Doxycycline use in patients with lymphangioleiomyomatosis: safety and efficacy in metalloproteinase blockade*

Doxiciclina em pacientes com linfangioleiomiomatose: segurança e eficácia no bloqueio de metaloproteinases

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Abstract

Objective: Lymphangioleiomyomatosis (LAM) is characterized by lung cysts, whose development is associated with matrix metalloproteinase (MMP) hyperactivity, principally that of MMP-2 and MMP-9. Our objective was to compare LAM patients and controls in terms of the levels of these MMPs, as well as to determine the safety and efficacy of treatment with doxycycline, a potent MMP inhibitor. **Methods:** Prospective clinical study involving female LAM patients who received doxycycline (100 mg/day) for six months. Urine and blood samples were collected for the quantification of MMP-2 and MMP-9 before and after the treatment period. Samples from 10 healthy women were also collected. **Results:** Of the 41 LAM patients who started the treatment, 34 completed the protocol. Serum and urinary MMP-9 levels were significantly lower in the controls than in the LAM patients ($p < 0.0001$). Comparing pre- and post-treatment values, we found that the median level of MMP-9 in serum decreased from 919 ng/mL to 871 ng/mL ($p = 0.05$), whereas that of MMP-9 in urine decreased from 11,558 pg/mL to 7,315 pg/mL ($p = 0.10$). After treatment, the median level of MMP-2 in serum was significantly lower ($p = 0.04$) and urinary MMP-2 levels were undetectable. Nausea, diarrhea, and epigastric pain were the most prevalent adverse affects and were often self-limiting. There was only one case in which the patient discontinued the treatment because of side effects. **Conclusions:** We have demonstrated, for the first time, a decrease in serum and urine levels of MMPs in LAM patients treated with doxycycline, which proved to be a safe medication, with mild and well-tolerated side effects.

Keywords: Lymphangioleiomyomatosis; Matrix metalloproteinases; Doxycycline.

Resumo

Objetivo: A linfangioleiomiomatose (LAM) é caracterizada pela presença de cistos pulmonares, cuja formação está associada à hiperreatividade de metaloproteinases de matriz (MMP), principalmente MMP-2 e MMP-9. Objetivamos comparar os níveis dessas MMPs entre pacientes com LAM e controles saudáveis, assim como avaliar, nas pacientes com LAM, a segurança e a eficácia do tratamento com doxiciclina, um potente inibidor de MMPs. **Métodos:** Estudo clínico prospectivo no qual as pacientes com LAM receberam doxiciclina (100 mg/dia) por seis meses, coletando-se amostras de urina e sangue para a dosagem de MMP-2 e MMP-9 antes e ao final do período. Foram ainda obtidas amostras de 10 mulheres saudáveis. **Resultados:** De 41 pacientes com LAM que iniciaram o tratamento, 34 concluíram o protocolo. Os níveis de MMP-9 sérica e urinária foram significativamente inferiores no grupo controle ($p < 0,0001$). Comparando-se os valores antes e após o tratamento, a mediana do nível sérico da MMP-9 reduziu de 919 ng/mL para 871 ng/mL ($p = 0,05$), enquanto a mediana da dosagem urinária de MMP-9 diminui de 11.558 pg/mL para 7.315 pg/mL ($p = 0,10$). A mediana da MMP-2 sérica apresentou um decréscimo significativo após o tratamento ($p = 0,04$). Não foram detectados níveis de MMP-2 urinária. Epigastralgia, náuseas e diarreia foram os efeitos adversos mais prevalentes, e geralmente autolimitados. Apenas 1 paciente interrompeu o tratamento devido a efeitos colaterais. **Conclusões:** Pela primeira vez, conseguiu-se evidenciar em pacientes com LAM a redução dos níveis séricos e urinários de MMPs após o uso de doxiciclina, que se mostrou uma medicação segura, com efeitos colaterais leves e toleráveis.

