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Hazard characterization of an anti-human tissue factor antibody by combining results of tissue cross-reactivity studies and distribution of hemorrhagic lesions in monkey toxicity studies

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ABSTRACT

Tissue cross-reactivity (TCR) studies are conducted when developing therapeutic antibodies, but their value is sometimes questioned because the positive organs often do not match the target organs of toxicity. We conducted TCR studies in human and cynomolgus monkey tissues for the development of an anti-human tissue factor antibody (TFAb) and also for a commercially available antibody, to clarify the true distribution of the target antigen. Tissue factor (TF) was found to be distributed in a wide variety of organs and tissues, including the heart and urinary bladder, in human and monkey. Administration of the TFAb to cynomolgus monkey caused hemorrhagic lesions mainly in the heart and urinary bladder in an incidental manner. This was thought to show the physiological role of TF in regulating hemostasis in these organs. Because the distribution of antigen in human and monkey was similar, the possibility that the TFAb would have similar effects in human was judged to be high, and because of the incidental nature of the effects, that they would be difficult to avoid. Thus it was possible to prospectively characterize the hazardous potential of a therapeutic antibody by accurately evaluating the tissue distribution of the target antigen and understanding its biological nature.

1. Introduction

Tissue cross-reactivity (TCR) studies are designed to identify the binding of therapeutic antibodies in tissues (Leach et al., 2010). Information on the distribution of this binding is crucial for estimating cells and organs that may be affected, and is thought to be valuable information for safety evaluation (Leach et al., 2010). Such studies are also used to support the relevance of species by comparing the binding in the species used in toxicity studies to that in human (Kato et al., 2009; Leach et al., 2010).

Because TCR studies obtain important information for safety evaluation, they are required for IND/CTA (investigational new drug application/clinical trial application) in support of first-in-human clinical trials (Leach et al., 2010). But the value of TCR studies is at times questioned because the positive organs often do not match the target organs of toxicity (Leach et al., 2010; Suzuki et al., 2015). One reason for this discrepancy is that therapeutic antibodies are not necessarily suitable for immunohistochemistry (Leach et al., 2010), so it is often important to know the true distribution of the target antigen to estimate the risk in human (Kato et al., 2009; Leach et al., 2010; Furukawa et al., 2017). Another reason for the discrepancy is the mode of action of the antibody or the biological features of the target antigen, because toxicity is thought to occur from a combination of the physiological activity of the target antigen and the modification of that activity by the therapeutic antibody (Kato et al., 2009; Suzuki et al., 2015). But there are few reports that address these matters in detail when interpreting TCR data for prospective hazard characterization in human (Leach et al., 2010).

We have attempted to develop an anti-human tissue factor antibody (TFAb) that inhibits tissue factor (TF) activity to target a wide variety of thrombotic disorders. TF is the substance that initiates extrinsic blood coagulation (Mitchell, 2010). Injury to vessel walls and surrounding tissue exposes the TF within the tissue to the coagulation factors in the blood, activates the blood coagulation system, and causes thrombosis (Tatsumi and Mackman, 2015; Witkowski et al., 2016). Due to this mechanism, the main role of TF is thought to be hemostasis and maintenance of blood vessels (Witkowski et al., 2016). On the other hand, high levels of TF have

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been related to cardiovascular diseases, such as arteriosclerosis, acute coronary syndrome, and venous thromboembolisms (Smiley and Becker, 2014), and TF was thought to be a promising target for therapy (Bode and Mackman, 2015).

TF is known to be expressed in a variety of organs and tissues, including brain, lung, placenta, heart, testis, and kidney (Drake et al., 1989; Bode and Mackman, 2015; Witkowski et al., 2016). It is also normally expressed in endothelial cells, pericytes, and fibroblasts (Drake et al., 1989; Bode and Mackman, 2015; Witkowski et al., 2016). Although the antigen is known to be widely expressed, there is little information on its physiological role in non-pathogenic states. Thus we deemed that extrinsic coagulation is only activated when there is tissue damage or inflammation and not under normal conditions, which led us to expect that there would be no severe toxic effects of the TFAb.

Based on the preliminary information, we conducted a series of non-clinical studies and obtained accurate target distribution data. Additionally, by considering the time and frequency of administration, we were able to comprehend the characteristics of toxicity caused by inhibiting the physiological activity of TF with the TFAb. Finally, we prospectively evaluated the possible toxicity in human by combining the non-clinical data.

