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Review

Complement activation as a bioequivalence issue relevant to the development of generic liposomes and other nanoparticulate drugs

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ABSTRACT

Liposomes are known to activate the complement (C) system, which can lead in vivo to a hypersensitivity syndrome called C activation-related pseudoallergy (CARPA). CARPA has been getting increasing attention as a safety risk of i.v. therapy with liposomes, whose testing is now recommended in bioequivalence evaluations of generic liposomal drug candidates. This review highlights the adverse consequences of C activation, the unique symptoms of CARPA triggered by essentially all i.v. administered liposomal drugs, and the various features of vesicles influencing this adverse immune effect. For the case of Doxil, we also address the mechanism of C activation and the opsonization vs. long circulation (stealth) paradox. In reviewing the methods of assessing C activation and CARPA, we delineate the most sensitive porcine model and an algorithm for stepwise evaluation of the CARPA risk of i.v. liposomes, which are proposed for standardization for preclinical toxicology evaluation of liposomal and other nanoparticulate drug candidates.

1. Introduction

Over the past 40 years, since they were first used in patients (1), liposomes matured to witness the rise and surge of generic products. Lipodox, a generic version of PEGylated liposomal doxorubicin (Doxil™) is the only product that was registered so far by the US Food and Drug Administration (FDA) (but not by the European Medicine Agency, EMA), while in many countries, e.g., Argentina, China, India, Iran, South Korea and Taiwan, a number of generic liposome products have been registered or are under development. Examples of generic Doxil include Doxisome™ and Sinadoxosome™, and those of generic AmBisome: Ambil™, Fungisome™, Fosome™, Lambin™, Lipholyn™, Amphonex™ [1–3].

The arrival of generic liposome formulations brought along a lot of unanswered questions regarding their bioequivalence with the innovator products, which questions are very difficult to answer in light of the multi-molecular, highly organized structure and complex manufacture of these nanopharmaceuticals. Their formulation procedure often involves a large variety of technological steps and intra-process controls, which render the structure of each liposomal formulation to be highly unique and thus difficult to reproduce. Minor deviations in manufacturing may lead to major changes in therapeutic efficacy and/or toxicity, which led the regulatory authorities to categorize liposomal drugs as ``non-biological complex drugs'' (NBCDs) [4–6] and implement special procedures for their handling in new drug (NDA) and accelerated new drug (ANDA) applications (or their European variants). The special procedures implies the requirement to conduct a great number of in vitro assays for proving bioequivalence, for example the European Medicines Agency (EMA) lists some 190 parameters for consideration in proving the bioequivalence of a generic intravenous liposomal product in comparison with an ``innovator liposomal product'' [7]. A detailed analysis of these tests and concepts behind them has been provided recently [1].

The critical importance of control over the manufacturing process is exemplified by several regulatory or voluntary actions having major impact on the manufacturing of liposomal drugs [1], the best known case being the shutdown of Ben Venue Laboratories, the only site where Doxil was produced [8,9]. That action led to a shortage of Doxil for almost 2 years, until the FDA approved Lipodox.

The above mentioned widely known and publicized, rightful concern about the clinical impact of liposomal structural variability should not overshadow another equally important factor affecting their therapeutic utility, namely, the individual patient

List of Abbreviations: ANDA, abbreviated new drug application; C, complement; CARPA, C activation-related pseudoallergy; FDA, US Food and Drug Administration; EMA, European Medicine Agency; HR, heart rate; HSR(s), hypersensitivity reaction (s); ISO, International Organization for Standardization; NBCD(s), non-biological complex drugs(s); NDA, new drug application; PAP, pulmonary arterial pressure; SAP, systemic arterial pressure; SRBC, sheep red blood cell

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variation in sensitivity and biological response to different liposomal therapies. The efficacy and toxicity of liposomal drugs also depend on anatomical, physiological and immunological features of the individual patient and his/her disease, which cannot be predicted or controlled at the level of manufacturing.