Descritores: Linfangioleiomiomatose; Metaloproteinases da matriz; Doxiciclina.

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Introduction

Pulmonary lymphangioleiomyomatosis (LAM) is a rare cystic disease that affects women of childbearing age, with an incidence of approximately 2.6 per million population.[1] It can occur sporadically or in association with the autosomal disorder known as tuberous sclerosis.[2]

The most common clinical profile is characterized by dyspnea, pneumothorax, and renal angiomyolipoma; however, involvement of the lymphatic system, in the form of chylothorax or lymphangioma, can also occur.[3-6] Chest CT scans reveal diffusely distributed nodular cysts with thin, well-defined walls.[7] Histology reveals clusters of immature smooth muscle cells, which can be spindle-shaped, located adjacent to the nodules, or epithelioid, running along the peribronchovascular bundle. Those cells are positive for smooth muscle alpha actin antibody, and those of epithelioid type also stain positive for immunoreactivity with human melanoma black 45 monoclonal antibody.[8] Hormone receptors for estrogen and progesterone have also been identified in LAM cells, being initially described by Brentani et al.[9]

Spindle cells express matrix metalloproteinases (MMPs), which are functional extracellular matrix (ECM) components responsible for lung remodeling and lymphangiogenesis and whose activity is regulated by tissue inhibitors of MMP (TIMPs).[10] In electron microscopy studies, degradation of elastic fibers has been observed in areas of smooth muscle cell proliferation in lung biopsies of LAM patients.[11] Immunohistochemical analyses have revealed significant MMP-2 and MMP-9 reactivity in the lung tissue of LAM patients, when compared with normal lung tissue.[12] Matsui et al. found intense MMP-2 activity in the cells of LAM patients, as well as intense membrane type 1 MMP activity, which is responsible for activating MMP-2 proenzyme conversion on the cell membrane surface.[13] In agreement with those findings, Odajima et al. reported that serum and plasma MMP-9 titers, as determined by zymography, were significantly higher in LAM patients than in controls.[14]

There has been growing interest in the relationship between the pathogenesis of cystic lung destruction and MMP activity in LAM, including the possibility that MMPs represent a therapeutic target. One case report described the use of doxycycline, a well-known MMP inhibitor, in a female LAM patient who was on the lung transplant list.[15] After four months of treatment, the patient showed an improvement in FEV_1 and gas exchange, accompanied by a significant reduction in urinary MMP levels. To date, there have been no prospective clinical trials evaluating the efficacy and safety of doxycycline use in MMP blockade in female LAM patients.

The objectives of the present study were to compare LAM patients and healthy females in terms of serum and urinary MMP-2 and MMP-9 levels; to evaluate the efficacy of doxycycline as an MMP inhibitor; and to determine the safety of doxycycline use in the treatment of LAM patients.

Methods

This was a prospective clinical study in which all female LAM patients (clinical and radiological diagnosis or histopathological diagnosis, or both) under follow-up treatment between November of 2006 and July of 2009 at the Outpatient Clinic for Interstitial Diseases of the *Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo* (HC-FMUSP, University of São Paulo School of Medicine *Hospital das Clínicas*), in the city of São Paulo, Brazil, were invited to participate. We also evaluated a control group comprising 10 women who had no respiratory disease, no other medical conditions, and no history of smoking. The study was approved by the HC-FMUSP Research Ethics Committee, and all participants gave written informed consent.

Urine and blood samples were collected from the LAM patients for the quantification of MMP-2 and MMP-9. Samples from were also collected from the controls. Subsequently, the LAM patients received doxycycline at a dose of 100 mg/day for six months, after which urine and blood samples were again collected. All blood and urine samples collected were centrifuged at 1,500 rpm for 10 min and stored at −80°C for subsequent analysis.

