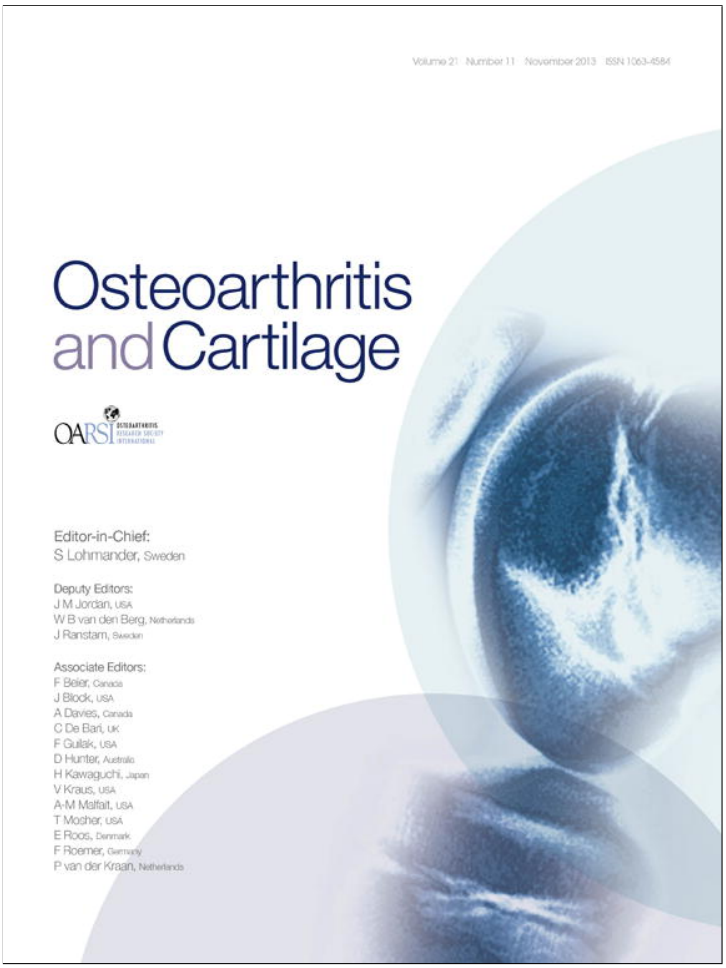

Osteoarthritis and Cartilage 21 (2013) 1807–1810

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Brief report

Targeting of ADAMTS5's ancillary domain with the recombinant mAb CRB0017 ameliorates disease progression in a spontaneous murine model of osteoarthritis

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ARTICLE INFO

Article history:
Received 17 May 2013
Accepted 7 August 2013

Keywords:
ADAMTS5
Monoclonal antibody
Osteoarthritis
Animal model
STR/ort mouse

SUMMARY

Objective: ADAMTS5 (aggrecanase-2) has been demonstrated to be crucial in the development of osteoarthritis (OA), by use of several mouse mutants carrying either truncated, catalytically inactive enzymes or aggrecanase-resistant mutant aggrecan. We have selected recombinant monoclonal antibodies directed against ADAMTS5, by using Intracellular Antibody Capture Technology (IACT). CRB0017 revealed very high affinity for the enzyme in Biacore analyses and very good specificity in a panel of binding assays. Therefore, we tested CRB0017 in a relevant spontaneous OA model, the STR/ort mouse.

Design: STR/ort male mice were recruited at 5 months of age, and treated intra-articularly in each knee with CRB0017 1.2 μg, CRB0017 12 μg, or vehicle. After 6 weeks, the intra-articular administration of CRB0017 was repeated with the same doses. After 3 months from recruitment, the animals were sacrificed and the femorotibial joints processed for histology and scored in a blind fashion according to both Mankin's and the OARSI methods.

Results and conclusions: All histological scores were significantly decreased in the CRB0017 12 μg/knee group compared to vehicle, while administration of CRB0017 1.2 μg was associated with a trend to a decrease in the same parameters. Therefore, CRB0017 administered twice in 3 months could modify the course of OA in the STR/ort mouse, by delaying cartilage breakdown as assessed histologically. The procedure of blind scoring of the histological samples clearly showed that knee intra-articular administration of CRB0017, an anti-ADAMTS5 antibody, dose-dependently improved disease progression in a relevant animal model of OA.

Introduction

The etiology of osteoarthritis (OA), the most widespread form of arthritis, is largely unknown. Breakdown of aggrecan, the most represented non-collagenous protein in articular cartilage, is an early event in the onset and progression of OA, and therefore since their discovery aggrecanases have been postulated to have a role in OA, which has been recently widely demonstrated in relevant animal models. Mouse mutants lacking functional ADAMTS5 (aggrecanase-2) are protected from articular cartilage loss in a relevant OA surgical paradigm[1], as are mice carrying aggrecanase-resistant mutant aggrecan[2]. As a consequence, ADAMTS5 has since been regarded as an appealing possible therapeutic target for OA[3].

