PERSPECTIVES IN HYPERTENSION

Are nanobodies the future of tissue-specific angiotensin AT_1-receptor blockers?

U. MUSCHA STECKELINGS
University of Southern Denmark, Odense (U.M.S.). Denmark

Angiotensin AT_1 receptor blockers (ARBs) are among the most frequently prescribed anti-hypertensive drugs. They combine high therapeutic efficacy with very good tolerability. Approved ARBs such as Losartan, Valsartan, Olmesartan, Candesartan, Irbesartan or Telmisartan are fully synthetic small molecules. While small molecule antagonists of G-protein coupled receptors (GPCRs) usually have the advantage of a high affinity for their target, they also have limitations such as undesired passage through the placental barrier due to their small size and chemical properties. Regarding ARBs, placental passage constitutes a significant problem since ARBs are fetotoxic, because intact AT_1 receptor (AT_1R) signalling is required for normal kidney development. Another problem of GPCR ligands is the often-limited selectivity for other receptors or receptor subtypes or tissues.

Antibodies generally possess much better selectivity than small molecule drugs. This could also apply to antibodies binding to receptors, because – unlike small molecule agonists or antagonists, which solely interact with structures within the receptor binding pocket - antibodies could be designed to additionally recognize epitopes outside of the orthosteric pocket in order to increase selectivity.[1] Pharmacologically, such antibodies could act as competitive or allosteric antagonists, or, in principle, agonists.

While there are several good reasons to develop therapeutic, GPCR-targeting antibodies, this type of antibody is still a rare exception with presently only two FDA-approved drugs of that kind.[2]

The groups of Andrew C. Kruse and Robert J. Lefkowitz have developed a method for discovery of GPCR-targeting antibodies and as one of the first targets they selected the angiotensin AT_1 receptor.[1,3] These AT_1R-targeting antibodies are of a special type, called nanobodies (Fig. 1).[4] Nanobodies consist of a single variable domain heavy chain derived from heavy-chain only antibodies (Fig. 1), the latter being endogenously present in very few species like for example in camelids (camels, dromedars, lamas, alpacas – nanobodies derived from camelids are called VHH nanobodies) or in sharks (so-called VNAR nanobodies). The prevailing type of nanobody in drug development projects is camelid VHH nanobodies. Such nanobodies can be generated by immunisation of camelids or of transgenic mice, which have been generated to produce heavy-chain-only antibodies. Alternatively, there are also techniques for fully synthetic production of nanobodies by cDNA recombinant technologies, i.e. not requiring in vivo immunisation. Large-scale production and amplification of nanobodies for therapeutic use in humans can be processed in microbial expression systems, whereas conventional antibodies are usually produced in mammalian cell cultures, the latter being more costly, yielding lower amounts and requiring more complex purification steps.[4,5] Nevertheless, for regulatory reasons and because of large production capacities based on mammalian cell cultures worldwide, mammalian expression systems are currently still often preferred for nanobodies. Nanobodies have a number of advantages over conventional antibodies such as extreme stability (potentially allowing oral

