Clinical Trial of Doxycycline for Matrix Metalloproteinase-9 Inhibition in Patients With an Abdominal Aneurysm

Doxycycline Selectively Depletes Aortic Wall Neutrophils and Cytotoxic T Cells

Jan H.N. Lindeman, MD, PhD; Hazem Abdul-Hussien, MD; J. Hajo van Bockel, MD, PhD; Ron Wolterbeek, MD; Robert Kleemann, PhD

Background—Doxycycline has been shown to effectively inhibit aneurysm formation in animal models of abdominal aortic aneurysm. Although this effect is ascribed to matrix metalloproteinase-9 inhibition, such an effect is unclear in human studies. We reevaluated the effect of doxycycline on aortic wall protease content in a clinical trial and found that doxycycline selectively reduces neutrophil-derived proteases. We thus hypothesized that doxycycline acts through an effect on vascular inflammation.

Methods and Results—Sixty patients scheduled for elective open aneurysmal repair were randomly assigned to 2 weeks of low-, medium-, or high-dose doxycycline (50, 100, or 300 mg/d, respectively) or no medication (control group). Aortic wall samples were collected at the time of operation, and the effect of doxycycline treatment on vascular inflammation was evaluated. Independently of its dose, doxycycline treatment resulted in a profound but selective suppression of aortic wall inflammation as reflected by a selective 72% reduction of the aortic wall neutrophils and a 95% reduction of the aortic wall cytotoxic T-cell content (median values; \( P < 0.00003 \)). Evaluation of major inflammatory pathways suggested that doxycycline treatment specifically quenched AP-1 and C/EBP proinflammatory transcription pathways (\( P < 0.0158 \), NS) and reduced vascular interleukin-6 (\( P < 0.00115 \)), interleukin-8 (\( P < 0.00246 \), NS), interleukin-13 (\( P < 0.0184 \), NS), and granulocyte colony-stimulating factor (\( P < 0.031 \), NS) protein levels. Doxycycline was well tolerated; there were no adverse effects.

Conclusions—A brief period of doxycycline treatment has a profound but selective effect on vascular inflammation and reduces aortic wall neutrophil and cytotoxic T-cell content. Results of this study are relevant for pharmaceutical stabilization of the abdominal aneurysm and possibly for other inflammatory conditions that involve neutrophils and/or cytotoxic T cells. (Circulation. 2009;119:2209-2216.)

Key Words: aneurysm ■ immunology ■ inflammation ■ molecular biology ■ trials

Pharmaceutical strategies inhibiting aneurysm growth, thereby reducing the need for surgical repair, could have major advantages for patients and socioeconomically.1,2 An abundance of matrix metalloproteinase-9 (MMP9) in growing abdominal aortic aneurysms (AAAs),3 along with the observation that disruption of MMP9 gene prevents AAA formation,4 led to the notion that MMP9 is critically involved in AAA formation.5 It was thus proposed that pharmaceutical inhibition of MMP9 could restore the balance between matrix degradation and deposition,5 thereby reducing aneurysmal growth.6

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Independently of its antibiotic properties, the tetracycline analogue doxycycline has been shown to reduce both MMP9 expression and activity,7 suggesting that doxycycline treatment may reduce aneurysmal growth. Indeed, doxycycline has been convincingly shown to prevent AAA formation in a variety of animal models,6,8–10 and the results from 2 small clinical studies suggest that doxycycline also reduces AAA expansion in humans.11,12 Remarkably, although the rationale behind doxycycline therapy is based on its putative effects on MMP9 expression and activity,8 the effects of doxycycline on MMPs in the human aneurysm are unclear, with published studies13,14 suggesting that doxycycline acts through a different mechanism.7

In vitro studies characterize doxycycline as a pleiotropic antiinflammatory and immunomodulatory agent.7 However, the relevance of this well-tolerated compound15 for modulat-
ing inflammation in human disease in general and AAA in particular is unclear. In this prospective clinical trial, we systematically examined the effect of 3 pharmacologically relevant doses (low [50 mg/d], regular [100 mg/d], or high [300 mg/d]) of doxycycline on MMP9 expression and inflammatory processes in the aneurysmal wall of patients scheduled for elective open aneurysm repair. Evaluation of the effect of doxycycline treatment showed that MMP9 was reduced on the protein but not on the mRNA level. Because aortic neutrophils carry large amounts of preformed MMP9 protein and lack de novo protein synthesis,16,17 we hypothesized that the observed reduction of MMP9 protein may relate to a change in the aortic content of neutrophils. The present study shows that a brief period of 2 weeks of doxycycline treatment significantly reduces the aortic content of neutrophils and of cytotoxic T cells, another cell type considered crucial for the process of AAA formation.18–21 Based on these findings on the cellular level, a molecular analysis on the level of relevant cytokines and transcription factors22 was performed next.