In order to evaluate a possible therapeutic approach to OA via biologic interference with ADAMTS5, we have used the proprietary Intracellular Antibody Capture Technology (IACT) to select a panel of antibody domains from large recombinant scFv SPLINT libraries[4–6]. The antibodies were selected using different and specifically engineered domains of ADAMTS5 as antigens.

In particular, mAb CRB0017[7] demonstrated very high affinity for the spacer domain of the enzyme (as revealed by Biacore assays) both in the single chain and in the IgG4 format. Moreover, the antibody was shown to reduce the proteolytic activity of the enzyme *in vitro* with an IC_{50} in the low nanomolar range. Therefore,

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http://dx.doi.org/10.1016/j.joca.2013.08.015

this antibody was selected for further drug development. To verify the hypothesis that interfering with the biological activity of ADAMTS5 could improve the course of OA, we tested mAb CRB0017 in STR/ort mice, which develop a spontaneous, age-related OA and are considered a relevant model for human OA[8–11].

Methods

mAb CRB0017 production and purification

Anti-ADAMTS5 CRB0017 scFv was reformatted to IgG4 antibody for expression in mammalian cells. CHO-S cells were stably transfected with plasmids encoding CRB0017 heavy and light chains. Clarified supernatant was loaded onto HiTrap protein A-sepharose column (GE Healthcare) previously equilibrated with PBS. The antibody was recovered by acidic elution (10 mM HCl pH 2.5) followed by immediate neutralization with 1 M Tris–HCl pH 8.5. Pooled fractions were dialyzed against PBS.

Animal model

Male STR/ort mice (Harlan, Italy) were housed with *ad libitum* access to food and water, in a temperature-controlled room with a 12 h light/dark cycle. All the experimental procedures described were in compliance with international laws and policies (Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

STR/ort mice were recruited at 5 months of age (n = 20–22/treatment group), randomly distributed by treatment in each cage, with four animals per cage, weighed and treated intra-articularly, each in both knees with single dosing of either CRB0017 1.2 μg, CRB0017 12 μg, or vehicle (in a final volume of 4 μl). The control group consisted only of vehicle treated mice as we never observed any significant differences between mice treated with an irrelevant IgG4 and vehicle treated mice in our previous experiments (data not shown). After 6 weeks the intra-articular administration of CRB0017 was repeated with the same doses. After 3 months from recruitment the animals were sacrificed and hind limbs explanted and fixed in formalin o/n. Knees were embedded in paraffin, 4-micron thick sections were produced and stained with toluidine blue and then scored in a blind fashion according to both Mankin's and the OARSI scores[12,13]. Total score represents the OARSI score plus cell loss plus GAG loss scores. This takes into account all parameters that were reported separately in order to provide the best detail, allowing a further and more complete view on OA progression as a whole.

Since OA develops independently in each joint of each single STR/ort mouse[8–11], both knees were injected. Accordingly, the joints were scored independently for each animal. To support this approach, during the setup phase of the analysis model, we investigated the presence of a correlation between the right and left knee scores in control groups of mice treated either with vehicle or with an irrelevant human IgG4 in three different experiments (including the control group of the present study, in total 75 mice). This analysis showed that the correlation between the pathology scores evaluated in right and left knees is very low, if any, and never statistically significant, in spite of the large number of animals involved (data not shown). Statistical analysis was performed by Kruskal–Wallis ANOVA on ranks followed by Dunn's test comparing each treatment group vs vehicle.

Results

As we aimed for a therapeutic protocol, and not a preventive one, 5-month-old male STR/ort mice were enrolled for this experiment. At this age, 100% of male STR/ort mice display some degree of OA. Mice were monitored daily for the duration of the experiment, and showed general well being; no deaths or weight loss were observed, nor other relevant signs of toxicity. Three months after the first injection, vehicle treated mice displayed severe OA with clefting, fibrillation and erosion of articular cartilage, sclerosis and focally necrosis of subchondral bone, osteochondrophytes and pathological changes in ligaments and capsula [Fig. 1(a)].

All OA histopathological scores were dose-dependently reduced following treatment with CRB0017, with the higher dose reaching a profound, statistically significant decrease vs vehicle, for all parameters [Fig.1(b) and 2]. This experiment was repeated twice with the same protocol, yielding the same results (data not shown).

