Neutrophil-mediated delivery of nanotherapeutics across blood vessel barrier

Xinyue Dong¹, Dafeng Chu¹ & Zhenjia Wang^{*,1}

¹Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Spokane, Washington 99202, USA

* Author for correspondence: zhenjia.wang@wsu.edu

Nanotherapeutics, nanoparticles (NPs) loaded with drugs, show the ability of tissue targeting, long circulation and low toxicity compared with free drugs. Endothelium lying the lumen of a blood vessel is a barrier to restrain tissue deposition of nanotherapeutics. Neutrophils, a type of white blood cells, migrate across endothelium during inflammation. There is an emerging concept that *in situ* targeting of neutrophils allows delivery of nanotherapeutics into deep tissues at disease sites. Here we summarize the recent advances in delivery of nanotherapeutics to inflammatory tissues or tumor microenvironments via neutrophil infiltration. The studies would shift the current paradigm of nanomedicine to biology-driven design of nanotherapeutics.

First draft submitted: 18 July 2017; Accepted for publication: 16 October 2017; Published online: 8 December 2017

Keywords: active delivery • cancer • inflammation • nanotherapeutics • neutrophil

The role of neutrophils in pathogenesis

- Neutrophils migrate from bloodstream to injured or infectious tissues during inflammation.
- Neutrophil infiltration is involved in the pathogenesis of most diseases.

Neutrophils are the critical component of the innate immune system because they are the first responders in circulation to arrive at inflammatory sites, eliminating pathogens from the body [1]. During inflammation, neutrophils cross the endothelial layer migrating from circulation to a site of pathogens, called neutrophil recruitment.

newlands
press

Figure 1. Neutrophil-mediated delivery of nanoparticles in inflamed lungs. (A) Scheme of neutrophil-mediated delivery of NPs across blood vessel barrier. NPs are internalized *in situ* by activated neutrophils that deliver NPs into a disease site. (B) Percentages of neutrophils internalizing BSA NPs in BALF. The data were analyzed from flow cytometry. (C) Depletion of neutrophils by anti-Gr-1 antibodies completely diminishes the transport of Cy5-BSA NPs. Cy5-BSA NPs in BALF were quantified using fluorescence. BALF: Bronchoalveolar lavage fluid; BSA: Bovine serum albumin; N.D.: Not detected; NP: Nanoparticle. Reprinted with permission from [11] © American Chemical Society (2016).

Neutrophil recruitment is initiated by the binding and adhesion of neutrophils to the surface of endothelium [2,3] and subsequently trans-endothelial migration is mediated by a gradient of cytokines and chemokines [3]. Neutrophil recruitment/infiltration is involved in many autoimmune and inflammatory disorders, such as arthritis [4], atherosclerosis [5], brain degenerative diseases [6], inflammatory bowel disease [7] and skin diseases [8]. Targeting the pathway of neutrophil infiltration might be a means to improve the treatment of autoimmune and inflammatory disorders.

Neutrophil-mediated delivery of nanotherapeutics

- *In situ* targeting of neutrophils is a key factor to deliver nanotherapeutics across blood vessel barrier.
- Several nanotherapeutic systems that can specifically target activated neutrophils *in vivo* have been reported.