2. Complement activation by liposomes and its consequences

One of the potentially perturbing factors in the patients' individual response to liposomal therapy is that liposomes may have intrinsic, payload-independent biological activities and toxicities that also need to be considered from the point of bioequivalence, since non-equivalence in these effects may be as impactful as non-equivalence in physicochemical properties. One prominent example of such potentially dangerous biological activity of liposomes is complement (C) activation, the main subject of this review. Complement activation by liposomes has been known since the late 1960s [10,11] as an intrinsic feature of most charged and/or liganded phospholipid bilayer vesicles [12,13]. Fig. 1, delineates how C activation may hurt the therapeutic potential of liposomes in three major ways: 1) it can lead to opsonization of vesicles, which, in turn, triggers their rapid clearance, 2) it can augment the immunogenicity of liposomes, which makes their repeated use problematic, and 3) it can lead to hypersensitivity reactions (HSRs), called C activation-related pseudoallergy (CARPA), which represents a safety issue with many `reactogenic' liposomes [19,78–81].

Based on these significant adverse consequences, the presence and extent of C activation are properties that may need to be carefully matched in generic formulations to that in the originator, in order to prevent any unexpected extra C activation in certain individuals with potential severe hypersensitivity reaction (HSRs) and to avoid changes in pharmacokinetics and immunogenicity.

Table 1 lists the main features of liposomes influencing C activation in vitro. From the fact that so many minor detail can influence the activation process, their variable combination in different liposomes entails countless and unpredictable outcome in terms of net C activating power. For this reason it is very difficult, if possible at all, to predict the C activating capability of liposome formulations based only on structural analysis. The activation may need to be experimentally quantitated for each liposome preparation if that is critical for any reason.

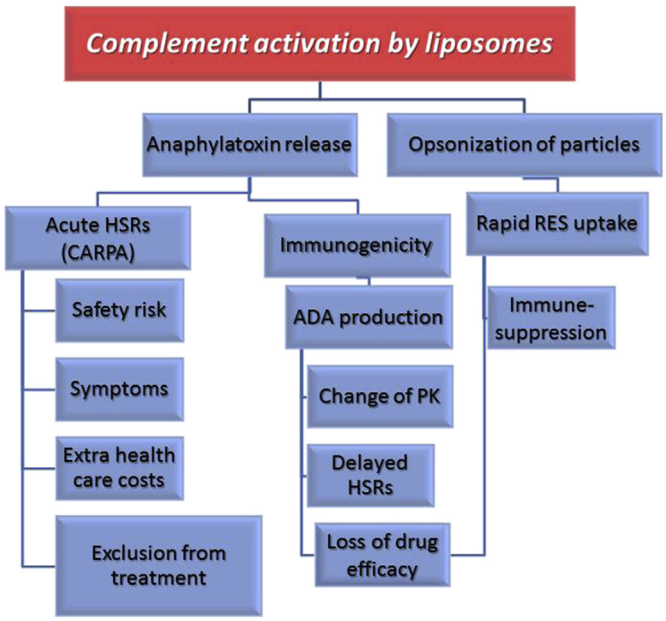

Fig. 1. Consequences of liposome-induced C activation.

3. Hypersensitivity reactions triggered by therapeutic liposomes

3.1. CARPA due to liposomal nanomedicines

Table 2 shows the marketed liposomal drugs that have been reported to cause HSRs along with some of their features and symptoms of hypersensitivity. Importantly, despite the wide variation of vesicle structure, the symptoms are very similar or identical. This fact, taken together with the large variety of other nanostructures causing CARPA implies the involvement of an adaptable afferent and invariant efferent mechanism in the phenomenon [19]. Although CARPA resolves in most patients within minutes or hours after stopping the infusion, the reaction may become life-threatening in a minority of patients (a few percent) and, occasionally, become even fatal (roughly at $\leq 0.01\%$).

The features of CARPA that distinguish them from classical IgE-mediated reactions include 1) the rise of symptoms at first exposure; 2) the diminution or disappearance of symptoms upon re-exposure; 3) their spontaneous resolution; 4) the dependence of reaction strength on the speed of infusion; 5) their response to steroid and antihistamine premedication; 6) the high reaction rate (2–10%) and, finally, 7) the negativity of standard allergy tests. It should be noted that there are patients in whom the reaction arises at the second or third treatment. However, in these cases immunogenicity of the liposome could be an aggravating factor, i.e., immunoglobulin (IgG and/or IgM) response to the first administration of the drug.