Discussion

Since the discovery of its importance in OA onset and progression, ADAMTS5 has been pursued as a target for therapies aimed at disease modification, with strategies employing design of small molecules to inhibit its protease activity[3,14]. Unfortunately this search has been rewarded with little success, likely because of the high homology of the catalytic domain across the diverse metalloprotease families, and the resulting difficulties at achieving specificity in order to avoid a burden of undesired effects. With the aim of developing a therapeutic approach to OA devoid of off-target side effects, we attempted to interfere with ADAMTS5's function by

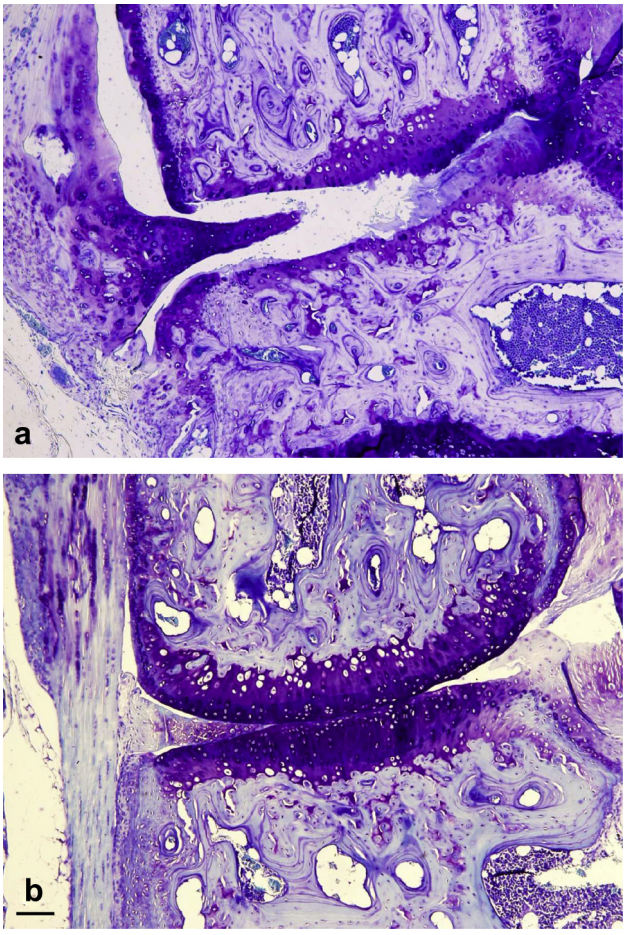

Fig. 1. Representative histology images of knee joints from 8-mo STR/ort mice treated with CRB0017 12 μg/knee (b) or vehicle (a) for 3 months. Bar, 0.2 mm.

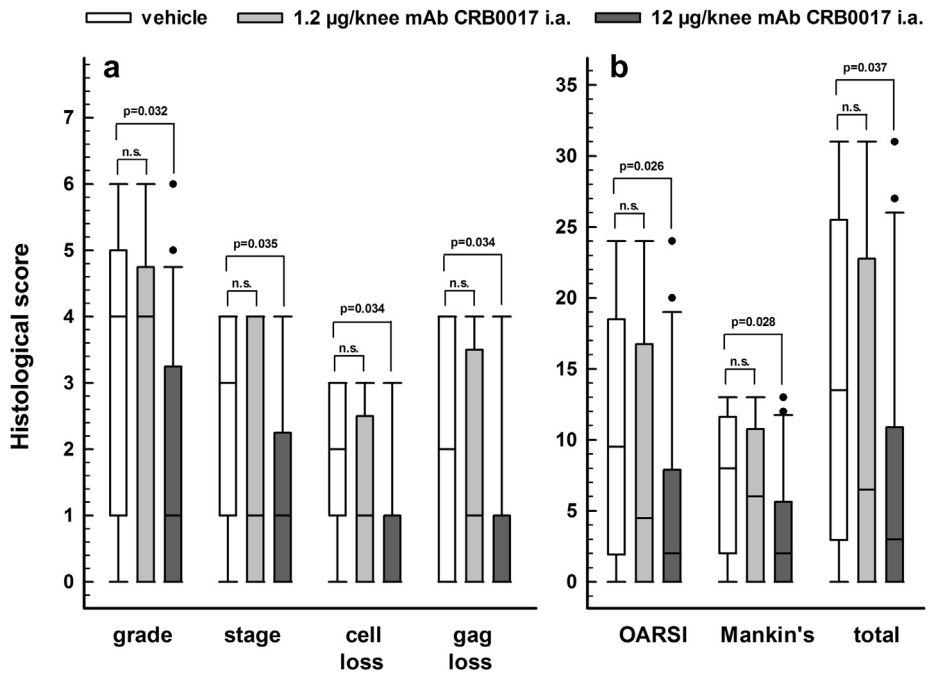

Fig. 2. Histopathological OA scoring of tibial medial plateaus from 8-mo STR/ort mice treated with CRB0017 12 µg/knee ($n = 20$, n. of knees = 34) or CRB0017 1.2 µg/knee ($n = 21$, n. of knees = 37) or vehicle ($n = 20$, n. of knees = 34) for 3 months, according to both the OARSI and Mankin's methods. Grade: scale 0–6. Stage: scale 0–4. Cell loss: scale 0–3. GAG loss: scale 0–4. Total score represents the OARSI score plus cell loss plus GAG loss scores. Statistics were performed by Kruskal–Wallis ANOVA on ranks followed by Dunn's test comparing all treatment groups vs vehicle. Box plots represent median and 5% and 95% confidence limits; bars are the most distant values from the median that are not outliers, while dots are the outliers.

