Current Topics

Present and Future of Therapeutic Drug Monitoring in New Fields

Review

Therapeutic Drug Monitoring of Antibody Drugs

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In recent years, many antibody drugs that play an important role in the pharmacotherapy of several diseases have been developed. Antibody drugs exhibit immunogenicity *in vivo* leading to the development of antibodies against the antibody drug (anti-drug antibody). Nonetheless, other factors also affect the pharmacokinetics of antibody drugs. Recently, therapeutic drug monitoring (TDM) of infliximab was introduced for personalized medicine. However, the usefulness of TDM in antibody therapy remains unclear. In addition to intervention studies, real-world data analysis is important. Unlike small-molecule drugs, antibody drugs do not have a uniform molecular weight; therefore, using the conventional analysis methods, it is impossible to determine the true pharmacokinetic outcomes of these agents. To analyze structural changes of antibody drugs in the body, new technologies are necessary. In the future, along with the development of new drugs, the establishment of novel analytical methods is essential to facilitate the promotion of personalized medicine.

Key words therapeutic drug monitoring; antibody drug; infliximab; MS

1. INTRODUCTION

In recent years, many biopharmaceuticals such as antibody drugs have been developed. Unlike small-molecule drugs, antibody drugs do not have uniform molecular weights because they are purified from cell-biosynthesized proteins. Post-translational modifications, such as glycosylation, inclusion of disulfide bonds, and amino acid modifications, cause heterogenicity, making them mixtures of molecules of various molecular weights.[1] Therefore, unlike for small-molecule drugs, quantification using mass spectrometry is not usually possible for antibody drugs. In many cases, immunochemical measurement methods are used to determine the various molecules in antibody drugs. However, using the aforementioned techniques, metabolites of antibody drugs cannot and have not been identified to date; therefore, there is no information on how antibody drugs cause changes in the body.[2] To analyze structural changes in antibody drugs, new characterization technologies are necessary.

2. ANTIBODY DRUGS

Antibodies produced by B cells are involved in biological defense mechanisms. The technology for producing hybridomas was established in the 1970s, and since then, it has become possible to produce monoclonal antibodies by creating immortalized B cells. In 1982, Miller *et al.*[3] used this technique to produce and administer a mouse monoclonal antibody that bound to a specific antigen (idiotype antibody) for patient-derived lymphoma. This was the first antibody-associated therapeutic agent for patients with cancer. Applying the aforementioned manufacturing technology to make chimeric antibodies finally led to the birth of rituximab.[4]

Human immunoglobulin, a protein of approximately 150 kDa, consists of two heavy chains and two light chains connected by disulfide bonds (Fig. 1). Light chains and the N-terminal side of heavy chains are called fragment antigen binding (Fab) regions and bind to antigens. The C-terminal side of the heavy chain is called the fragment crystallizable (Fc) region and binds to Fc receptors. A carbohydrate is attached to the Fc region on the Fab side, which can affect the binding ability of the Fc receptors. In addition, immunoglobulins are classified into five classes based on the type of the heavy chain: immunoglobulin G (IgG), IgM, IgD, IgE, and IgA. All antibody drugs approved so far are of the IgG type. They are further divided into IgG1, IgG2, IgG3 (no approved drug), and IgG4. Molecular characteristics, such as antibody-dependent cellular cytotoxicity, differ among these classes. Most antibody drugs approved for market in recent years are human antibodies (-umab) or humanized antibodies (-zumab). Previously, human and mouse chimeric antibodies (-ximab), such as rituximab, infliximab (IFX), and cetuximab have also