3.2. Hypersensitivity reactions to Doxil and the role of complement

The presence of 2K-PEG on the surface of Doxil reduces interactions with plasma proteins [20,21] and prolongs its circulation time ($T_{1/2}$ in blood: ~55 h [22–24]. These effects also imply reduced opsonization, which contradicts to Doxil being an efficient C activator. Nevertheless, C activation by Doxil has been shown in several in vitro and in vivo studies performed by independent laboratories [17,25–30], and the main questions regarding the phenomenon today are 1) what is the mechanism of activation and 2) how can it be reconciled with the long circulation time of these liposomes.

As for the mechanism of C activation by Doxil, we have shown earlier that it proceeds via both the classical and the alternative pathways [25], and that it is associated with the deposition of large C3b complexes on the liposome surface, a sign of effective opsonization (Fig. 2).

Regarding the C activation-related opsonization versus PEG-induced long circulation time of PEGylated liposomes (stealth paradox), it has been shown that association of cationic peptides with PEGylated liposomes does occur [32], that phospholipase A2 hydrolyzes the PC of PEGylated liposomes [33], and that anti-PEG antibodies bind to Doxil and other PEGylated liposomes [34–41]. These facts attest to an absence of interference by PEG with effective protein binding to liposomes. Thus, the widely believed reduction of protein binding by PEG may be selective, not valid for all proteins. Further explanations of the stealth paradox include: 1) only a small fraction of Doxil activate C and gets opsonized, with the overwhelming majority of vesicles remaining C3b-free and, hence, stealth; 2) it is PEG's steric hindrance of the ligation of iC3b to CR3 receptor-bearing RES macrophages that explains the reduced phagocytosis; 3) there is a competition for CR3 [31] between surface-bound and free-iC3b, and the deposition of iC3b on Doxil at cryptic sites are inaccessible to CR3 [42]; 4) an anti-

Table 1
Features of liposomes influencing C activation[a].

- Most liposomes can activate C in human serum or plasma, but not in all samples.
- There are liposomes which do not activate C even in a large number of human samples, but this does not mean that they are fully reactivity free.
- In addition to vesicle features, C activation by liposomes in human serum or plasma depends on individual sensitivity and experimental conditions.
- Certain liposomes activate in all, while others only in a few percentage of human samples.
- Individual sensitivity to liposomal C activation is vesicle type specific.
- C activation by liposomes may proceed on all 3 pathways of activation: classical, alternative and lectin.
- C activation by liposomes can be triggered by the binding of IgG, IgM, C3, CRP, and C1q.
- C activation by liposomes is enhanced by
  - ≻Positive or negative surface charge.
  - ≻Large versus small size (multilamellar vs. unilamellar vesicles).
  - ≻Inhomogeneity.
  - ≻Endotoxin contamination.
  - ≻Presence of aggregates.
  - ≻Presence of doxorubicin (or, assumedly, similar amphipathic weak base) in the extra-liposome medium that promotes aggregation.
  - ≻Oversaturating (> 50%) amounts of cholesterol in the membrane.
  - ≻PEGylation of liposomes via negatively charged phospholipid anchor (e.g. phosphatidyethanolamine).
  - ≻Coating of liposomes with polyamino acids.

[a] Based on refs. [12–18].

phagocytic serum factor (called dysopsonins) suppresses particle recognition by phagocytic cells [43,44].

4. Lack of ability of standardized complement and immunotoxicological tests in evaluating CARPA

4.1. In vitro tests

The C assays recommended by the regulatory authorities today for different human use applications for drug candidates have no, or limited usefulness in assessing the risk of CARPA. In particular, the International Organization for Standardization (ISO) issued in 2002, and then updated in 2009 ``ISO 10993-4'' [45], a guidance that included a list of C assays. However, these assays were designed solely for biocompatibility evaluation of medical devices made of solid artificial material, which get in direct contact with blood. Such devices include endovascular grafts, shunts, rings, patches, heart valves, balloon pumps, stents, pacemakers and hemopheresis filters. The absence of C activation by these devices is so essential that a separate guidance was issued for measuring C activation via the alternative pathway [46]. The tested devices, or their pieces, are incubated with human sera and C activation is measured by established chemical, immunochemical (C3a, C5a, C4d, Bb, SC5b-9, iC3b ELISAs) or the CH50 assay of C activation [47]. Nevertheless, none of these assays were tailored to measure liposome, or other nanomedicine-induced C activation. Although appropriate adaptation of these tests to measure C activation by liposomes is not a problem, such adaptation has not been validated or regulated.

