

P-07

Complement Activation-Related Pseudoallergy (CARPA) in Rats: The Example of Liposomal Amphotericin-B (AmBisome)

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1. Introduction

Many intravenously administered nanodrugs are successful in their applications, but they share one particular problem, an immune reaction afflicting a small percentage of patients. The phenomenon is referred to by many names, e.g., hypersensitivity reactions (HSRs), infusion and anaphylactic reactions or pseudoallergy (1). Complement (C) activation is a possible explanation for these reactions, leading to the term, C activation-related pseudoallergy (CARPA). However, there are also observations on C-independent pseudoallergy (CIPA), whereupon HSRs occurred without measurable signs of C activation (2). The aim of the current study was to characterize HSRs in rats with focus on the role of C activation in the hemodynamic and blood cell changes that follow i.v. administration of the amphotericin B-containing liposome formulation (AmBisome), which is known to cause HSRs in man. We used cobra venom factor (CVF) and zymosan as C activator positive controls.

2. Materials and methods

Male Wistar rats (Toxicoop Ltd. (Budapest, Hungary)) were anesthetized with pentobarbital (60 mg/kg, i.p.). The left carotid artery and the left femoral artery and vein were cannulated for measuring mean arterial blood pressure (MABP), heart rate and for collecting blood samples before and at 1, 3, 10 and 30 min after treatments. A 10 min control period was recorded at least to confirm steady-state blood pressure and heart rate. Saline, the liposomal amphotericin B formulations, AmBisome (2.2 and 22 mg/kg phospholipid, PL) and Abelcet

(20 mg/kg), cobra venom factor (10 U/kg) and zymosan (10 mg/kg) were injected over 1 min into the left femoral vein. Complement activation was assessed by measuring C3 consumption, using the Pan-C3 ELISA kit (Quidel). Blood cell counts were measured using an Abacus vet5 hematological analyzer.

3. Results

As shown in *Figure 1*, the two C activators, zymosan and CVF caused a major (> 60%) drop in MABP, followed by partial recovery. Liposomal amphotericin B (AmBisome) also decreased MABP in a dose-dependent manner (*Figure 1*), but the effect was less expressed compared to the C activators.

As expected, both zymosan and CVF caused massive C consumption in rat blood, although the extent and kinetics of changes were different for the two activators (*Figure 2*) as CVF caused almost complete C3 depletion while the effect of zymosan reached a plateau earlier and at a lower level. AmBisome, at high dose, also caused significant C activation (*Figure 2*), which was similar to that caused by zymosan.

The lowest MABP values and C3 consumption showed highly significant correlation (*Figure 3*) in the animals treated with zymosan, CVF and high dose of AmBisome.

Zymosan, CVF and AmBisome also caused significant thrombocytopenia that seemed to be proportional with C3 consumption (not shown). AmBisome at low dose caused a small transient leukopenia, while zymosan induced a large but transient drop in white blood cell count that returned to the baseline at 30 min after treatment. However, CVF and the high dose of AmBisome induced leukopenia with compensatory leukocytosis by the

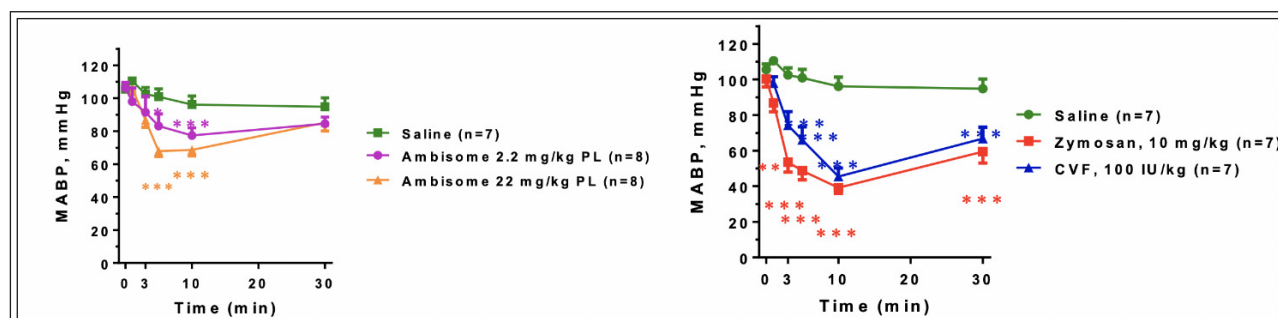


Figure 1 Blood pressure changes following i.v. administration of amphotericin B-containing liposomes and direct complement activators. The effects of AmBisome at two doses (left) and zymosan and CVF (right). Significant differences (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) are shown relative to the group treated with saline (NS).