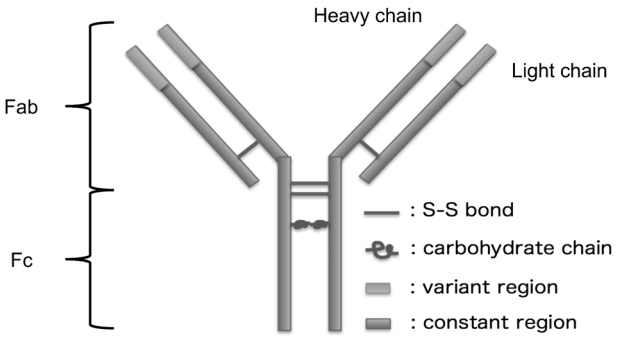

Fig. 1. Structure of Antibody Drugs

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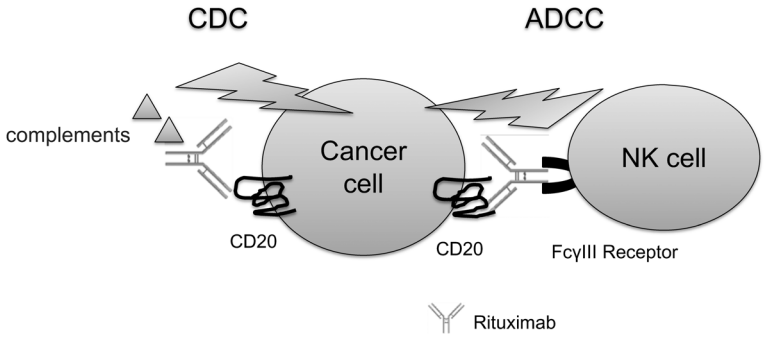

Fig. 2. Pharmacodynamic Mechanisms of Antibody Drugs in Cancer Treatment

ADCC: antibody-dependent cellular cytotoxicity, *CDC:* complement-dependent cellular cytotoxicity.

been approved for use and are currently on the market.

Two major mechanisms of action of antibody drugs are behind their therapeutic efficacy. One is neutralizing activity, in which the antibody binds to the target molecule and inhibits the function of the molecule. In this molecular mechanism, antibody drugs can exert their efficacy by simply binding to an antigen. On the other hand, complex mechanisms are involved in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cellular cytotoxicity (CDC) (Fig. 2). For example, rituximab binds to CD20, which is expressed on the surface of cancer cells. However, it does not inhibit the activity of CD20. The Fc region of rituximab bound to CD20 is recognized by the FcγIII receptor on natural killer cells and releases interferon γ and perforin/granzyme to kill cancer cells. Similarly, in CDC, complement factors bind to the Fc region of rituximab, which then binds to CD20 and activates the complement pathway. Not only does the antibody drug bind to the antigen to elicit a response, but the activation of another mechanism *via* the Fc region is important. In addition, carboxylate chains attached to antibody drugs are vital for ADCC activation. It has been reported that ADCC activity increases 100-fold in antibodies in which fucose has been removed.[5] Therefore, carboxylate chains have been shown to play an important role in drug efficacy.

3. PHARMACOKINETICS OF THERAPEUTIC ANTIBODIES

Unlike small-molecule drugs, antibody drugs do not disappear following hepatic metabolism or urinary excretion. Generally, proteins are taken up by various cells, such as vascular endothelial cells, *via* endocytosis, and are transported to lysosomes for degradation.[6] The half-life of antibody drugs is very long (approximately two weeks). The fetal Fc receptor FcRn is localized on the endocytic cell membrane and when the antibody drug binds to FcRn, it is excreted into the blood *via* a recycling mechanism without being transferred to the lysosomes. The action of FcRn is considered to contribute to the long half-life of antibody drugs. Therefore, if FcRn is not functional, the half-life of the drug is approximately 2–3 d.[7]

Because an antibody drug is a large molecule, it exhibits immunogenicity *in vivo*. Antibodies against antibody drugs (anti-drug antibodies) are produced. In particular, chimeric antibodies are highly immunogenic and anti-drug antibodies frequently appear following their administration.[8,9] When an anti-drug antibody is produced, the blood concentration of the antibody drug is significantly decreased, leading to a reduction in therapeutic efficacy. Antibody drugs may be used for a prolonged duration in the treatment of autoimmune diseases. However, the frequency of anti-drug antibody production increases, which has become a clinical issue as a cause of secondary ineffectiveness.