Apart from these assays of C activation by medical devices, the only standardized C test that we are aware of is the measurement of the anticomplementary activity (ACA) in therapeutic intravenous immunoglobulin preparations with a CH50 assay using guinea pig serum [48]. The assay, which has no use in assessing CARPA, is detailed in the European Pharmacopoiea (6, 20.6.17, 2008).

The above information point to a lack of standardized assays for measuring C activation by liposomes or other nanomedicines in vitro. The result is a wide variety of C tests and conditions used in different laboratories engaged in drug development all over the world, reporting a wide variety of data that are often inconsistent and impossible to relate to each other. Table 3 lists the experimental conditions that influence the outcome of C activation tests using liposomes or other nanomedicines as reaction triggers. In

Table 2
Hypersensitivity reactions triggered by clinically used liposomal drugs.

Brand name (manufacturer)	Active ingredient	Indication	Type of particle (Size)	Symptoms[a]
Doxil, Caelyx (Johnson & Johnson)	Doxorubicin	Ovarian cancer, Kaposi sarcoma, myeloma multiplex, breast cancer	Liposomes (80–100 nm)	Flushing, shortness of breath, facial swelling, headache, chills, back pain, tightness in the chest or throat, hypotension
Myocet (Elan)				Flushing, dyspnea, fever, facial swelling, headache, back pain, chills, tightness in the chest and throat, hypotension
Abelcet (Elan, Enzon)	Amphotericin B	Fungal infections	Solid microparticles (1.6–11 mm)	Shortness of breath, change in blood pressure
Ambisome (Gilead, Fujisawa)			Liposomes (45–80 nm)	Chills, rigors, fever, nausea, vomiting, cardiorespiratory events
Amphotec, Amphocyl (Elan)			Disk shape solid nanoparticles (115 nm)	Hypotension, tachycardia, bronchospasm, dyspnea, hypoxia, hyperventilation
DaunoXome (Gilead)	Daunorubicin	Kaposi sarcoma	Liposomes (45 nm)	Back pain, flushing, chest tightness
Visudyne (Novartis)	Verteporfin	Age-related macular degeneration	Multilamellar liposomes (multimicron)	Chest pain, syncope, sweating, dizziness, rash, dyspnea, flushing, changes in blood pressure and heart rate, back pain

[a] Listed in the safety warnings of the package information. Table modified from Ref. [47] with permission.