Serum and urinary levels of MMP-2 and MMP-9 were measured by ELISA (R&D System, Inc., Minneapolis, MN, USA), in accordance with the manufacturer instructions. We employed 96-well plates (Costar; Corning Inc., Cambridge,

MA, USA), which were sensitized with 100 µL of monoclonal antibody and incubated for 18 h at 4°C. Subsequently, in order to prevent nonspecific bindings, the plates were blocked with 300 µL of 2% BSA and incubated for 2 h at 37°C. After blocking, sample and standards diluted in PBS were added at 100 µL/well. The plates were again incubated for 18 h at 4°C, after which 100 µL of biotinylated conjugated antibody were added. We then added 100 µL of streptavidin-conjugated horseradish peroxidase (1:250) to each well and incubated the plates for another 30 min at 37°C. For color development, we added 100 µL of developer (hydrogen peroxide and tetramethylbenzidine) per well, and the plates were further incubated for 5 to 30 min, depending on the protein, at 37°C. The reaction was stopped by the addition of 50 µL of 30% sulfuric acid to each well, followed by gentle agitation of the plates.

The MMP titers were determined by optical density comparison in an ELISA reader (Power Wave; Bio-Tek Instruments Inc., Winooski, VT, USA) with a 450-nm filter. The detection limit for MMP-2 was 156.2 pg/mL, whereas that for MMP-9 was 15.6 pg/mL.

The statistical analysis was performed with the Statistical Package for the Social Sciences, version 15.0 (SPSS. Inc., Chicago, IL, USA). Parametric variables, defined by the normal curve on the histogram, were compared by Student's t-test and are expressed as means and standard deviations. Nonparametric variables are expressed as medians and interquartile ranges (IQRs). Unpaired nonparametric variables were compared by the Wilcoxon test, whereas paired nonparametric variables were compared by the Mann-Whitney test. The level of statistical significance was set at $p < 0.05$.

Results

All 42 female LAM patients under follow-up at our facility during the study period agreed to participate in the protocol. Of those, only 1 was excluded, because she had undergone lung transplantation. Therefore, a total of 41 patients were started on treatment with doxycycline (100 mg/day). The mean age of the patients was 41 ± 9 years, whereas that of the controls was 40 ± 4 years. Although none of the patients were current smokers, 10 (24%) were former smokers. Of the 41 patients, 27 (66%) had a history of pneumothorax. The diagnosis of LAM was established by histopathology in 37 patients (93%), being based on lung biopsy in 35 and on biopsy of another site in 2. In the remaining 4 patients, the diagnosis was made on the basis of clinical and radiological evidence (renal angiomyolipoma and lung cysts, respectively).

There were 7 patients who did not complete the six months of treatment. The reasons were as follows: worsening of respiratory symptoms, in 1 (who was listed for lung transplantation four weeks after initiation of the protocol); symptoms related to the side effects associated with doxycycline (acute colitis) after one month of treatment, in 1; dropout, in 1; and failure to report for MMP quantification at six months, in 4 (who nevertheless remained on doxycycline and did not experience any adverse effects). A total of 34 patients completed the six months of treatment and underwent quantification of MMP-2 and MMP-9 in blood and urine. The clinical and functional characteristics of this group are shown in Tables 1 and 2, respectively. There was no statistical difference between the study group and the control group in terms of age ($p = 0.29$).

In the control group, the median values of MMP-9 in serum and urine were 89.6 ng/mL (IQR: 80 to 102) and 200 pg/mL (IQR: 89 to 263), respectively. The comparison of these serum and urinary values with those of the LAM patients revealed significant differences for both ($p < 0.0001$), as shown in Figures 1 and 2. Serum and urinary MMP-2 levels were below the detection limit in the controls.

After six months of treatment with doxycycline, there was an overall reduction in the median serum levels of MMP-9 in the LAM patients—from 919 ng/mL (IQR: 742 to 1,268) to 871 ng/mL (IQR: 564 to 1,053; $p = 0.05$)—as shown in Figure 1. In 20 of those patients (59%), the proportional change in the median, in relation to baseline levels, was −34% (IQR: −45% to −15%), whereas, in the remaining 14 patients, there was stabilization or an increase, with a median of 8% (IQR: 3% to 35%).