4. METHODS TO DETERMINE THE THERAPEUTIC EFFICACY OF ANTIBODY DRUGS

The blood concentrations of antibody drugs have been measured in clinical trials of drug development. Enzyme-linked immunosorbent assay (ELISA) is the gold standard for the quantification of therapeutic proteins in support of pharmacokinetics data. In the ELISA method, a molecule that binds to an antibody drug is immobilized on a plate and the target molecule is trapped. In many cases, an antibody that recognizes a specific sequence of an antibody drug (mainly the Fab region) is immobilized on the plate. Owing to this, all the molecules to which the antibody can bind are measured. Even if part of the carboxylate chain is changed or the Fc region is modified, it is still detected as the same. Recently, a method for measuring blood concentrations of antibody drugs using LC-MS was developed using a simple pretreatment method.[10] The nano-surface and molecular orientation limited proteolysis (nSMOL) method is one such technique that has been applied for LC-MS bioanalysis of antibody drugs.[11] Unlike the ELISA method, it is not necessary to construct a measurement method for each antibody drug. Simultaneous measurements of various antibody drugs can be performed.[12]

5. THERAPEUTIC DRUG MONITORING (TDM) OF THERAPEUTIC ANTIBODIES

IFX is a chimeric monoclonal antibody composed of human constant and murine variable regions that specifically bind to tumor necrosis factor alpha (TNF-α). IFX therapy has substantially improved the treatment of rheumatoid arthritis (RA), inflammatory bowel disease, and psoriasis. Previous studies have shown that approximately 10–60% of patients with RA receiving IFX developed anti-infliximab antibodies (ADAs) within the first 6 months.[13] In addition to ADAs, baseline TNF-α levels were another factor that was reported to reduce serum IFX levels.[14] A prospective, randomized, double-blind study (the RISING study) reported a significant correlation between serum IFX levels and disease activity score in 28 joints

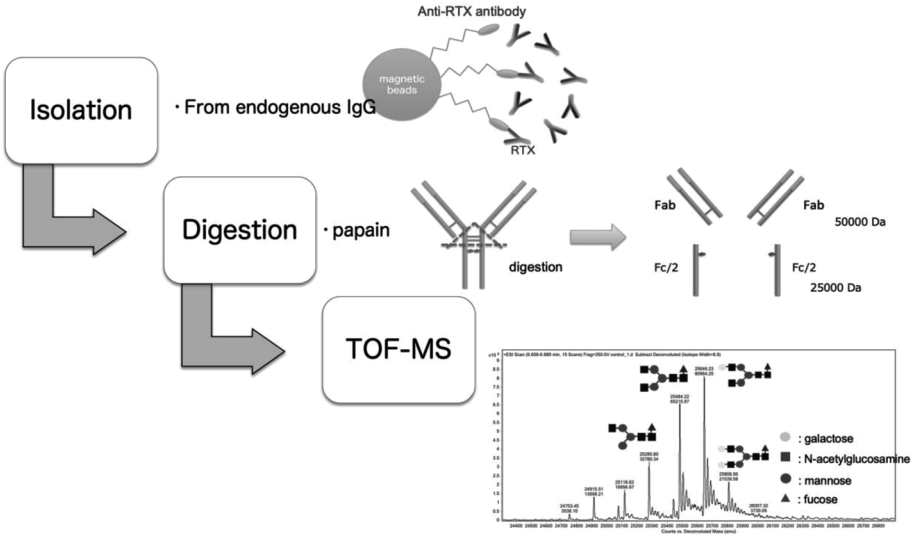

Fig. 3. Novel Method to Analyze the Structures of Antibody Drugs Using MS

TOF-MS: time-of-flight mass spectrometry.