Due to transmigration of neutrophils from bloodstream to inflammatory diseases, targeting activated neutrophils by NPs is a critical step to hijack neutrophils for targeted drug delivery [9]. It was reported that bovine serum albumin nanoparticles (albumin NPs) are able to specifically bind to activated neutrophils *in situ* [10]. Inspired by the study, Chu *et al.* proposed to examine whether neutrophils can mediate the transport of albumin NPs across endothelial barrier (Figure 1A) [11]. To prove this concept, they used a mouse model of acute lung inflammation because the lung has a unique property with many tiny air sacs (called alveoli) surrounded by capillaries to form an interface between blood circulation and airspace. After LPS (lipopolysaccharide) causes inflammation in the mouse lung, neutrophils transmigrate from bloodstream to the lung airspace. Analysis of albumin NPs in lung bronchoalveolar lavage fluid (BALF) allows to evaluate whether neutrophils can carry albumin NPs across blood vessel barrier. The data on flow cytometry showed that neutrophils in bronchoalveolar lavage fluid contained NPs (Figure 1B). The neutrophil infiltration in lungs was observed at 2 h and the neutrophils contained albumin NPs that were intravenously injected. The neutrophil infiltration increased with the time and at 20 h there were 30% of neutrophils that contained albumin NPs. The time-dependence of neutrophil movement is consistent with the time course of neutrophil transmigration during inflammation. When neutrophils were depleted using anti-Gr-1 antibodies (Abs), the transport of albumin NPs in lungs was completely abolished (Figure 1C). The results clearly indicate that neutrophils are carriers to mediate the transport of NPs across blood vessel barrier. The detailed discussion and experiments have been reported in the [11].

To test the usefulness in targeted drug delivery using this approach, albumin NPs loaded with 2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide (TPCA-1), an anti-inflammation drug, showed the dramatic decrease of lung inflammation and injury because of the decrease in number of neutrophils

and amount of inflammation factors, IL-6 [12] and TNF- α [13] in the lungs (data not shown here). In addition, albumin NPs loaded with cefoperazone acid (an antibiotic) demonstrate the anti-bacterial effect in a mouse model of *Pseudomonas aeruginosa*-infection. Taken together, NP targeting of neutrophils is a new and novel strategy to deliver nanotherapeutics in a site of diseases.

Tumor microenvironments are complex and communicate with the immune system. The link between inflammation and cancer can be traced back in the 19th century when Rudolf Virchow observed leukocytes within tumors [14]. Similarly, tumor-associated neutrophils exist in tumor microenvironments, and they stimulate angiogenesis to support tumor growth and metastasis [15]. The movement of neutrophils in tumors is random, thus it is hard to target neutrophils for drug delivery. However, a novel concept is proposed in which acute and temporary inflammation is generated in tumor tissues, and afterward neutrophils will infiltrate in the tumor. Two recent studies show that after tumor tissues are stimulated by Abs [16] or photosensitization (PS) [17], the inflammation rapidly occurs and intravenous administration of engineered NPs can increase cancer therapies via neutrophil-mediated delivery of nanotherapeutics.

TA99 is a monoclonal Ab that specifically binds a gp75 antigen, which is highly expressed on melanoma cells. When TA99 Abs bind the gp75 antigens, recruitment of neutrophils is initiated via a mechanism of antibody-dependent cell-mediated cytotoxicity (ADCC). In the study [16], administration of TA99 activated neutrophil infiltration and albumin NPs were injected subsequently to bind activated neutrophils. The confocal images showed the internalization of NPs by neutrophils in tumors when co-administration of TA99/NPs, but there was no existence of NPs in neutrophils without administration of TA99 (Figure 2A). The authors quantitatively measured the amount of albumin NPs in tumors (Figure 2B), and found that transport of NPs dramatically decreased when neutrophils were systemically depleted using anti-Gr-1. The result indicates that the movement of NPs is dependent on neutrophil tumor infiltration. When albumin NPs loaded with pyropheophorbide-a (Pp-a), a photosensitizer, were injected into mice implanted with melanoma, the tumor was suppressed and the mouse survival dramatically increased (Figure 2C).