At baseline, the median level of MMP-9 in urine was 11,558 pg/mL (IQR: 5,551 to 23,563), whereas, after six months of treatment with doxycycline, it was 7,315 pg/mL (IQR: 2,393 to 16,683; $p = 0.10$), as shown in Figure 2. The MMP titers decreased in 21 patients (62%), there

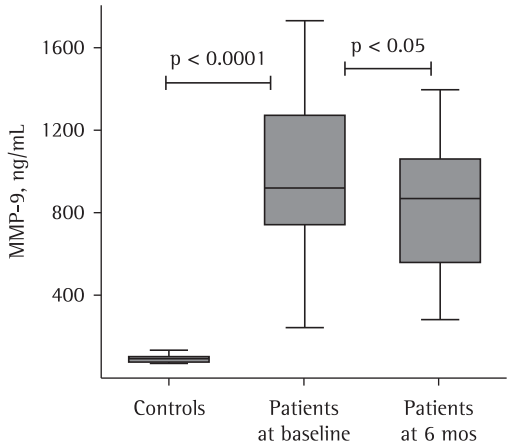

Figure 1 - Serum levels of matrix metalloproteinase 9 (MMP-9) in 10 controls and in 34 patients with lymphangioleiomyomatosis, before and after doxycycline use.

being, in relation to the baseline value, a change of −58% in the median (IQR: −90% to −45%), whereas, in the remaining 13 patients, there was an increase of 65% in the median (IQR: 23% to 79%).

The median titers of MMP-2 in serum were below the detection limit at baseline (75th percentile: 833 pg/mL) and also after treatment with doxycycline (75th percentile: 592 pg/mL; p = 0.04). The MMP titers decreased in 11 patients (32%), there being a change of −12% in the median (IQR: −75% to −12%), whereas, in the remaining 23 patients, there was stabilization

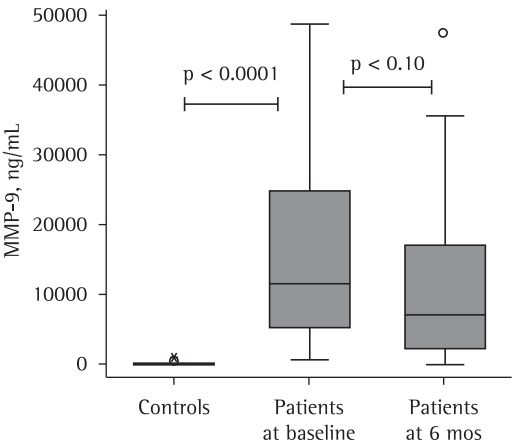

Figure 2 - Urinary levels of matrix metalloproteinase 9 (MMP-9) in 10 controls and in 34 patients with lymphangioleiomyomatosis, before and after doxycycline use.

or an increase, the median and IQR being below the detection limit. All urinary MMP-2 levels were below the detection limit.

During treatment with doxycycline, none of the patients had to discontinue the drug because of side effects, except for one patient who had acute colitis related to doxycycline. Some patients experienced adverse effects, affecting the gastrointestinal tract in most cases. The most prevalent such effect was epigastric pain (in 47% of the cases), which was typically mild and disappeared within two weeks. Proton pump inhibitors were used in half of the patients. We also observed nausea (in 21%) and diarrhea (in 21%), which were mild and self-limiting. Two patients complained of pruritus and photosensitivity reaction. Both symptoms promptly resolved with sunscreen use and avoidance of excessive sun exposure.

Discussion

The present study showed that the serum and urinary levels of MMP-9 were higher in a group of LAM patients than in a group of healthy controls. Urinary MMP-2 titers were undetectable in both groups, and serum MMP-2 titers were undetectable in the control group.