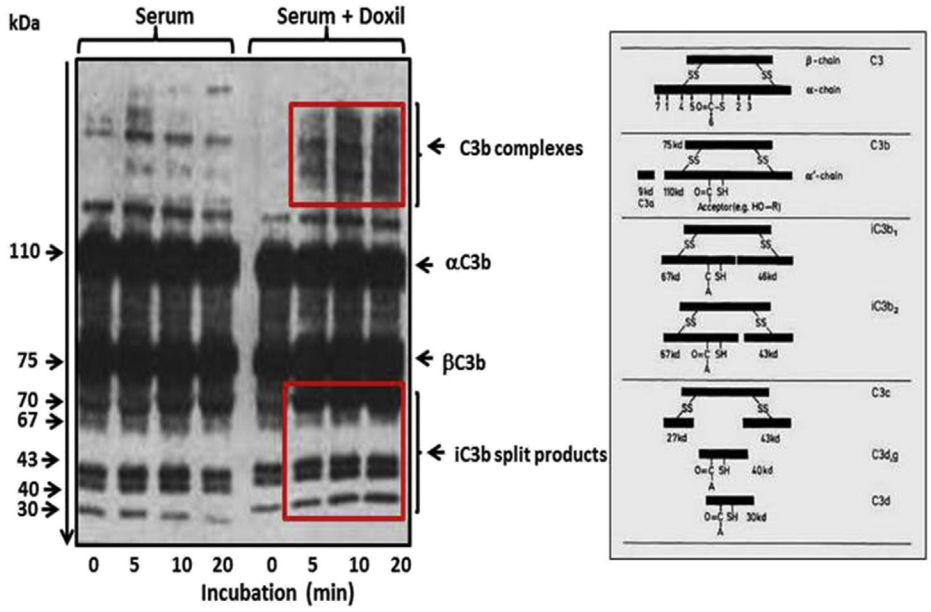

Fig. 2. Doxil-triggered C3 conversion in normal human serum in vitro. Doxil (0.2 mg Dox/mL) was incubated with 20% normal human serum supplemented with ^{125}I-labeled C3 for the indicated times at 37 °C. C3 conversion was analyzed by SDS PAGE as described in Ref. [21]. The right panel is a scheme of C3 degradation in serum that helps identifying the degradation products by their molecular weight. Figure reproduced from Ref. [30] with permission.

order to obtain consistent and inter-laboratory reproducible data, all listed conditions need to be kept constant.

4.2. In vivo tests

The standard immunotoxicological tests recommended by the regulatory authorities today for different human use applications have also limited usefulness in assessing the risk of CARPA. Some tests can *a priori* be ruled out as they assess immune functions that play no direct role in CARPA. These assays include the mouse popliteal lymph node assay (PLNA) [49,50], the mouse ear-swelling test [51–53] and the murine local lymph node assay (MLLA) [54]. The guinea pig tests, i.e., the ``maximization" test (GPMT) and the occluded patch test (Buehler's test) [54,55] were shown to be useless in predicting systemic hypersensitivity [56]. Likewise, measurement of lymph node weight or B, T or other immune cell stimulation gives no direct information on CARPA, whose complex pathomechanism was recently detailed in Ref. [19].

5. Dedicated tests to measure C activation and CARPA triggered by liposomes

5.1. In vitro tests

As mentioned above, the C tests listed in ``ISO 10993-4" can be used for liposomes and other nanomedicines on condition that the vesicles or nanoparticles get appropriate dispersion in NHS or plasma. Activation is then quantitated by one or more C split product ELISAs (C3a, C5a, SC5b-9, Bb, C4d, iC3b). The sheep red blood cell (SRBC) hemolysis assay is also usable and is less expensive, but it is also less sensitive than the ELISAs. The above ELISA assays are specific for humans, but a recently commercialized ELISA (PAN-C3) measures animal C3, e.g., in pigs, dogs, rats, mice, and in essentially all blooded species that utilizes C3 as a central protein in C activation [57]. Another development in the field of C methodology is the use of international C standards, which provide reference for establishing the absolute values of C proteins and split products in human test sera or plasma [58,59].

5.2. Cellular tests

Cellular tests can measure anaphylatoxin activity in blood, serum or plasma, and are based on the known biological effects of anaphylatoxins on blood cells, e.g., granulocytes, platelets, monocytes [60]. In fact, aggregation, chemotaxis, adherence or other motions of these blood cells have been used for a long time to quantitate anaphylatoxin activity in body fluids. Another relevant blood cell assay in predicting CARPA is the basophil activation assay, which, for example, quantitates basophil CD203c upregulation by FACS as a model of mast cell response to (pseudo)allergens [61–63]. We reported preliminary, promising results with this assay reproducing the occurrence of liposome reactions [19],

Table 3
Experimental conditions influencing C activation by liposomes and other nanomedicines.[a]

- Use of C source: normal human serum (NHS) or anticoagulated plasma or anticoagulated whole blood.
- Freshness and storage conditions of NHS, plasma and blood.
- The nature, source and concentration of anticoagulant used in case of plasma studies.
- Diluent of reaction trigger liposomal or other drug.
- The extent of dilution of serum/plasma/blood upon incubation.
- The conditions of incubation (temperature, length, shaking speed)
- Concentration of reaction trigger liposomal or other drug.
- Assay endpoint
- Source of ELISA kit

[a] Based on refs. [12–18].

however, further dedicated studies will be needed to confirm the predictive value of the basophil test for the occurrence of CARPA.

Taken the in vitro C assays together, it needs to be pointed out that they provide only partial, semiquantitative evaluation of the risk of CARPA, since they report only on the activity of afferent arm of the process, i.e., the extent of anaphylatoxin formation. The efferent arm, the body's response to anaphylatoxins, remains unknown. These can be measured only in *in vivo* animal models.

