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The Monoclonal Antibody Panobacumab (KBPA101) improves *Pseudomonas aeruginosa* Opsonophagocytosis by THP-1 Monocytes

Hugues Jacqmin, Julien M. Buyck, Paul M. Tulkens, Françoise Van Bambeke[1]

Cellular and Molecular Pharmacology, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium

Mailing address:
P.M. Tulkens
Louvain Drug
Research Institute
av. Mounier 73 Bte B1.73.05
1200 Brussels – Belgium,
tulkens@facm.ucl.ac.be

Abstract (edited)

Background: Panobacumab (KBPA101) is a fully human IgM monoclonal antibody antibody derived from an immortalized human lymphocyte raised against the O11 serotype PA, with successful multicentre, open pilot Phase 2a clinical trial in critical patients with nosocomial PA O11 pneumonia (Lu et al. J Antimicrob Chemother 2011; 66:1110–16). We have examined its effect on opsonophagocytosis of PA in a model of human THP-1 monocytes.

Methods: PA ATCC 27313 serotype O11 was used. To mimic the situation occurring in patients, bacteria were first exposed to human non-specific serum (from healthy volunteers), and phagocytosis was thereafter allowed for up to 2 h (multiplicity of infection: 10) in medium containing human serum (0.5 or 1 %) and increasing concentrations of KBPA101 (controls: no serum and no KBPA101). Extracellular bacteria were eliminated by a 1 h incubation with gentamicin (100 µg/ml). Monocytes were thereafter immediately washed and cell-associated CFU were counted using cell protein content as a reference.

Results: The table shows that KBPA101 increases phagocytosis of PA (in addition to what is obtained by human serum alone) in a concentration-dependent manner, which becomes significant as from 50 ng/ml for each of the 2 conditions tested.

KBPA101 (ng/ml)	Increase in *P. aeruginosa* phagocytosis at 30 min (Δlog CFU vs control [no serum])			
	0.5 % human serum		1 % human serum	
0	0.21 ± 0.07	a, A	0.26 ± 0.09	a, A
20	0.20 ± 0.08	a, A	0.33 ± 0.08	a, A
50	0.46 ± 0.11	a, A	0.39 ± 0.08	a, A
100	0.52 ± 0.09	b, A	0.65 ± 0.09	b, A
200	0.56 ± 0.16	b, A	0.78 ± 0.14	b, A

Stat. analysis (ANOVA, Tukey post hoc test): values with different letters are significantly different from one another ($p < 0.05$); small letters: analysis per column; caps letters: analysis per row.

Conclusions: KBPA101 improves PA phagocytosis by human monocytes beyond what can be obtained by human serum alone. As the necessary concentration of KBPA101 is the range of that observed in humans in the published Phase2a trial (10-35 ng/mL), opsonophagoytosis may have contributed to the therapeutic efficacy observed in this trial.

References

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Background and Aim

Conventional antibiotics (i.e., acting as inhibitors of key bacterial metabolic reactions) have now increasingly found their limits due to (i) the remarkable ability of the main pathogens to evade their action (resistance), and (ii) the difficulty of discovering still unexploited but essential targets [1]. In this context, immunotherapy, developed with modern tools, is now an important avenue for active exploration by both Industry and clinicians [2;3].

Infections caused by *Pseudomonas aeruginosa* may be amongst those for which such novel approach may be most critical, since this highly successful opportunistic pathogen displays multiple intrinsic and acquired multidrug resistance mechanisms that, today, severely limit the usefulness of currently available antibiotics [4].

Panobacumab (KBPA101) is a fully human monoclonal antibody of the IgM/k isotype directed against the LPS O-polysaccharide moiety of *P. aeruginosa* serotype IATS O11. Panobacumab demonstrated effectiveness in animal models (murine burn wound sepsis and lung infection) [5], was well tolerated in phase I studies, and was shown recently to be associated with high clinical cure and survival rates in patients developing nosocomial *P. aeruginosa* O11 pneumonia [6].

The aim of the present study was to assess the effect of panobacumab on the opsonophagocytic activity of macrophages towards *P. aeruginosa*. The rationale for such study stems from (i) the observation that *P. aeruginosa* can invade eucaryotic cells where it can be subject to destruction by host defense mechanisms; (ii) that panobacumab could exert part of its antipseudomonal effect by increasing phagocytosis of its target organism [5].

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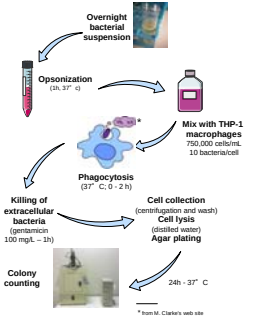
Methods

We used the *P. aeruginosa* strain ATCC 27313 and a model of human THP-1 macrophages in which the internalization and intracellular survival of this strain had been previously demonstrated [7]. The model and the main experimental steps are depicted in the figure.

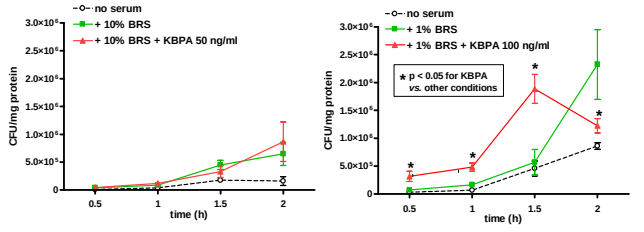


Opsonization was tested in the presence of control human serum or of baby rabbit serum (devoid of anti-*Pseudomonas* antibodies).

Bacterial loads were expressed as no. of CFUs per mg of eukaryotic cell protein (determined by the Folin-Ciocalteu/biuret) method) to obtain a more reliable denominator values than macrophage counting.

Results

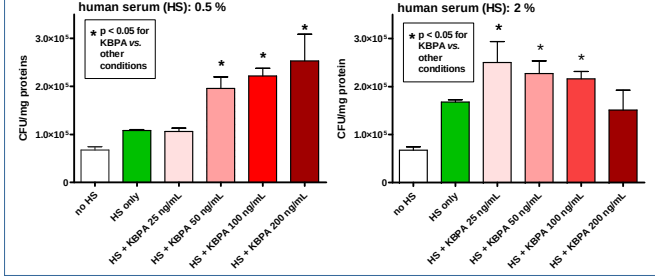
1. Influence of time, baby rabbit serum (BRS) and panobacumab (KBPA) concentrations



At 10% BRS (left), no specific effect of panobacumab could be demonstrated at 50 ng/mL.

Reducing BRS to 1% and increasing panobacumab to 100 ng/mL (right) resulted in significant increase in phagocytosis up to 90 min of contact.

2. Influence of panobacumab (KBPA) and of human serum (HS) concentrations at 30 min



At low HS concentration (left), panobacumab increases phagocytosis on a concentration-dependent manner as from of 50 ng/mL.

At larger HS concentration (right), a significant effect is seen at lower panobacumab concentration (25 ng/mL), but this effect decreases upon increasing its concentration.

Conclusions and Discussion

1. Panubacumab increases the phagocytosis of *Pseudomonas aeruginosa* by human macrophages, but the experimental conditions (serum and panomacumab concentrations, time of exposure) appear very critical.
2. The concentrations of panubacumab at which a significant effect can be demonstrated are pertinent of those observed in human serum after its systemic administration (see ref. 6). However, to be therapeutically useful in the target indication (nosocomial pneumonia), increase in phagocytosis would need to take place in the alveolar and interstitial milieu rather than in serum, which has not been explored here.
3. Attention should also be paid to detect not only viable but also dead and/or aggregated bacteria as the technique used here (colony counting) does not allow to detect them.