2. Materials and methods

2.1. Tissue cross-reactivity studies

2.1.1. Sample preparation

Normal human tissues that had been obtained via autopsy or surgical biopsy and frozen on dry ice at collection were purchased from commercial vendors. The tissues were anonymized and transferred to the test facility (Charles River Laboratories, Pathology Associates, Frederick, MD), where they were embedded into O.C.T. medium (Sakura Finetek Japan, Co., Ltd., Chuoku, Tokyo, Japan) and stored in the tissue bank at the test facility. Cynomolgus monkey tissues were obtained from necropsies performed at various facilities of Charles River Laboratories, and after being prepared by the same methods as those used for human tissues, were stored in the tissue bank of the test facility. All human tissues used in the human study were obtained according to the guidelines of the tissue supplier, which include the necessity of informed consent and anonymity procedures to protect donor confidentiality. The cynomolgus monkey tissues were obtained by procedures approved by the Institutional Animal Care and Use Committee at each Charles River Laboratories facility. Positive control samples included O.C.T.-embedded cell blocks of J82 human bladder transitional cell carcinoma cells (ATCC No. HTB-1, American Type Culture Collection, Gaithersberg, MD) and recombinant full-length human TF protein (amino acids 1–263 [non-lipidated], American Diagnostica Inc. Greenwich, CT), which was UV-adhered to UV-activated resin slides. UV-adhered human parathyroid hormone-reactive protein (amino acids 1–34, Sigma, St. Louis, MO) was used as a negative control sample.

2.1.2. Test system

The TFAb, a humanized IgG4k monoclonal antibody directed against human TF, was manufactured at Chugai Pharmaceutical Co., Ltd. (Gotemba, Shizuoka, Japan). In addition to the TFAb, a commercially available mouse monoclonal antibody raised against human TF (American Diagnostica Inc.) was applied as a positive control antibody (CAb). Isotype-matched negative control antibodies were incorporated for both assays. An indirect immunoperoxidase staining procedure was performed. Acetone-fixed cryosections were used for staining. Each primary antibody was applied and subsequently the biotinylated secondary antibodies (biotinylated mouse anti-human IgG4, Southern Biotechnology Associates, Inc., Birmingham, AL or biotinylated goat anti-mouse IgG1, F (ab')$_2$ fragment specific, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), and then the slides were treated with the ABC Elite reagent (Avidin-biotin-peroxidase kit, Vector Laboratories, Burlingame, CA). The reaction was visualized with a 3,3′-diaminobenzidine solution (Sigma, St. Louis, MO), counterstained with hematoxylin, coverslipped, and read under a light microscope.

2.2. Toxicity studies designed to evaluate the modifying effects on target activity in cynomolgus monkey

2.2.1. Animals

For the single-dose toxicity study, cynomolgus monkeys imported from China were assigned to the study at Chugai Pharmaceutical Co., Ltd. at the age of 4 years. The animals were housed in stainless steel cages under conditions of 24 °C ± 2 °C, humidity of 55% ± 10%, air change rate 14–16 times per hour, and a 14/10 h light-dark-cycle. The animals were fed 70 mg of monkey diet (Certified diet PS, Oriental Yeast Co., Ltd., Itabashi, Tokyo, Japan) and 1/2 of a banana per day, with tap water supplied ad libitum.

For the intermittent-dose toxicity study, cynomolgus monkeys imported from Vietnam were assigned to the study at LSI Medience Corporation (Kashima, Ibaraki, Japan) between the ages of 3–4 years. The animals were housed in stainless steel cages under conditions of 26 °C ± 2 °C, humidity of 555 ± 15%, air change rate 6–25 times per hour, and a 12 h light-dark-cycle. The animals were fed 100 mg of monkey diet (CMK-1α, CLEA Japan, Inc., Meguro, Tokyo, Japan) per day, and given sterilized water ad libitum.

The study protocols were approved by the animal welfare committees at the study facilities, and the studies were conducted in compliance with the necessary guidelines.

2.2.2. Study designs

For the single-dose study, 15 male and 15 female animals were subjected to the study. Three animals of each sex were given single intravenous doses of 0, 3, 10, 30, or 100 mg/kg of the TFAb (Fig. 1). Two animals from each dose group were necropsied at Day 14, and 1 animal per group at Day 28. For the intermittent-dose study, 15

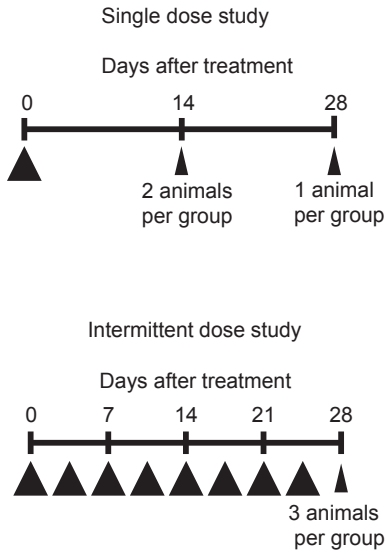

Fig. 1. Toxicity studies designed to evaluate the modifying effects of TF on target activity in cynomolgus monkey.
Pathology data from a single-dose study and an intermittent-dose study were examined. Arrows, administration of the TFAb. Arrow heads, necropsy.