In the literature, MMP hyperactivity has been reported in cystic lung diseases. In patients with Langerhans cell histiocytosis, Hayashi et al. demonstrated, by confocal microscopy, the existence of moderate to intense MMP-2 activity in the areas where there were Langerhans cells, together with injury to the alveolar epithelial basement membrane.[16] It has been reported that, in patients with light chain deposition disease, there is ECM degradation, accompanied by expression of MMP-1, MMP-2, MMP-9, and MMP-14, together with a lack of TIMP-1 and TIMP-2, in the lung parenchyma.[17]

The imbalance between MMPs and their inhibitors has also been implicated in the pathogenesis of other lung diseases, such as emphysema and asthma.[18] Urinary MMP-2 levels have been associated with disease activity and treatment response in a variety of cancers.[19] It has been reported that there is MMP-2 and MMP-9 hyperactivity in the lung tissue of female LAM patients, when compared with that of controls.[12] It has also been shown that serum

Table 1 - Clinical and demographic characteristics of the 34 female patients with lymphangioleiomyomatosis who completed the study.

Characteristic	n = 34
Age, years, mean ± SD	43.0 ± 8.6
Smoking history, n (%)	8 (24)
Tuberous sclerosis, n (%)	5 (15)
Dyspnea, n (%)	29 (85)
Chylous effusion, n (%)	4 (12)
Pneumothorax, n (%)	23 (68)
Angiomyolipoma, n (%)	14 (41)
Diagnosis	
Histopathological, n (%)	31 (91)
Clinical and radiological, n (%)	3 (9)

titers of MMP-9 are higher in LAM patients than in controls,[14] and our study corroborates this finding.

Doxycycline, which belongs to the tetracycline family, is used in clinical practice because of its antimicrobial effect. When administered at low doses, doxycycline acts on ECM remodeling, as well as on cell migration and in vitro cell proliferation.[20] This effect is partly due to the inhibition of MMPs, which belong to a family of enzymes that are regulated by zymogen activation and can degrade ECM substrates, such as gelatin, laminin, and elastin, as well as type I and type IV collagen.[21]

Doxycycline has been used as an MMP inhibitor in experimental models, and MMP-mediated anti-angiogenesis activity has been found in the brains of rats after the use of this medication.[22] Tetracycline derivatives reduce tissue degradation in aortic aneurysms, as well as inhibiting the local invasion of neoplastic cells and their metastasis.[23,24] Chang et al. demonstrated the in vitro effect of doxycycline on LAM cell adhesion, as well as on MMP-2 and MMP-9, revealing a reduction in cell proliferation, although very high doses of doxycycline were required and there was no significant blockade of MMP expression.[25] These findings, however, are questionable, because, to date, there have been no pathophysiological studies involving animal models of the LAM lung. Although the mechanism of action of doxycycline remains unclear, it is speculated that it inhibits MMPs by inducing TIMP activity.[26] However, we did not quantify TIMPs in our protocol.

Table 2 - Pulmonary function test results of the 34 female patients with lymphangioleiomyomatosis who completed the study.[a]

Variable	Result
FVC, L	3.2 ± 0.6
FVC, % of predicted	93 ± 14
FEV_1, L	2.2 ± 0.7
FEV_1, % of predicted	78 ± 25
FEV_1/FVC	0.7 ± 0.2
TLC, L	5.0 ± 0.8
TLC, % of predicted	105 ± 14
RV, L	1.8 ± 0.6
RV, % of predicted	133 ± 49
RV/TLC	0.35 ± 0.08
DLCO, mL/min/mmHg	16.2 ± 6.7
DLCO, % of predicted	62 ± 25

[a]Values expressed as mean ± SD.

We found that, after six months of doxycycline use, there were reductions in the serum and urinary titers of MMP-9, as well as in the serum levels of MMP-2, in our LAM patients. The blockade of serum MMP-9 found in our study was borderline (p = 0.05), suggesting that the use of doxycycline for a longer period could intensify this blockade. The reduction found in the serum levels of MMP-2 was statistically significant. There was a decrease in the urinary titers of MMP-9, this difference also being borderline significant (p = 0.10).

