liposomes and all tachyphylactic CARPA triggers arise as a consequence of double hit on CARPA-mediating cells, i.e., concurrent stimulation of these cells by anaphylatoxins plus non-covalent (physical) binding (or uptake) of liposomes through the mechanisms mentioned above. When the response to one (or both) hit(s) weaken(s), which may occur as a consequence of self-limiting receptor signaling, i.e., down-regulation, tachyphylaxis may develop.

8.2. The possible role of PIM cells

As for the localization and identity of CARPA-mediating cells in pigs, the very short interval (few minutes) between liposome application and the appearance of pulmonary symptoms points to a possible key role of the pulmonary intravascular macrophages (PIM cells, PIMs), which are present in large numbers in pigs [57,58]. Besides alveolar, interstitial, pleural and free vascular macrophages, PIMs residing in the pulmonary capillaries are part of the macrophage system of the lung. Like the Kupffer cells in the liver, their main task is to rapidly and directly remove blood-borne particles, such as cellular debris, bacteria, viruses, endotoxins, etc. [59–62]. PIM cells, like all macrophages, express anaphylatoxin receptors (ATR) and PRR, and, with their extended, glycoalyx-covered pseudopods and spikes, they efficiently trap live or dead cells and particles [59,63–65]. These cells also display high phagocytic activity and immediate and significant secretory reactions [59,63–65], which is in keeping with the rapid response to i.v. administered liposomes and other reaction inducer particles.

There are two important characteristics of PIM cells from the point of CARPA. One is that desmosome-like intercellular adhesion plaques that differ from tight and gap junctions, firmly attach these cells to the capillary endothelium. Thus, PIM cells probably represent “static” resident cells in the lung of pigs, not temporary voyager macrophages whose previous history determines their activation state and, hence, reactivity [66]. This would explain the high sensitivity and consistency of the pigs’ lung to react to nanoparticles. The second key feature is that these cells express, in addition to ATR, PRR [67,68], which may recognize the PEG corona of Doxil. Following the activation of these
9. Clinical relevance of the porcine CARPA model

Because of the extreme sensitivity of pulmonary response to nanoparticles, the clinical relevance of the porcine CARPA model has been a subject of intense debate. Clearly, the dramatic cardiopulmonary reaction of pigs to i.v. administered nanodrugs, drug carriers, polymers and other particulate agents does not reproduce the “normal” human physiological response to such medication, mostly characterized as being tolerable, or negligible, representing mostly an annoyance, not a major concern or safety risk. What the porcine CARPA reaction does reproduce is the severe, occasionally lethal HSR to i.v. medications, which occurs very rarely and which involves major signs of cardiopulmonary distress, e.g., shock, arrhythmias, myocardial infarction, ventricular fibrillation with, in the worst case, lethal cardiac arrest. The porcine CARPA is thus an in vivo model of a rare extreme condition that represents the most feared adverse effect of i.v. pharmacotherapy. We propose that the model may have best use for studying the mechanism of the latter condition, and, importantly, in the quantitative evaluation of the potential reactogenicity of (nano)drugs, drug carriers, polymers and other particulate agents in a hypersensitive individual.

10. Evaluation of the porcine CARPA test as a screening assay for in vivo reactogenicity of nanoparticles

In extending the above thought on the use of the porcine CARPA model as a screening test for the in vivo reactogenicity of different i.v. medications in an occasional hypersensitive individual, it should be emphasized that the model has unique advantages vis-à-vis other currently applied approaches of reactogenicity prediction. In particular, 1) its high sensitivity reduces the risk of false negative results, i.e., that a CARPAgentic medicine would not be detected by the assay; 2) the individual variation of the pulmonary reaction is small, ensuring relatively unambiguous answers relating to the presence or absence of reactivity, using minimal number of animals. However, on the other side of the balance, the study with pigs, a large animal, requires special skill, instrumentation and facility, and, in the case of tachyphylactic responses, only first-dose reactions are relevant. Future studies will hopefully expand our first understanding of the use of this unique model of a unique adverse human condition.

Acknowledgment

We thank Dr. Carl Alving for launching and expanding, and Dr. Chezy Barenholz for continuing and maintaining support for the porcine studies discussed in the review. The financial support from USUHS CRADA G170XH, TAMOP-4-2-1/B-09/1/KMKR-2010-0001 and TAMOP-4.2.1.B-10/2/KONV-2010-0001 in the framework of the New Hungarian Development Plan, supported by the European Union, and co-financed by the European Social Fund is gratefully acknowledged. The financial support for the Nanomedicine Research and Education Center by Gedeon Richter NyRT is also gratefully acknowledged.

References

Complement activation-related pseudoallergy: mechanism of anaphylaxis


In vitro and in vivo complement activation and related anaphylactic effects associated with blood, ANSI/AAMI/ISO 10993

Animal models of complement-mediated hypersensitivity reactions to liposomes