5.3. In vivo tests

Studies over the past 16 years provided ample evidence that pigs provide a useful, highly sensitive model of CARPA, particularly for its most serious, life threatening cardiopulmonary and hemodynamic manifestations [64–71]. Fig. 3 illustrates the endpoints that the model offers, and the instruments used for their measurement. The symptoms observed during CARPA in pigs include hemodynamic, hematological; biochemical and skin changes, referred to as ``CARPA tetrad'' [68–74].

Among the hemodynamic symptoms, the rise of pulmonary arterial pressure (PAP) is the most prominent and reproducible measure of CARPA, which is invariably present with all reactogenic liposomes and other nanoparticles. However, depending on the reaction trigger and intensity of reactions, the wave forms of PAP curves, as well as those of systemic arterial pressure (SAP) and heart rate (HR), can substantially differ (Fig. 4), which reflects the complexity of underlying pathomechanism.

The pulmonary reaction in pigs has been attributed to the presence of special intravascular macrophages (PIM cells) in the lung of these animals [75], as these cells are directly exposed to blood and their function is to screen blood from particulate pathogens. They can be activated both by anaphylatoxins and via particle binding to their surface receptors, and they respond to activation with massive secretion of vasoactive mediators [75]. The changes in SAP are more variable; it can drop, rise, display no change or undulate. The hematological changes typically include initial leukopenia followed by protracted leukocytosis and thrombocytopenia: among these the leukopenic effect is the most frequent. Among the biochemical changes, a rise of TXB2 is often striking, as it was found to show massive alterations in CARPA [64].

6. Algorithm for CARPA testing

As discussed above, the currently applied ``industry standard'' C and immune toxicology assays are not applicable for CARPA without adaptation for (nano)particle dispersions. On the other hand, even after ``adaptation'', the in vitro C tests can be used only for a semiquantitative assessment of the risk of CARPA, since C activation is only the afferent arm of the reaction; the efferent arm is mainly patient and conditions dependent. The unique significance of the porcine CARPA model is that the pigs' standard response mimics the worst-case human scenario, when the patient is hypersensitive and reacts to the drug with a life-threating anaphylactoid reaction that can lead to shock. In other words, the pigs standardize the efferent arm of CARPA with highest possible sensitivity. The porcine CARPA test is, however, neither simple, nor inexpensive; it cannot be used for high-throughput screening, as favored in many efficacy and toxicity studies in drug discovery. Accordingly, an optimized CARPA assessment procedure should combine the high-throughput, affordable C screening assay with the highly sensitive pig model to make the evaluation most definitive. Also, the process should allow regulatory validation and standardization to ensure consistency. To achieve the above goals, a decision tree was suggested recently [19] to guide through the available ELISAs and the pig test to tell whether a drug candidate carries a significant risk for CARPA and how to handle this risk (Fig. 5). According to this scheme, the test agent (drug candidate) is first incubated with a few normal human serum (NHS) samples to explore possible major C activation. If the result is positive, the agent is likely to carry a high risk for CARPA in vivo. As for the threshold for considering C activation as ``major'', an activation factor (for example a rise of SC5b-9 above baseline over 20–30 min incubation at 37 °C) of 5 to 10-fold may be a realistic predictor for the occurrence of clinical reaction, as such rises (of SC5b-9) were shown to correlate with clinical symptoms of patients treated with Doxil [17]. However, the