Of the 41 patients recruited for the protocol, 7 (17%) were not reassessed at six months. Of those 7 patients, 4 failed report for the six-month follow-up evaluation because they resided in another state. However, those patients continued to use the medication and did not experience any adverse effects. Doxycycline proved to be safe and well tolerated. The most common side effects were those affecting the gastrointestinal tract. The symptoms were typically self-limiting, and, in patients requiring proton pump inhibitors, there was satisfactory resolution of symptoms. Only 1 patient had to be withdrawn from the protocol, because of acute colitis, which reversed immediately after doxycycline was discontinued.

We have demonstrated, for the first time, that LAM patients have high MMP levels in blood and urine, as well as that low-dose daily

use of doxycycline for six months reduces those levels, there being a significant reduction in serum levels of MMP-2, together with a borderline reduction in serum and urinary levels of MMP-9. Doxycycline proved to be a safe medication, with mild, reversible side effects that were well-tolerated.

To date, there is no curative treatment for LAM. Although several studies in the last two decades have contributed information critical to the understanding of the pathogenesis of this disease, there is a lack of prospective randomized studies of LAM treatments. A recent randomized, double-blind clinical trial of rapamycin, an inhibitor of the mammalian target of rapamycin, demonstrated stabilization of pulmonary function and improvement in quality of life in LAM patients.[27] The establishment of national and international registries is also key to enabling access to patients for participation in studies.[28] The results obtained in our study support the development of a prospective randomized protocol to assess the functional effects of doxycycline and survival in LAM patients treated with this drug.