Methods

The present randomized, dose-ranging study was performed in patients awaiting open aneurysm repair. The trial included 3 treatment groups and 1 nontreated (control) group. Randomization was performed by block randomization (blocks of 20 patients). Randomization lists were generated by the Leiden University Medical Center Department of Medical Statistics, and randomization was performed by the Leiden University Medical Center pharmacy. The study was performed in an investigator-blinded fashion. Planned open aneurysm repair of an infrarenal aortic aneurysm was the primary inclusion criterion. Decision for open repair was based on anatomic

Specific Immunocapture MMP Activity Assays

Because of the rapid inactivation of active enzymes by endogenous inhibitors released after cell destruction by homogenization, direct measurement of active MMPs in aortic tissue homogenates is not feasible.24 Therefore, MMP activities were measured only after in vitro activation (p-aminophenylmercuric acetate) of captured latent proenzymes. Activity was determined in established MMP8 and MMP9 immunocapture protease activity assays (Amersham Biosciences, Buckinghamshire, UK).

Western Blot Analysis

Western blot analyses was performed essentially as described previously22 with antibodies specific for the human forms of perforin (sc-7417; Santa Cruz Biotechnology, Heerhugowaard, the Netherlands), granzyme A (M1791; Sanquin, Amsterdam, the Netherlands), p65-nuclear factor (NF)-κB (nonactive conformation; sc-8008), p65-NFκB (active conformation of p65-NFκB; Chemicon, Billerica, Mass; MAB3026), c-Jun (sc-45), phospho(Ser73)-c-Jun (sc-7981), C/EBPα; Chemicon, Billerica, Mass; MAB3026), c-Jun (sc-45), phosphoSer73)c-Jun (sc-7981), C/EBPα (sc-9315), C/EBPβ (sc-150), C/EBPδ (sc-636), and STAT3 (sc-7179) (all Santa Cruz Biotechnology); phospho-STAT3 (Epitomics PS727, Huissen, the Netherlands); and β-actin (sc-1615; Santa Cruz Biotechnology).

Corresponding secondary antibodies were obtained from Santa Cruz Biotechnology (donkey anti-goat) and Pierce (Ettne-Leur, the Netherlands; goat anti-rabbit and goat anti-mouse). Immunoblots were visualized and quantified with the Super Signal West Dura Extended Duration Substrate (Perbio Science, Ettne-Leur, the Netherlands), LabWorks 4.6 software, and a luminescent image workstation (UVP, Cambridge, UK). Protein expression levels were standardized for β-actin, and separate anti–β-actin immunoblots were performed for each sample.
Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Evaluable patients, n</th>
<th>15</th>
<th>13</th>
<th>15</th>
<th>15</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range), y</td>
<td>74.8 (69–84)</td>
<td>72.7 (62–85)</td>
<td>74.1 (50–88)</td>
<td>72.1 (58–87)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean AAA diameter, cm</td>
<td>6.7</td>
<td>6.5</td>
<td>6.3</td>
<td>6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mean time between diagnosis and surgery, mo</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Female sex, n</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Current smoker, n</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Statin use, n</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>NS</td>
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<tr>
<td>Antihypertensives, n</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Antiplatelet therapy, n</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Statistical Analysis

Power calculation for this study was based on data published in Reference 13. In that publication, doxycycline therapy reduced relative MMP9 mRNA expression from 7.2 (SE, 3.1) to 1.3 (SE, 0.5) units. The present study was designed to have a 90% power to detect a 50% reduction of MMP9 mRNA levels at a significance level of 0.05.

All values are expressed as means (SD) for normally distributed data or medians [interquartile range] for nonnormally distributed data (skewed). The level of statistical significance was dictated by Bonferroni-Holm’s correction (33 variables).

One-way ANOVA for normally distributed data or the Kruskal–Wallis test for nonnormally distributed data did not indicate a difference between the 3 doxycycline doses for all variables studied (all P>0.16; detailed data available from the corresponding author on request). To increase the power of the study, all doxycycline-treated individuals were therefore evaluated as a single group.