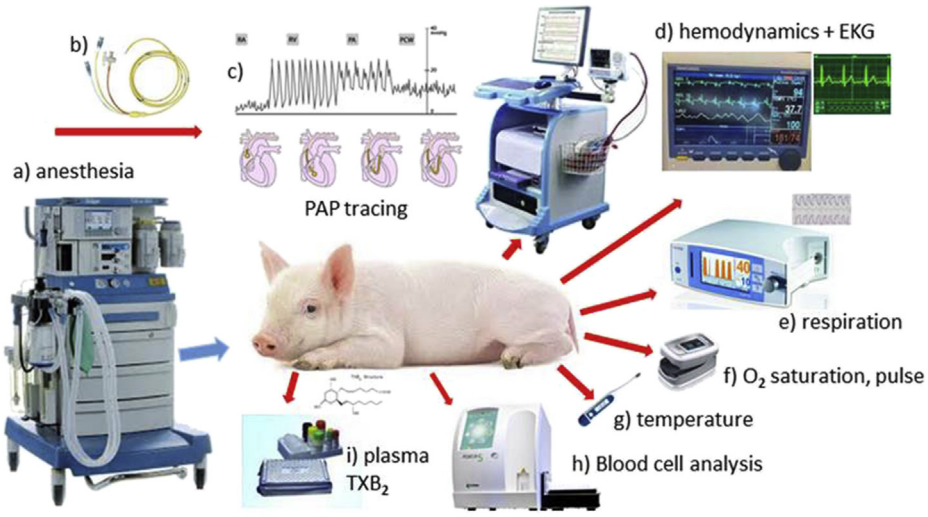

Fig. 3. Instruments and endpoints measured in the porcine CARPA model. a) anesthesia machine; b) Swan-Ganz catheter; c) blood pressure wave forms directing the passage of the tip of the Swan-Ganz catheter via the right atrium (RA), right ventricle (RV) and pulmonary artery (PA) until being wedged into the pulmonary capillary bed; d) computerized multiple parameter hemodynamic monitoring system (1000 Hz sampling rate). From the continuous recording of SAP and PAP signals online averaging is performed and recorded, together with the heart rate, derived from SAP signal; e) capnograph connected to the tracheal tube to measure respiratory rate, $etCO_2$ and $inCO_2$; f) pulse oximeter (fixed on the tail) measures O_2 saturation in blood and pulse rate; g) temperature is measured with a thermometer placed in the rectum; h) veterinary hematology analyzer measuring all blood cell counts and WBC differential; i) ELISA for measuring biomarkers of allergic/inflammatory reactions, e.g., TXB2, histamine, leukotrienes, adenosine, tryptase and C3 levels, etc. The figure was reproduced from Ref. [71] with permission.

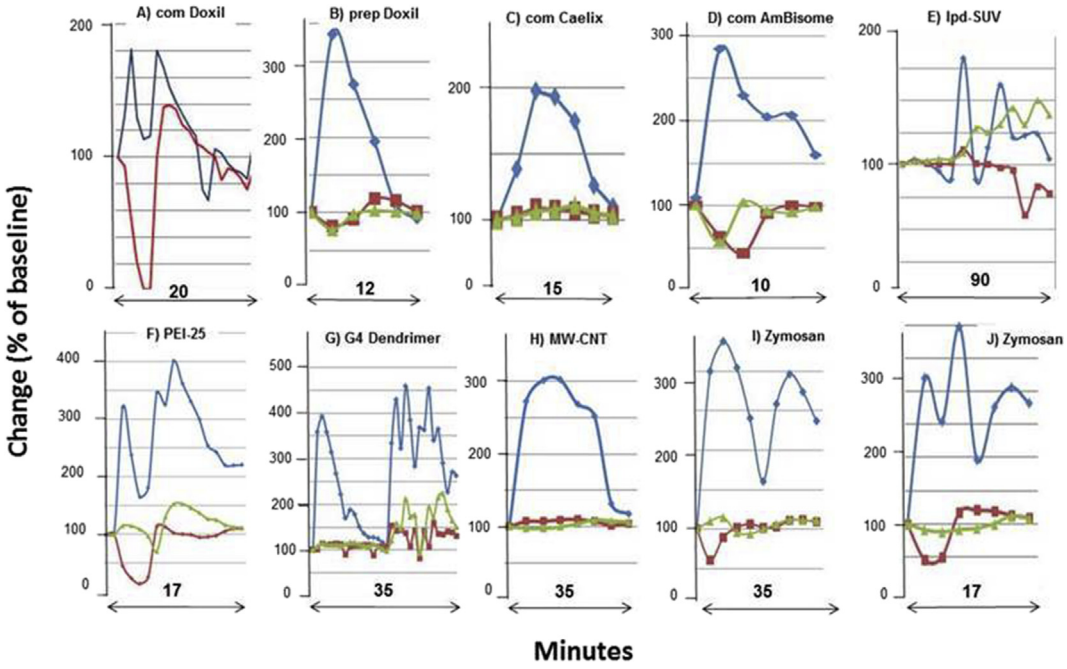

Fig. 4. Variation of pulmonary and systemic blood pressure and heart rate wave forms immediately after i.v. bolus administration of different nanoparticles in pigs. Minutes indicate the timespan of reactions. Blue, red and green are PAP, SAP and heart rate curves respectively. Changes are shown in % of baseline. Abbreviations only here: com, commercial; prep, self-prepared; lpd, lipophilic prodrug-containing liposomes; PEI25, 25kD pegylated poly(ethylene imine); G4 dendrimer, 4th generation dendrimer; MW-CNT, multiwall carbon nanotube. The figure was reproduced from Ref. [71] with permission.