means of a biological agent. Therefore, we undertook extensive selective screening of recombinant scFv libraries to isolate specific antibodies directed against single domains of ADAMTS5. The best candidate for therapeutic development was mAb CRB0017, which demonstrated excellent affinity (K_D in low nanomolar range), and high selectivity for its antigen[7]. A previous study has highlighted the possibility of targeting ADAMTS5's exosites in its ancillary domain as a possible therapeutic strategy to reduce its activity[15]. In order to obtain a proof of concept for this approach, we studied the effects of CRB0017 in our animal model of choice for OA, the STR/ort mouse. We selected intra-articular administration in view of its compliance in osteoarthritic patients for a treatment that is assumed to be once in several weeks, and arguably should reduce the risk of side effects in virtue of the low amount of compound used.

As a possible limitation of the STR/ort model it is to be acknowledged that in mice at 5 months of age (i.e., at the beginning of the follow-up) it is not possible to have a baseline observation nor to select a signal joint (i.e., the worst joint) that could be followed during the pathology progression, as usually done in human studies. Nevertheless, as above detailed, we demonstrated the absence of any statistically significant correlation between the pathology scores evaluated in right and left knees in control animals treated with vehicle or with an irrelevant human IgG4. On these bases, we believe that in these specific experimental conditions considering each joint as independent does not bias the validity of the results. Therefore, both knees were injected, and scored independently for each animal.

Another possible limitation of the study is that it is not possible to determine how much of the activity observed can be due to the local activity of CRB0017 injected or to a systemic activity of the mAb that possibly leaks from the synovial space, since no quantitative bioanalytical methods were applied to detect the circulating CRB0017. Although, due to the high local concentration of mAb, the former hypothesis seems more probable, this issue will be addressed in further studies in which the effect of a systemic administration will be compared to that of an intra-articular injection with the same dose level, in addition assessing the mAb circulating levels with a properly validated method.

In conclusion, mAb CRB0017 administered twice in 3 months indeed modified the course of OA in the STR/ort mouse, by delaying cartilage breakdown as assessed histologically. The procedure of blind scoring of the histological samples clearly showed a dose dependent effect of the antibody in reducing the severity of the osteoarthritic pathology in the STR/ort mice. Taken together, these data show that knee intra-articular administration of mAb CRB0017, an anti-ADAMTS5 monoclonal antibody targeting an ancillary domain, dose-dependently improved disease progression in a relevant animal model of OA. To the best of our knowledge, this is the first report on the efficacy of a recombinant mAb against ADAMTS5 in OA disease modification; we believe that the data here presented suggest a possible novel therapeutic approach for OA.

Contributions

RC, MaV, CG, CC, SC, and MV contributed in the process of data acquisition, in drafting the article, and in its final approval. RC, LM, SC, ML, GU, GC and MV contributed in the analysis and interpretation of data, in the critical revision of the article, and in its final approval. RC, ML, GC, LCR and MV contributed in the conception and design of the study, in the critical revision of the article, and in the final approval of the version to be submitted. GC, LCR and MV, contributed also in the obtaining of funding.

RC (riccardo.chiusaroli@rottapharm.com), ML (marco.lanza@rottapharm.com), GC (gianfranco.caselli@rottapharm.com), LCR (lucio.rovati@rottapharm.com), MV (michela.visintin@rottapharm.com) declare to take responsibility for the integrity of the work as a whole, from the inception to finished article.

Role of the funding source
Funding was supported by Regione FVG L.R. 30/84 capo VIII (decreto 1903/2005 N.1276), by Regione FVG POR FESR 2007-2013 (decreto 1382/2011 N. 451/FESR) and by the Rottapharm group. However, the Rottapharm group as a corporate entity had no role in the conduct of the study; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest
SC declares that she has no competing interests. All the other authors are scientists from the research unit of the Rottapharm group.

Acknowledgments

The authors wish to thank Dr Giampaolo Giacovelli for statistical advice, and Mrs Adriana Grotti and Mrs Rosanna Cavagnoli for their technical support. The authors would also like to acknowledge Dr Louis Boon and Dr Marcel den Hartog at Bioceros BV for contributions to this work.

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