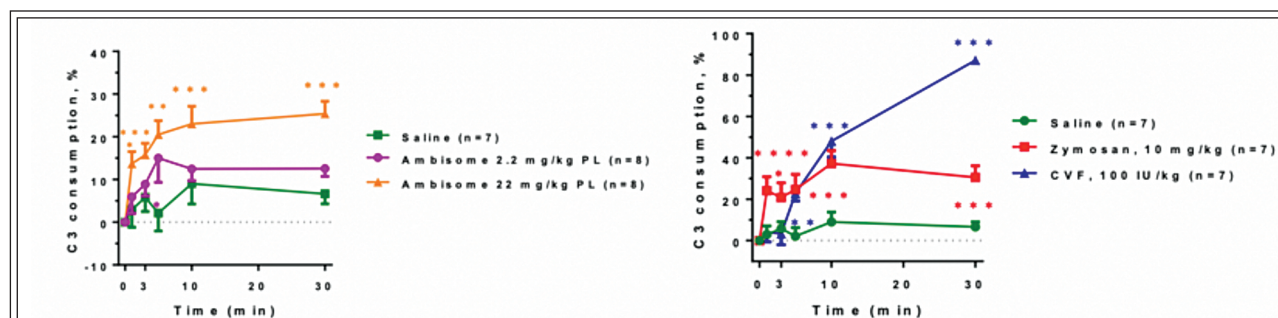


Figure 2 Effects of AmBisome at 2 doses (left) and direct complement activators (right) on the complement system in anesthetized rats. Complement activation was assessed as complement C3 consumption expressed as percentage decreases in C3 concentration relative to the baseline (time 0). Significant differences (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) are shown vs. the saline treated group

end of the observation period (not shown).

4. Conclusions

The observations that AmBisome caused dose-dependent hypotension, blood cell changes and C3 consumption in rats with similar kinetics on the minute time scale as did the known C activators provide evidence for the involvement of C activation in the effects of liposomes. Furthermore, the dose dependence of AmBisome's C-consuming and adverse physiological effects, taken together with the proportional C3 consumption and hypotensive effects of liposomes and C activators suggest that the extent of C activation is rate-limiting in the physiological changes, thus, the adverse reaction to AmBisome represents mainly CARPA.

5. Acknowledgements

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References

1. Szebeni, J.; Simberg, D.; Gonzalez-Fernandez, A.; Barenholz, Y.; Dobrovolskaia, M.A., Roadmap and strategy for overcoming infusion reactions to nanomedicines. *Nat. Nanotechnol.*, 13: 1100–1108 (2018).
2. Szebeni, J., Mechanism of nanoparticle-induced hypersensitivity in pigs: Complement or not complement? *Drug Discov. Today*, 23: 487–492 (2018).
3. Dézsi, L., Mészáros, T., Órfi, E., Fülöp, T.G., Hennies, M., Rosivall, L., Hamar, P., Szebeni, J., Szentácsi, G., Complement Activation-Related Pathophysiological Changes in Anesthetized Rats: Activator-Dependent Variations of Symptoms and Mediators of Pseudoallergy, *Molecules*, 24: 3283 (2019)
4. Dezső, L., Fülöp, T., Mészáros, T., Szentácsi, G., Urbanics, R., Vazsonyi, C., Órfi, E., Rosivall, L., Nemes, R., Kok, R.J., et al., Features of complement activation-related pseudoallergy to liposomes with different surface charge and PEGylation: Comparison of the porcine and rat responses. *J. Control. Release* 195: 2–10 (2014)