The sequence of our analyses was as follows. We first analyzed the effect of doxycycline on MMP9 mRNA and protein levels. Because of the selective reduction of MMP9 protein only, we analyzed putative changes in the aortic wall neutrophil content. On observing an effect on neutrophil content, we performed a further evaluation. This analysis showed that doxycycline treatment selectively reduces aortic wall neutrophil and cytotoxic T-cell content. On the basis of this cellular evaluation, a subsequent analysis of relevant upstream- and downstream-acting cytokines and transcription factors was performed.

Differences between the control group and the doxycycline-treated groups were evaluated in 1-way ANOVA with contrasts (for the normally distributed data) or by the Wilcoxon–Mann–Whitney test in the case of nonnormally distributed continuous data. Possible associations between aortic wall IL-6 and IL-8 levels and cellular content were evaluated by Pearson’s correlation. All analyses were performed with SPSS 16.0 (SPSS Inc, Chicago, Ill).

Results

Patients

Three relevant doses of doxycycline (low [50 mg/d], regular [100 mg/d], or high [300 mg/d]) were evaluated. A total of 60 patients were enrolled in the study. For logistical reasons, aneurysm repair had to be postponed in 2 patients; hence, a total of 58 patients could be analyzed. Doxycycline was well tolerated, and there were no withdrawals.

Patient characteristics are shown in Table 1. All 4 groups are comparable with regard to age, sex, statin use, and AAA diameter.

Statistical analysis did not indicate a difference between the 3 doxycycline doses for all variables studied; hence, all 3 doxycycline doses were combined. Probability values are for the combined doxycycline group versus the control group.

Doxycycline and MMP9 Expression

Evaluation of the effect of 2 weeks of doxycycline therapy indicated a trend toward reduced MMP9 proenzyme levels (P=0.00263, NS; Figure 1A) but did not influence MMP9 mRNA expression (P<0.206; Table 2).

Doxycycline and the Inflammatory Responses in AAA

Because of the selective effect of doxycycline treatment on MMP9 protein levels but not on mRNA expression, we next analyzed the aortic wall neutrophil content. Figure 1B and 1C show that doxycycline markedly reduced the aortic wall neutrophil content (76% reduction of the median value; P<0.000025). Further evaluation of other cell types in the aorta also revealed a profound reduction of the aortic wall cytotoxic (CD8+ T-cell content (96%) of the median value; P<0.0000001; Figure 1C and 1D) but not on other cell types (macrophages [CD68+]; T-helper cells [CD4+], B cells [CD20+], and plasma cells [CD138+]; Figure 1E). Figure 1B and 1D illustrate that the effects of the individual doxycycline doses are equivalent.

Tissue distribution of these cells as assessed by immunohistochemistry was not influenced by doxycycline.

The decrease in neutrophil and cytotoxic T-cell count was paralleled by a reduction of granzyme A (P<0.00001; Figure
1F), a specific marker of cytotoxic T-cell activation. The effect on the neutrophil-specific marker neutrophil collagenase (MMP8; \( P < 0.0053 \); Figure 1A) and the cytotoxic T-cell marker perforin (\( P < 0.0158 \)) did not reach statistical significance.

mRNA levels of markers of monocyte/macrophage activation (ie, macrophage elastase [MMP12] and cathepsin K) were not affected by doxycycline therapy. Similarly, except for a small reduction of IgG heavy-chain expression, doxycycline therapy...
Table 2. mRNA Expression

<table>
<thead>
<tr>
<th></th>
<th>Nontreated Control Subjects</th>
<th>Doxycycline-Treated Subjects</th>
<th>P*</th>
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</thead>
<tbody>
<tr>
<td>MMP9</td>
<td>−1.21 (0.44)</td>
<td>−1.40 (0.61)</td>
<td>0.206 (NS)</td>
</tr>
<tr>
<td>MMP12</td>
<td>−2.57 (3.30 to −1.97)</td>
<td>−2.18 (3.28 to −1.55)</td>
<td>0.326 (NS)</td>
</tr>
<tr>
<td>Cathepsin K</td>
<td>−1.74 (0.69)</td>
<td>−1.85 (0.49)</td>
<td>0.486 (NS)</td>
</tr>
<tr>
<td>BLIMP-1</td>
<td>−1.64 (0.59)</td>
<td>−1.81 (0.48)</td>
<td>0.265 (NS)</td>
</tr>
<tr>
<td>MAD-4</td>
<td>−1.11 (0.36)</td>
<td>−1.11 (0.25)</td>
<td>0.890 (NS)</td>
</tr>
<tr>
<td>Ig linker protein</td>
<td>0.34 (0.39)</td>
<td>0.28 (0.48)</td>
<td>0.714 (NS)</td>
</tr>
<tr>
<td>IgG heavy chain</td>
<td>1.73 (0.60)</td>
<td>1.36 (0.48)</td>
<td>0.020 (NS)</td>
</tr>
</tbody>
</table>