To target integrin β_2 on activated neutrophils, a new drug-delivery platform was designed in which NPs were coated with anti-CD11b Abs [17]. In this study, PS was operated by shining light on a tumor after photosensitizers (pyropheophorbide-a [Ppa]) were intravenously injected, and acute inflammation-mediated neutrophil infiltration into the tumor [18]. Intravital microscopy on tumors demonstrated that the transport of NPs was mediated by neutrophils after iv. injection of NPs. The results showed that NPs-CD11b were present outside of blood vessels when the tumor tissue was imaged using intravital microscopy. In the controls, PEG (polyethylene glycol)-coated NPs or no PS on tumors did not mediate the transport of NPs, demonstrating that anti-CD11b coating and PS are required for the movement of NPs into the tumor (Figure 3A). When neutrophils were systemically depleted using anti-Gr-1 Abs (neutrophil specific Abs), the movement of NPs inhibited, clearly showing that neutrophils are carriers that mediate the transport of NPs (Figure 3A). The quantitative analysis using flow cytometry and direct measurement of tumor deposition of NPs indicated that anti-CD11b-coated NPs hijacked activated neutrophils for their movement into tumors. Anti-CD11b-decorated gold nanorods (GNRs-CD11b) were exploited in the laser-induced photothermal therapy to tumors. GNRs-CD11b suppressed the tumor growth (Figure 3B) and displayed a significant increase of survival (Figure 3C).

Conclusion & future perspective

Here we describe three representative studies that show a principle concept of neutrophil-mediated drug delivery, but many opportunities in this direction will be explored. Design of NPs that can *in situ* target activated neutrophils is critical and important. Many receptors expressed on neutrophils could be targeted when Abs are decorated to NPs. This strategy would enable materials scientists and clinicians to develop new therapeutic platforms.

By the inspiration of neutrophil-mediated nanotherapeutic delivery, other immune cells could also be utilized for targeted delivery because immune cells exist in tumor microenvironments and inflammatory tissues. For example, lipid nanocapsules were reported to be internalized by T lymphocytes (T cells) and T cells chaperoned the drug-loaded nanocapsules into tumor sites in the lymphoid mouse model [19]. It is shown that monocytes are able to take up PEGylated-single-walled carbon nanotubes *in situ*, improving the accumulation of NPs in tumors [20]. Real-time imaging on how NPs bind to and are internalized by neutrophils in live animals is very important to reveal new mechanisms of NPs trafficking [21]. Therefore, intravital microscopy is a promising tool to visualize in real-time individual NPs and subcellular compartments of single cells in many live organs.

Figure 2. Neutrophil-mediated delivery of albumin nanoparticles in tumors via antibody-dependent cell-mediated cytotoxicity. (A) Confocal fluorescence microscopy of neutrophils (green) and Cy5-BSA NPs (red) in tumors when TA99 or no TA99 was injected, and subsequently albumin NPs were administrated in mice implanted with melanoma. Nucleus was stained by DAPI (blue). (B) The concentrations of Cy5-BSA NPs in tumors after administration of buffer, TA99 or TA99/neutrophil depletion antibody Gr-1, respectively. (C) Drug-loaded albumin NPs after TA99 treatment increased the survival of mice bearing melanoma after photodynamic therapy under laser radiation at 660 nm. DAPI: 4',6-diamidino-2-phenylindole; NP: Nanoparticle. Reprinted with permission from [16] © Wiley (2015).

While *in situ* NPs targeting of activated neutrophils has been demonstrated, there are several interesting questions to be addressed. 30–50% of activated neutrophils can take up the NPs, and studies have shown the enhanced therapies of anti-inflammation and cancers [11,16,17], but the percentage of administrated NPs in neutrophils is not analyzed. Another concern is that the binding and uptake of NPs should not affect neutrophil transmigration. In three studies presented here [11,16,17], the agents (such as photosensitizers or gold NPs) used are not toxic and do not inhibit neutrophil movement. A recent report showed a surprising study on neutrophil-mediated delivery of paclitaxel in a brain tumor after neutrophils were *ex vivo* loaded with liposomal paclitaxel NPs [22]. Normally, liposomes cannot prevent the release of paclitaxel and paclitaxel is very toxic to neutrophils, therefore this study [22]