correlation between C activation in vitro and the occurrence of clinical symptoms in vivo remains to be established in the future with higher precision.

If the in vitro C assay in NHS is not showing C activation, based on the substantial individual variation of C response, testing in a much larger number of NHS (in the range of 10–100) can be recommended, and/or testing in pigs using bolus administration. The reactogenicity in these models can be quantitated, among others by using the cardiac abnormality score (CAS) [66]. In case of low reactogenicity (CAS score 1–2), the test agent may carry a small, but not negligible risk for CARPA in a small percentage of hypersensitive individuals. In case of strong reactivity (CAS = 3–5), the risk of CARPA is great(er).

If the test drug does not cause C activation in a large number of NHS and it does not cause reactions in a relatively low number (n = 4–6) of pigs, it may be considered as CARPA-free, although

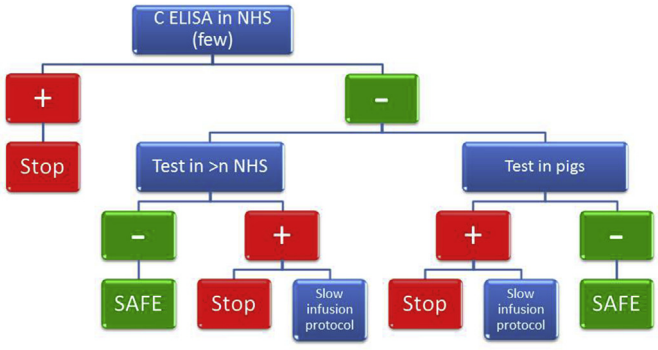

Fig. 5. Decision tree to predict the risk of CARPA induction. Abbreviations: NHS, normal human sera; blue entries are tests: C ELISA = ELISA of C activation byproducts (C3a, C5a, sC5b-9, Bb, C4d); "+" and "–" signs are major reaction and no reaction, respectively, where major is defined in the text. > n = large number of human NHS; SAFE mean that the tested drug candidate is unlikely to cause CARPA, while "slow infusion" means the possibility to develop a safe administration protocol by slow infusion. STOP means high risk for CARPA. The figure was reproduced from Ref. [19] with permission.

obviously the experimental conditions need to be relevant and the tests technically valid. But even if bolus administration leads to HSR in the animal model, or C is activated in NHS, desensitization, premedications and inhibition of C activation can be used to decrease the risk of clinical reactions [31,69,76–78]. Alternatively, slow administration protocols can be developed that secure safe human use of (mildly C activator) liposomal nanomedicine (or `liposomal drug') candidates.

7. Summary and outlook

Generic versions of liposomal drugs are now being introduced on the market, bringing up the difficulty of establishing bioequivalence between highly complex vesicular nanostructures manufactured by different developers. The current approach of regulatory agencies to solving this problem is the mandating of a great number of physicochemical tests in preclinical bioequivalence studies, one of which is directed to assessing C activation and CARPA. However, unlike with physicochemical assays, the state-of-art technology for measuring C activation and CARPA triggered by liposomal nanomedicines has not crystallized yet. The widely used and regulatory-approved in vitro and in vivo immune tests are irrelevant, while the available, relevant tests are not yet accredited for use in regulatory toxicology.

The present review of liposomal C activation and CARPA, and analysis of their measurements, will hopefully help in better understanding of this uncharted cross section of pharmacology, liposome technology, immunology and toxicology. In particular, we hope that the outlined scheme of CARPA risk assessment and management will get feedback from the scientific community and regulatory agencies to make it useful in the safety evaluation of liposomal drugs and other nanomedicines.

Conflict of interest

None.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.177.

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