NS indicates level of significance not reached after Bonferroni-Holm’s correction. Log transcript level relative to GAPDH (GAPDH=0) of markers of monocyte/macrophage activation (MMP9 and MMP12, cathepsin K) and B-cell/plasma cell activation (BLIMP-1, MAD-4, Ig linker protein, IgG heavy chain). Values are mean (SD) or median (interquartile range) as appropriate.

*Probability value is for the comparison of the combined doxycycline groups versus the nontreated control group.

Effects of Doxycycline on Cytokine and Chemokine Expression

Doxycycline treatment resulted in a selective suppression of aneurysmal wall cytokine and chemokine protein levels (Table 3). IL-6 hyperexpression, a characteristic feature of AAA,22 was reduced from median values of 462 to 148 pg/g protein (IL-6; P<0.00115). The reduction of IL-8 levels (median values in the control group and the treated group, 165 and 70 pg/g protein, respectively) did not reach statistical significance (P<0.00246). The strong positive relationship between neutrotactic chemokine IL-8 and aortic wall neutrophil content in the control group (r=0.84, P<0.00016) was not found in the doxycycline-treated group (r=−0.037, P<0.819).

Consistent with the absence of an effect on monocyte/macrophage activation markers, doxycycline therapy did not influence the levels of inflammatory cytokines and chemokines that are associated predominantly with monocytes/macrophages, ie, IL-1β (P<0.352), tumor necrosis factor-α (0.579), MCP-1 (CCL-2; P<0.329), and macrophage inflammatory protein-1β (CCL4; P<0.166; Table 3).

Effects of Doxycycline on Mechanistically Relevant Inflammatory Transcription Factors

Doxycycline reduced the aortic wall protein concentrations of cytokines/chemokines that are regulated by AP-1 (IL-6 and IL-8) and/or C/EBP (IL-8, G-CSF) but did not affect the level of cytokines/chemokines that are controlled predominantly by NFκB (eg, IL-1β and MCP-1). These observations suggest that doxycycline therapy specifically quenches the inflammatory transcription factors AP-1 and/or C/EBP. Evaluation of AP-1, C/EBP, and NFκB protein expression levels and their activation status (active phosphorylated c-Jun for AP-1, active p65-NFκB protein confirmation for NFκB; Figure 2A and 2B) provided indications for an effect on the protein levels of all 3 C/EBP isomers (a/β/8; all P<0.00117; Figure 2C) and possibly on the activation of the AP-1 system (phosphory-
MMP9 expression in AAA,3,5,6 along with resistance of and progressive weakening of the aortic wall. Prominent the disease is responsible for the increased matrix turnover.

Indeed, doxycycline has shown to forestall AAA formation and growth in various animal models of the disease.6,8–10 Results from 2 preliminary human studies suggest that these experimental findings may also apply to human disease.11,12 Remarkably, although the rationale behind doxycycline therapy is based on its putative effects on MMP9 expression and activity,2,6 the effects are discussed controversially in published human studies.13,14 In the first published study, Curci et al.13 evaluated the effects of preoperative doxycycline treatment (100 mg orally twice a day for 7 days) in 8 patients with an AAA and compared the results with 7 nontreated controls. They showed that doxycycline treatment resulted in a 2.5-fold reduction of MMP9 protein levels (immunoblot) and an 82% reduction of MMP9 mRNA expression (determined by a semiquantitative method, competitive real-time polymerase chain reaction). In a later placebo-controlled study, Ding et al.14 tested the effect of 1 month of doxycycline treatment (100 mg once a day) or placebo on MMP mRNA expression (real-time polymerase chain reaction) and protein levels (both ELISA and immunocapture assays) in 51 patients scheduled for open repair. Unlike the initial study by Curci et al.13 no effect was found on MMP mRNA or protein expression.