Figure 3. Neutrophil-mediated delivery of anti-CD11b-coated nanoparticles in tumors after photosensitization on the tumors. (A) Intravital microscopy shows that transport of NPs is mediated by neutrophil tumor infiltration. PS represents photosensitization by the light at 660 nm on a tumor. There are four cases: (1) PS and NPs-CD11b (green); (2) PS and NPs-PEG (green); (3) NPs-CD11b only and (4) PS and NPs-CD11b after neutrophil depletion. Neutrophils were stained by Alexa Fluor-647-labeled anti-mouse LY-6G Abs (pink). Cy3-BSA (red) was injected to mice for labeling of blood vessels. Tumor sizes **(B)** and survival rates **(C)** of mice bearing tumor after the injection of GNRs-PEG and GNRs-CD11b with or without PS, or the injection of GNRs-CD11b after neutrophil depletion. PBS (the buffer only, without PS and laser irradiation at 808 nm), PS (PS but without laser irradiation at 808 nm), PS/808 nm laser and PEG/808 nm laser (with PS or PEG, and laser irradiation at 808 nm).

NP: Nanoparticle; PS: Photosensitization.

Reprinted with permission from [17] © Wiley (2017).

is needed to be rigorously re-examined and evaluated. The rational design of NP delivery systems is needed when toxic agents are delivered via neutrophils in cancer therapies.

In summary, we have discussed three studies that demonstrate a new strategy to target activated neutrophils using NPs, thus leading to the transport of NPs across blood vessel barrier. Using this novel concept and approach, nanotherapeutics will be efficiently delivered to various disease sites, therefore improving therapeutic outcomes. Compared with current platforms in targeted drug delivery [23–26], neutrophil-mediated delivery of nanotherapeutics is a new paradigm in nanomedicine that would shift to biology-driven design of nanotherapeutics.

Executive summary

- The blood vessel barrier is the key factor preventing nanotherapeutics from tissue deposition.
- Neutrophils are vehicles to deliver nanocargos across the blood vessel barrier via neutrophil infiltration.
- Three nanoparticle systems demonstrate the feasibility of neutrophil-mediated delivery of nanotherapeutics in acute lung inflammation/injury and cancers.

Financial & competing interests disclosure

This work was sponsored by National Institutes of Health grants RO1GM116823 to Z Wang. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as: • of interest