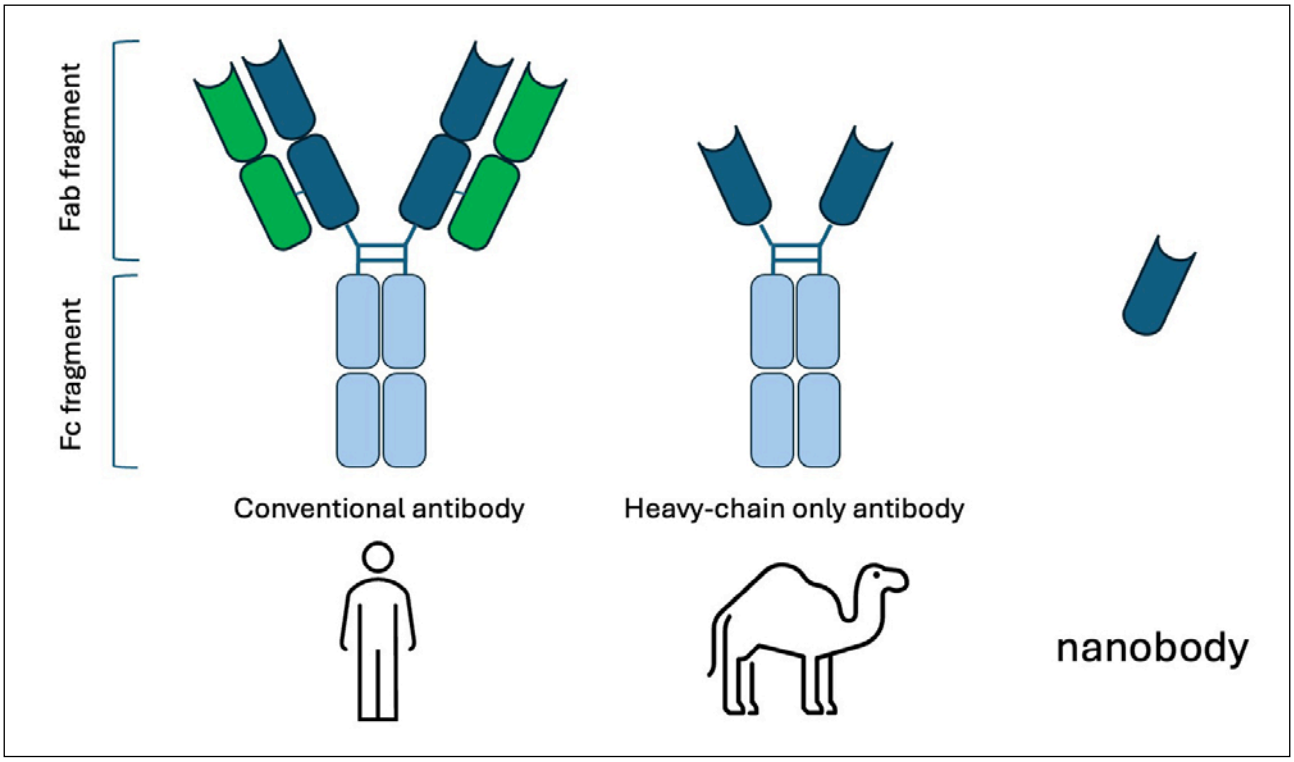

Figure 1: Conventional IgG antibody (left), camelid heavy-chain-only antibody (middle), nanobody (right). Dark blue: heavy chain, Green: light chain

application), low immunogenicity (especially when humanised), less posttranslational modifications and a much smaller size, which enables fast distribution and deep tissue penetration.[4,5]

Despite their small size, nanobodies retain high specificity and possess a higher antigen binding affinity for their target antigens than conventional antibodies. The first nanobody-based drug (Caplacizumab), a bivalent nanobody for the treatment of acquired thrombotic thrombocytopenic purpura, was approved by the FDA in 2018.[6] Currently (July 2024), there are three more nanobody-based drugs approved and in clinical use, two of them approved by FDA (Caplacizumab, Ciltacabtagene), one approved in China (Envafolimab) and one approved in Japan (Ozoralizumab) (https://www.biochempeg.com/article/375.html). At least 20 more of such drugs are in clinical development.

The AT_1R-targeting antibody developed by the Lefkowitz/Kruse groups is derived from a yeast-displayed library of fully synthetic nanobodies, i.e. it does not require animal immunisation. The initial lead, the AT118 nanobody, was selected based on its ability to compete with angiotensin II and the ARB Olmesartan for binding into the AT_1R binding pocket.[3] Modification of AT118 resulted in the higher affinity nanobody AT118-A, which was further modified to yield a humanised variant termed AT118-H. The Kruse group took these modifications further, starting with the generation of AT118-H variants with reduced non-specific binding (AT118-L).[1] Next steps served to reduce renal filtration and increase plasma half-life by fusion of the nanobody to a human IgG1 Fc and by dimerising this fusion-protein. In a final step, the Fc's neonatal Fc receptor (FcRn) binding site was mutated to prevent transport of the antibody across the placental barrier into the foetal circulation. Various tests revealed that concentrations of the fused nanobody were indeed minimal in the foetal circulation in a mouse model, while its ability to antagonise AT_1R-mediated Gαq signalling and lower Ang II-induced hypertension in mice was retained thus making this nanobody a potential candidate for treating maternal hypertension in pregnancy by AT_1R blockade without the teratogenic risk.

Interestingly, extensive additional cryo-electron microscopy studies revealed that the interaction of the nanobodies with the AT_1R differs from

that of small molecule AT_1R antagonist: like AT_1R antagonists, they "freeze" the intracellular pocket in an inactive state that does not allow receptor signalling, whereas – unlike AT_1R antagonists - the extracellular domain is stabilised in an active-like state.[1]

Collectively, these studies by the Kruse and Lefkowitz groups introduced a new modality for AT_1R-targeting drugs. While (modified) nanobodies will with high certainty not replace the well-established small molecule AT_1R antagonists as treatment for "conventional" hypertension, they may open up new possibilities for treating hypertension under conditions which require a tissue specific effect such as AT_1R blockade in maternal but not foetal tissue during pregnancy.

U. Muscha Steckelings – usteckelings@health.sdu.dk

References:

1. Skiba MA, Sterling SM, Rawson S, Zhang S, Xu H, Jiang H, et al. Antibodies expand the scope of angiotensin receptor pharmacology. Nat Chem Biol. 2024 May 14;

2. CUSABIO [Internet]. [cited 2024 Jul 11]. The Collection of Progress of Anti-GPCR Antibody Drugs. Available from: https://www.cusabio.com/c-21061.html

3. McMahon C, Staus DP, Wingler LM, Wang J, Skiba MA, Elgeti M, et al. Synthetic nanobodies as angiotensin receptor blockers. Proc Natl Acad Sci U S A. 2020 Aug 18;117(33):20284–91.

4. Jin BK, Odongo S, Radwanska M, Magez S. NANOBODIES®: A Review of Diagnostic and Therapeutic Applications. Int J Mol Sci. 2023 Mar 22;24(6):5994.

5. Kunz S, Durandy M, Seguin L, Feral CC. NANOBODY® Molecule, a Giga Medical Tool in Nanodimensions. Int J Mol Sci. 2023 Aug 25;24(17):13229.

6. Scully M, Cataland SR, Peyvandi F, Coppo P, Knöbl P, Kremer Hovinga JA, et al. Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. N Engl J Med. 2019 Jan 24;380(4):335–46.