Our findings suggest an effect of doxycycline therapy on MMP9 protein levels (P<0.00263), as reported by Curci et al.13 Yet, in line with Ding et al.14 we did not find an effect of doxycycline on MMP9 mRNA expression levels (P=0.206). The reducing effect of doxycycline on MMP9 protein but not on mRNA may be explained by reduced aneurysmal wall neutrophil content. Neutrophil MMP9 is produced (transcribed and translated) during neutrophil maturation in bone marrow, and the protein is subsequently stored in the secondary granules. MMP9 mRNA expression in circulating or infiltrated neutrophils is negligible.15,16 The absence of an effect of doxycycline on aortic wall MMP9 mRNA expression suggests that treatment does not affect MMP9 expression in other cell types (eg, macrophages). Together, these observations may suggest that the reduction of MMP9 protein can be ascribed mainly to the disappearance of aortic wall neutrophils and that the effects of doxycycline therapy relate to an antiinflammatory effect.

This study shows that the immune modulatory effects of doxycycline in AAA are profound and potentially therapeutically relevant and that these effects are highly selective; a brief period of 2 weeks of doxycycline treatment suffices to strongly reduce aortic wall neutrophil and cytotoxic T-cell content. These cellular effects were paralleled by reduced levels of cytotoxic T-cell–specific marker granzyme A. The effects on perforin (P<0.0158), another cytotoxic T-cell activation marker, and the neutrophil markers neutrophil collagenase (MMP8) and neutrophil gelatinase (MMP9) (P=0.0053 and P=0.00263, respectively) did

Discussion

In this clinical trial, we demonstrate that doxycycline treatment has strong, highly selective effects on vascular inflammation in the AAA. The lowest doxycycline dose tested (50 mg/d) led to a profound reduction of aneurysmal wall neutrophil and cytotoxic T-cell content, 2 cell types considered crucial for the process of aneurysm formation.18–21

AAA is a common pathology and a major cause of death resulting from rupture. The hallmark pathology of AAA is a localized, chronic inflammatory response accompanied by a proteolytic imbalance.5,22,23 Increased proteolytic activity in the disease is responsible for the increased matrix turnover and progressive weakening of the aortic wall. Prominent MMP9 expression in AAA,3,5,6 along with resistance of MMP9 knockout mice to aneurysm formation,4 led to the concept of MMP9 inhibition as a means of reducing AAA formation and expansion.2 Independently of their antimicrobial properties, members of the tetracycline family have been shown to inhibit MMP9 expression7 and activity25 and to effectively prevent tissue injury in animal models of gingivitis, arthritis, and other inflammatory disorders.7 On the basis of these and similar studies, it was proposed that the tetracycline analogue doxycycline may inhibit AAA progression.2

Figure 3. The effect of doxycycline treatment on basal STAT-3 levels and STAT-3 phosphorylation, the primary downstream effector pathway of IL-6 signaling. Doxycycline does not influence basal STAT-3 levels (P=0.3428), but a trend toward reduced STAT-3 phosphorylation (p-STAT-3; P<0.0268) suggests reduced IL-6 signaling. □ indicates nontreated control subjects, ■, doxycycline-treated subjects.

To test whether the doxycycline-mediated suppression of IL-6 levels is sufficient to impair the IL-6 signaling pathway, we evaluated the levels of STAT-3, the primary downstream transcriptional effector of the IL-6 signaling pathway. Basal STAT-3 expression levels were comparable in doxycycline-treated and nontreated individuals (P<0.3428, NS), but a trend (P<0.0268, NS) toward reduced STAT-3 phosphorylation (ie, the activated form of this transcription factor) suggests diminished IL-6 signaling on doxycycline therapy (Figure 3).
confounding factors that may affect bioavailability. For example, we specifically instructed patients to avoid simultaneous ingestion of doxycycline and cation-containing preparations and excluded patients with kidney dysfunction.

The absence of a dose-response relationship in our study presumably indicates that the antiinflammatory effects in AAA are already maximal at the lowest dose of doxycycline used in this study (50 mg). Consistent with this, subantimicrobial doses of doxycycline (20 mg BID) are approved by the Food and Drug Administration for inhibition of excess MMP8 and MMP9 activity in periodontitis,26 showing that low-dose systemic doxycycline treatment has clinical effects. For logistical reasons, the treatment period in this study was relatively short (2 weeks), yet observations from other chronic inflammatory conditions involving neutrophils such as periodontal disease, acne, and rosacea7 suggest that the effects of doxycycline persist during prolonged treatment.