- 1 Phillipson M, Kubes P. The neutrophil in vascular inflammation. *Nat. Med.* 17(11), 1381–1390 (2011).
- 2 Sadik CD, Kim ND, Luster AD. Neutrophils cascading their way to inflammation. *Trends Immunol.* 32(10), 452–460 (2011).
- 3 Kruger P, Saffarzadeh M, Weber ANR *et al.* Neutrophils: between host defence, immune modulation, and tissue injury. *PLoS Pathog.* 11(3), (2015).
- 4 Cascao R, Rosario HS, Souto-Carneiro MM, Fonseca JE. Neutrophils in rheumatoid arthritis: more than simple final effectors. *Autoimmun. Rev.* 9(8), 531–535 (2010).
- 5 Soehnlein O. Multiple roles for neutrophils in atherosclerosis. *Circ. Res.* 110(6), 875–888 (2012).
- 6 Ferrari CC, Tarelli R. Parkinson's disease and systemic inflammation. *Parkinsons Dis.* doi:Unsp 43681310.4061/2011/436813 (2011) (Epub ahead of print).
- 7 Kucharzik T, Walsh SV, Chen J, Parkos CA, Nusrat A. Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. *Am. J. Pathol.* 159(6), 2001–2009 (2001).
- 8 Cohen PR. Neutrophilic dermatoses a review of current treatment options. *Am. J. Clin. Dermatol.* 10(5), 301–312 (2009).
- 9 Dong XY, Chu DF, Wang ZJ. Leukocyte-mediated delivery of nanotherapeutics in inflammatory and tumor sites. *Theranostics* 7(3), 751–763 (2017).
- 10 Wang ZJ, Li J, Cho J, Malik AB. Prevention of vascular inflammation by nanoparticle targeting of adherent neutrophils. *Nat. Nanotechnol.* 9(3), 204–210 (2014).
- 11 Chu D, Gao J, Wang Z. Neutrophil-mediated delivery of therapeutic nanoparticles across blood vessel barrier for treatment of inflammation and infection. *ACS Nano* 9(12), 11800–11811 (2015).
- **Demonstrates a principle concept of neutrophil-mediated delivery of nanoparticles using intravital microscopy and the mouse lung inflammation model.**
- 12 Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Bba-Mol. Cell. Res.* 1813(5), 878–888 (2011).
- 13 Bradley JR. TNF-mediated inflammatory disease. *J. Pathol.* 214(2), 149–160 (2008).
- 14 Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 140(6), 883–899 (2010).
- 15 Singel KL, Segal BH. Neutrophils in the tumor microenvironment: trying to heal the wound that cannot heal. *Immunol. Rev.* 273(1), 329–343 (2016).
- 16 Chu D, Zhao Q, Yu J, Zhang F, Zhang H, Wang Z. Nanoparticle targeting of neutrophils for improved cancer immunotherapy. *Adv. Healthc. Mater.* 5(9), 1088–1093 (2016).
- **Demonstrates that antibodies of TA99 activate neutrophil tumor infiltration and using this pathway the authors delivered a photodynamic therapy (PDT) agent via neutrophils.**
- 17 Chu D, Dong X, Zhao Q, Gu J, Wang Z. Photosensitization priming of tumor microenvironments improves delivery of nanotherapeutics via neutrophil infiltration. *Adv. Mater.* 29(27), (2017).
- **Demonstrates that photosensitization activates acute inflammation on the tumor, and subsequently neutrophils mediate the delivery of gold nanoparticles in the tumor. This is a new approach to deliver nanotherapeutics to treat cancers.**
- 18 Castano AP, Mroz P, Hamblin MR. Photodynamic therapy and anti-tumour immunity. *Nat. Rev. Cancer* 6(7), 535–545 (2006).
- 19 Huang B, Abraham WD, Zheng Y, Bustamante Lopez SC, Luo SS, Irvine DJ. Active targeting of chemotherapy to disseminated tumors using nanoparticle-carrying T cells. *Sci. Transl. Med.* 7(291), 291–294 (2015).
- 20 Smith BR, Ghosn EE, Rallapalli H *et al.* Selective uptake of single-walled carbon nanotubes by circulating monocytes for enhanced tumour delivery. *Nat. Nanotechnol.* 9(6), 481–487 (2014).
- 21 Wang Z. Imaging nanotherapeutics in inflamed vasculature by intravital microscopy. *Theranostics* 6(13), 2431–2438 (2016).
- 22 Xue J, Zhao Z, Zhang L *et al.* Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence. *Nat. Nanotechnol.* 12(7), 692–700 (2017).
- 23 Gao J, Chu DF, Wang ZJ. Cell membrane-formed nanovesicles for disease-targeted delivery. *J. Control. Rel.* 224 208–216 (2016).
- 24 Chu DF, Curdy C, Riebesehl B, Zhang Y, Beck-Broichsitter M, Kissel T. Enzyme-responsive surface erosion of poly(ethylene carbonate) for controlled drug release. *Eur. J. Pharm. Biopharm.* 85(3), 1232–1237 (2013).

- 25 Liu WH, Song JL, Liu K, Chu DF, Li YX. Preparation and *in vitro* and *in vivo* release studies of Huperzine A loaded microspheres for the treatment of Alzheimer's disease. *J. Control. Rel.* 107(3), 417–427 (2005).
- 26 Wang Z, Tiruppathi C, Cho J, Minshall RD, Malik AB. Delivery of nanoparticle: complexed drugs across the vascular endothelial barrier via caveolae. *IUBMB Life* 63(8), 659–667 (2011).