(DAS28)-remission.[15] Our real-world cohort data analysis suggested that primary ineffectiveness can be avoided in clinical practice.[16] Conversely, some patients showed a secondary loss of response to IFX following a lapse in continuous use. We thus proposed the development of a treatment algorithm based on IFX TDM, wherein IFX therapeutic efficacy can be extensively re-evaluated when blood IFX concentrations are low under continuous use. In Japan, Remicheck®, a quantitative assay by which IFX concentrations can be examined, was approved for patients with RA in 2017. In clinical practice, TDM of antibody drugs has therefore been launched.

6. METHODS TO ANALYZE THERAPEUTIC ANTIBODIES IN WHOLE BLOOD

A method for measuring the blood concentration of antibody drugs using LC-MS has been developed using a simple pretreatment method,[10] as alluded to above. However, because the drug concentration was determined using only the fragment peptide, changes in the carboxylate chains could not be detected, with these potentially being important for drug efficacy. Therefore, pharmacokinetic analysis techniques of antibody drugs that determine the parameters of the entire active moiety, such as those applied in small-molecule drugs, are desired.

Since antibody drugs possess a similar sequence to immunoglobulins in the living body, it is necessary to isolate them from other IgGs during mass spectrometry analysis. Consequently, we established a novel antibody drug analytical method.[17] For example, in the analysis of rituximab, we isolated it from serum using magnetic beads bound to anti-rituximab antibody, treated it with papain, and subjected the resultant sample to Liquid Chromatography/Time-of-Flight Mass Spectrometry (LC/TOF-MS) analysis (Fig. 3). As a result, peaks corresponding to the Fab region, including the antigen recognition site, the reduced fragment crystallizable (Fc/2) region, as well as the carboxylate chain structure were detected. Fab has the same molecular weight as its theoretical value.

We attempted to analyze human blood samples using this method.[18] Structural analysis of rituximab was performed using samples from 20 patients who received the drug for treatment of non-Hodgkin's B-cell lymphoma (UMIN000016713). The total blood rituximab concentration decreased over time, and 17 patients showed a drug half-life of approximately two weeks. In contrast, an extreme decrease in blood concentration was observed in three cases, with a half-life of approximately three days. One of them presented with urinary protein and thus the drug was assumed to have been excreted in the urine; however, the causes of the low half-life in the other two cases was unknown. When structural analysis was carried out 1 h and 3 weeks after administration, the proportion of some carboxylate chains had significantly changed. In particular, extreme changes were observed in cases where the blood levels changed significantly. Since FcRn has a great effect on the pharmacokinetics of antibody drugs,[7] it is assumed that changes in carboxylate chains are also involved in drug pharmacokinetics. In the future, a detailed analysis behind this postulated mechanism is required.

7. CONCLUSION

Various new antibody drugs, modified protein drugs, nucleic acid drugs, and cellular drugs are being developed. Using conventional analysis methods for small-molecule drugs, it is impossible to determine the true pharmacokinetics of the former molecules. In addition, the usefulness of TDM for antibody therapy remains unclear. In addition to intervention studies, real-world data analysis is important. In the future, along with the development of new drugs, the establishment of novel analytical methods is essential to streamline and promote personalized medicine.

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Conflict of Interest The author declares no conflict of interest.