A molecular explanation for the reduction of the aortic wall neutrophil content after doxycycline treatment may be the reduction of neutrophil chemoattractants such as IL-827 in the aneurysm wall. This notion is supported by the robust correlation between aortic wall IL-8 levels and aortic wall neutrophil content that is present in the nontreated controls and that is lost after doxycycline treatment. The possible reduction of G-CSF levels (P<0.00308, NS) may contribute to this effect because G-CSF reportedly stimulates survival, differentiation, and function of neutrophil precursors and mature neutrophils.28,29 Available preclinical studies suggest that doxycycline may also dose dependently interfere with neutrophil migration.30 Yet, the relevance of this effect has been questioned because of the results of an earlier clinical study.31

The selective reduction of CD8+ T cells, along with their activation markers perforin and granzyme A, after doxycycline treatment has not been reported previously. Our data demonstrate that the observed reduction of CD8+ T cells is selective because there was no effect on CD4+ T cells. To the best of our knowledge, T-cell trafficking and homing signals concurred. In the absence of an effect of doxycycline treatment on CD4+ T cells, we hypothesize that the reduction presumably reflects a direct effect on CD8+ T cells. There is evidence for diverging roles of specific signaling pathways in CD8+ T-cell and CD4+ T-cell biology. For example, specific roles for the AP-132 and IL-6/STAT333 signaling pathways have been reported in the context of cytotoxic T-cell proliferation (AP-1) and survival (IL-6/STAT3). In the absence of known direct toxic effect of doxycycline on cytotoxic T cells,17 we speculate that the selective reduction of cytotoxic T cells on doxycycline treatment may be mechanistically linked to the reduced AP-1 and/or STAT-3 activation after doxycycline therapy.

Direct inhibition of the AP-1 pathway has been shown to prevent and even reverse aneurysm formation in animal models of aneurysmal disease.34 This published report showed not only that AP-1 is critically involved in the inflammatory cascade and the proteolytic imbalance in AAA but also that reduced AP-1 signaling resulted in increased matrix deposition.

We found that doxycycline therapy reduced aortic wall G-CSF levels. G-CSF expression is under primary control of C/EBP transcription factors. All 3 isoforms (α, β, δ) are elevated in AAA,22 and increased aortic C/EBP expression is positively associated with aortic wall inflammation in atherosclerotic occlusive disease.35 Antiinflammatory strategies that reduce C/EBP expression have been described to also reduce atherosclerotic disease.36 Yet, little is known about the pathophysiological role of C/EBP in AAA A molecular explanation for the reduction of the aortic wall neutrophil content after doxycycline treatment may be the reduction of neutrophil chemoattractants such as IL-827 in the aneurysm wall. This notion is supported by the robust correlation between aortic wall IL-8 levels and aortic wall neutrophil content that is present in the nontreated controls and that is lost after doxycycline treatment. The possible reduction of G-CSF levels (P<0.00308, NS) may contribute to this effect because G-CSF reportedly stimulates survival, differentiation, and function of neutrophil precursors and mature neutrophils.28,29 Available preclinical studies suggest that doxycycline may also dose dependently interfere with neutrophil migration.30 Yet, the relevance of this effect has been questioned because of the results of an earlier clinical study.31

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Conclusions

We report that a period of 2 weeks of doxycycline treatment in patients with advanced abdominal aneurysms results in a profound and selective reduction of aortic wall neutrophil and cytotoxic T-cell content. This cellular effect is paralleled by a selective suppression of inflammation on the molecular level, ie, cytokines (IL-6, IL-8) and transcription factors (AP-1, C/EBP, STAT3), which are relevant for neutrophil and cytotoxic T-cell inflammation, as well as by a specific reduction of neutrophil-derived proteases. Our findings show that doxycycline therapy may be a useful strategy to selectively quench specific cellular and molecular aspects of vascular inflammation in AAA. The ability of doxycycline to reduce aneurysmal growth awaits confirmation in a sufficiently powered clinical trial. These data provide a promising avenue for further research evaluating the efficacy of doxycycline in other chronic vascular and nonvascular inflammatory conditions involving neutrophils and/or cytotoxic T cells such as Kawasaki disease37 and chronic obstructive pulmonary disease.38

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Disclosures

None.

References

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