References

1. Taveira-DaSilva AM, Steagall WK, Moss J. Lymphangioleiomyomatosis. Cancer Control. 2006;13(4):276-85.
2. Costello LC, Hartman TE, Ryu JH. High frequency of pulmonary lymphangioleiomyomatosis in women with tuberous sclerosis complex. Mayo Clin Proc. 2000;75(6):591-4.
3. Kitaichi M, Nishimura K, Itoh H, Izumi T. Pulmonary lymphangioleiomyomatosis: a report of 46 patients including a clinicopathologic study of prognostic factors. Am J Respir Crit Care Med. 1995;151(2 Pt 1):527-33.
4. Chu SC, Horiba K, Usuki J, Avila NA, Chen CC, Travis WD, et al. Comprehensive evaluation of 35 patients with lymphangioleiomyomatosis. Chest. 1999;115(4):1041-52.
5. Johnson SR, Tattersfield AE. Clinical experience of lymphangioleiomyomatosis in the UK. Thorax. 2000;55(12):1052-7.
6. Ryu JH, Moss J, Beck GJ, Lee JC, Brown KK, Chapman JT, et al. The NHLBI lymphangioleiomyomatosis registry: characteristics of 230 patients at enrollment. Am J Respir Crit Care Med. 2006;173(1):105-11.
7. Aberle DR, Hansell DM, Brown K, Tashkin DP. Lymphangiomyomatosis: CT, chest radiographic, and functional correlations. Radiology. 1990;176(2):381-7.
8. Ferrans VJ, Yu ZX, Nelson WK, Valencia JC, Tatsuguchi A, Avila NA, et al. Lymphangioleiomyomatosis (LAM): a review of clinical and morphological features. J Nippon Med Sch. 2000;67(5):311-29.
9. Brentani MM, Carvalho CR, Saldiva PH, Pacheco MM, Oshima CT. Steroid receptors in pulmonary lymphangiomyomatosis. Chest. 1984;85(1):96-9.
10. Ji RC. Lymphatic endothelial cells, lymphangiogenesis, and extracellular matrix. Lymphat Res Biol. 2006;4(2):83-100.
11. Fukuda Y, Kawamoto M, Yamamoto A, Ishizaki M, Basset F, Masugi Y. Role of elastic fiber degradation in emphysema-like lesions of pulmonary lymphangiomyomatosis. Hum Pathol. 1990;21(12):1252-61.
12. Hayashi T, Fleming MV, Stetler-Stevenson WG, Liotta LA, Moss J, Ferrans VJ, et al. Immunohistochemical study of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in pulmonary lymphangioleiomyomatosis (LAM). Hum Pathol. 1997;28(9):1071-8.
13. Matsui K, Takeda K, Yu ZX, Travis WD, Moss J, Ferrans VJ. Role for activation of matrix metalloproteinases in the pathogenesis of pulmonary lymphangioleiomyomatosis. Arch Pathol Lab Med. 2000;124(2):267-75.
14. Odajima N, Betsuyaku T, Nasuhara Y, Inoue H, Seyama K, Nishimura M. Matrix metalloproteinases in blood from patients with LAM. Respir Med. 2009;103(1):124-9.
15. Moses MA, Harper J, Folkman J. Doxycycline treatment for lymphangioleiomyomatosis with urinary monitoring for MMPs. N Engl J Med. 2006;354(24):2621-2.
16. Hayashi T, Rush WL, Travis WD, Liotta LA, Stetler-Stevenson WG, Ferrans VJ. Immunohistochemical study of matrix metalloproteinases and their tissue inhibitors in pulmonary Langerhans' cell granulomatosis. Arch Pathol Lab Med. 1997;121(9):930-7.
17. Colombat M, Caudroy S, Lagonotte E, Mal H, Danel C, Stern M, et al. Pathomechanisms of cyst formation in pulmonary light chain deposition disease. Eur Respir J. 2008;32(5):1399-403.
18. Vignola AM, Paganin F, Capieu L, Scichilone N, Bellia M, Maakel L, et al. Airway remodelling assessed by sputum and high-resolution computed tomography in asthma and COPD. Eur Respir J. 2004;24(6):910-7.
19. Moses MA, Wiederschain D, Loughlin KR, Zurakowski D, Lamb CC, Freeman MR. Increased incidence of matrix metalloproteinases in urine of cancer patients. Cancer Res. 1998;58(7):1395-9.
20. Nelson ML. Chemical and biological dynamics of tetracyclines. Adv Dent Res. 1998;12(2):5-11.
21. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92(8):827-39.
22. Lee CZ, Xu B, Hashimoto T, McCulloch CE, Yang GY, Young WL. Doxycycline suppresses cerebral matrix metalloproteinase-9 and angiogenesis induced by focal hyperstimulation of vascular endothelial growth factor in a mouse model. Stroke. 2004;35(7):1715-9.
23. Fife RS, Sledge GW Jr. Effects of doxycycline on in vitro growth, migration, and gelatinase activity of breast carcinoma cells. J Lab Clin Med. 1995;125(3):407-11.
24. Tamargo RJ, Bok RA, Brem H. Angiogenesis inhibition by minocycline. Cancer Res. 1991;51(2):672-5.
25. Chang WY, Clements D, Johnson SR. Effect of doxycycline on proliferation, MMP production, and adhesion in LAM-related cells. Am J Physiol Lung Cell Mol Physiol. 2010;299(3):L393-400.
26. Yao JS, Shen F, Young WL, Yang GY. Comparison of doxycycline and minocycline in the inhibition of VEGF-

induced smooth muscle cell migration. Neurochem Int. 2007;50(3):524-30.
27. McCormack FX, Inoue Y, Moss J, Singer LG, Strange C, Nakata K, et al. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. N Engl J Med. 2011;364(17):1595-606.
28. Nurok M, Eslick I, Carvalho CR, Costabel U, D'Armiento J, Glanville AR, et al. The International LAM Registry: a component of an innovative web-based clinician, researcher, and patient-driven rare disease research platform. Lymphat Res Biol. 2010;8(1):81-7.

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