REFERENCES

1) Walsh G, Jefferis R. Post-translational modifications in the context of therapeutic proteins. *Nat. Biotechnol.*, **24**, 1241–1252 (2006).
2) Hamuro LL, Kishnani NS. Metabolism of biologics: biotherapeutic proteins. *Bioanalysis*, **4**, 189–195 (2012).
3) Miller RA, Maloney DG, Warnke R, Levy R. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. *N. Engl. J. Med.*, **306**, 517–522 (1982).
4) Levy R. A brief personal history of cancer immunotherapy at Stanford: if these walls could talk…. *Immunol. Res.*, **58**, 277–281 (2014).
5) Mössner E, Brünker P, Moser S, *et al.* Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood*, **115**, 4393–4402 (2010).
6) Dostalek M, Gardner I, Gurbaxani BM, Rose RH, Chetty M. Pharmacokinetics, pharmacodynamics and physiologically-based pharmacokinetic modelling of monoclonal antibodies. *Clin. Pharmacokinet.*, **52**, 83–124 (2013).
7) Garg A, Balthasar JP. Physiologically-based pharmacokinetic (PBPK) model to predict igg tissue kinetics in wild-type and fcrn-knockout mice. *J. Pharmacokinet. Pharmacodyn.*, **34**, 687–709 (2007).
8) Baert F, Noman M, Vermeire S, Van Assche G, D' Haens G, Carbonez A, Rutgeerts P. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N. Engl. J. Med.*, **348**, 601–608 (2003).
9) Kalden JR, Schulze-Koops H. Immunogenicity and loss of response to TNF inhibitors: implications for rheumatoid arthritis treatment. *Nat. Rev. Rheumatol.*, **13**, 707–718 (2017).
10) Neubert H, Alley SC, Lee A, *et al.* 2020 White paper on recent issues in bioanalysis: BMV of hybrid assays, acoustic MS, HRMS, data integrity, endogenous compounds, microsampling and microbiome. *Bioanalysis*, **13**, 203–238 (2021).
11) Iwamoto N, Shimada T, Umino Y, Aoki C, Aoki Y, Sato TA, Hamada A, Nakagama H. Selective detection of complementarity-determining regions of monoclonal antibody by limiting protease access to the substrate: nano-surface and molecular-orientation limited proteolysis. *Analyst*, **139**, 576–580 (2014).
12) Iwamoto N, Takanashi M, Yokoyama K, Yonezawa A, Denda M, Hashimoto M, Tanaka M, Ito H, Matsuura M, Yamamoto S, Honzawa Y, Matsubara K, Shimada T. Multiplexed monitoring of therapeutic antibodies for inflammatory diseases using Fab-selective proteolysis nsmol coupled with LC-MS. *J. Immunol. Methods*, **472**, 44–54 (2019).
13) Strand V, Balsa A, Al-Saleh J, Barile-Fabris L, Horiuchi T, Takeuchi T, Lula S, Hawes C, Kola B, Marshall L. Immunogenicity of biologics in chronic inflammatory diseases: a systematic review. *BioDrugs*, **31**, 299–316 (2017).
14) Takeuchi T, Miyasaka N, Tatsuki Y, Yano T, Yoshinari T, Abe T, Koike T. Baseline tumour necrosis factor alpha levels predict the necessity for dose escalation of infliximab therapy in patients with rheumatoid arthritis. *Ann. Rheum. Dis.*, **70**, 1208–1215 (2011).
15) Takeuchi T, Miyasaka N, Inoue K, Abe T, Koike T. Impact of trough serum level on radiographic and clinical response to infliximab plus methotrexate in patients with rheumatoid arthritis: results from the RISING study. *Mod. Rheumatol.*, **19**, 478–487 (2009).
16) Nakae K, Masui S, Yonezawa A, *et al.* Potential application of measuring serum infliximab levels in rheumatoid arthritis management: a retrospective study based on KURAMA cohort data. *PLOS ONE*, **16**, e0258601 (2021).
17) Otani Y, Yonezawa A, Tsuda M, Imai S, Ikemi Y, Nakagawa S, Omura T, Nakagawa T, Yano I, Matsubara K. Time-dependent structural alteration of rituximab analyzed by LC/TOF-MS after a systemic administration to rats. *PLOS ONE*, **12**, e0169588 (2017).
18) Yonezawa A, Otani Y, Kitano T, Mori M, Masui S, Isomoto Y, Tsuda M, Imai S, Ikemi Y, Denda M, Sato Y, Nakagawa S, Omura T, Nakagawa T, Yano I, Hayakari M, Takaori-Kondo A, Matsubara K. Concentration and glycoform of rituximab in plasma of patients with B cell non-Hodgkin's lymphoma. *Pharm. Res.*, **36**, 82 (2019).