ARTICLE IN PRESS
+ MODEL
Journal of Dental Sciences xxx (xxxx) xxx

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.e-jds.com

Original Article

Prevention of medication-related osteonecrosis of the jaw after tooth extraction by local administration of antibiotics and atelocollagen sponge: A preliminary study

Natsumi Nakamura [a], Sakiko Soutome [a*], Akira Imakiire [a], Satoshi Rokutanda [b], Seigo Ohba [c], Shunsuke Sawada [d], Yuka Kojima [d], Yuki Sakamoto [e], Yoshiko Yamamura [f], Madoka Funahara [g], Mitsunobu Otsuru [b], Masahiro Umeda [b]

[a] *Department of Oral Health, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[b] *Department of Clinical Oral Oncology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[c] *Department of Regenerative Oral Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[d] *Department of Dentistry and Oral Surgery, Kansai Medical University, Osaka, Japan*
[e] *Department of Oral Surgery, Kansai Medical University Medical Center, Osaka, Japan*
[f] *Department of Oral Surgery, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan*
[g] *School of Oral Health Sciences, Kyushu Dental University, Fukuoka, Japan*

Received 29 January 2024; Final revision received 9 April 2024
Available online ■ ■ ■

KEYWORDS
Medication-related osteonecrosis of the jaw;
Tooth extraction;
Atelocollagen;
Minocycline

Abstract *Background/purpose:* Medication-related osteonecrosis of the jaw (MRONJ) often develops after extraction of a tooth with a local infection. Therefore, it is necessary to develop extraction methods that can prevent MRONJ. Before examining whether antibiotics and atelocollagen administered in the extraction socket can prevent the development of MRONJ after tooth extraction, this study was conducted to determine the appropriate antibiotics concentration and to conduct a clinical study with a small number of cases as a preliminary study.

* Corresponding author. Department of Oral Health, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki-City, Nagasaki-Prefecture, 852-8588. Japan.
E-mail address: sakiko@nagasaki-u.ac.jp (S. Soutome).

https://doi.org/10.1016/j.jds.2024.04.006

Materials and methods: First, a mixture of minocycline and atelocollagen at different concentrations was implanted into the bone cavity formed in the rabbit head, and the local minocycline concentration was measured after 24 and 48 h. Next, the incidences of MRONJ after tooth extraction in patients receiving high-dose antiresorptive agents were compared between the atelocollagen and atelocollagen/minocycline mixture groups. A group that did not undergo transplantation was also compared as a historical control.
Results: In animal studies, a mixture of 10 mg/ml minocycline injected into collagen and implanted into the bone cavity showed sufficiently high antimicrobial concentrations, even after 48 h. Post-extraction MRONJ occurred in 3 of the 13 control groups (23.1%), 3 of the 13 atelocollagen groups (23.1%), and 1 of the 13 atelocollagen/minocycline groups (7.7%).
Conclusion: Atelocollagen functions as a carrier for retaining antibiotics for a certain period. Although this was a study with a limited number of cases, it suggested that the local administration of atelocollagen/minocycline in the extraction socket may reduce the risk of MRONJ following tooth extraction.

Introduction

Medication-related osteonecrosis of the jaw (MRONJ), first reported by Marx in 2003, is a refractory osteonecrosis of the jaw caused by antiresorptive drugs (ARAs). Conservative treatment has been recommended as the first-line treatment for MRONJ; however, in recent years, favorable treatment results have been reported with surgical treatment.[1–5] It is experienced that many osteoporotic patients are elderly, and cancer patients with bone metastases are often in poor general condition or have a short-predicted life prognosis, and they do not wish to undergo invasive surgical treatment. Therefore, it is important to establish methods for prevention of MRONJ.

There are many reports that MRONJ is triggered by tooth extraction, so tooth extraction is avoided in patients receiving ARA, especially cancer patients receiving high-dose agent.[6–9] However, teeth that require extraction often already have dental infections, such as severe periodontal disease and apical lesions; therefore, even if tooth extraction is avoided and conservative measures are taken, MRONJ may develop from these dental diseases.[10,11] Soutome et al. reported that MRONJ develops in approximately 20% of high-dose ARA-treated patients after extraction of infected tooth; however, the subsequent incidence of MRONJ was even higher when the tooth was preserved, using propensity score matching analysis.[12]

In a multicenter study by Hasegawa et al., the incidence of MRONJ after tooth extraction in patients receiving high-dose ARA was reported to be as high as 41 out of 163 teeth (25.2%) and 39 out of 136 teeth (28.7%) in patients receiving high-dose denosumab (DMB).[13,14] Some of the authors (SS and MU) participated in these Hasegawa's multicenter clinical studies and experienced a very high incidence of MRONJ after tooth extraction in patients receiving high-dose ARA. Therefore, we felt that it is important to establish extraction methods that prevent the development of MRONJ.

Several extraction methods have been proposed to prevent development of MRONJ,[15–19] though none have a high level of evidence.[20] If local infection is one of the important causes of MRONJ development, it may be prevented by treating the local infection. Although antibiotics are administered systemically at the time of tooth extraction, the concentration of antibiotics in the extraction socket may not be sufficient due to decreased blood flow in the bone in patients receiving ARAs.[21] Therefore, it is desirable to locally administer antimicrobials to the extraction socket. However, there have been no reports of studies that have attempted to prevent the development of MRONJ after tooth extractions by topical administration of antibiotics. Therefore, we attempted to evaluate whether topical administration of antibiotics into the extraction socket reduces the risk of developing MRONJ following tooth extraction. First, it was decided to confirm the minimum antimicrobial concentration required for topical administration by animal experiments. Since animals are generally more metabolically active than humans, we thought that if we could maintain an effective local concentration for more than 48 h in animal experiments, we could apply it to humans. The purpose of this study was to confirm, through animal experiments, whether a sufficient concentration of antibiotics could be maintained for 48 h by mixing minocycline injection with atelocollagen as a carrier and inserting it into the extraction socket, and to confirm its safety and efficacy in a preliminary study by implanting it following tooth extraction in human patients using the similar method.

Materials and methods

Animal experiment

This study was conducted according to Act on Welfare and Management of Animals.[22] Animal experimentation protocols are based on The ARRIVE guidelines 2.0,[23] and they

were approved by the Animal Welfare Committee of Nagasaki University (#2111051758). Surgery was performed under monitored anesthesia and all precautions were taken to minimize suffering.

Animal studies were first conducted to determine the usefulness of atelocollagen as a carrier for the localized retention of antibiotics. Bovine dermal atelocollagen (Teruplug®; Olympus Terumo Biomaterials Corporation, Tokyo, Japan) was used as atelocollagen. Intravenous minocycline hydrochloride (Minocycline; Nichi-Iko Pharmaceutical Co., Ltd., Toyama-city, Japan) was used as an antibiotic and dissolved in saline at four different concentrations: 10 mg/ml, 1 mg/ml, 0.1 mg/ml, and 0.01 ml/mg. Japanese white rabbits of 11-week-old (Kitayama LaBes Co., Ltd., Ina-city, Japan) weighing 2—3 kg were anesthetized with pentobarbital, and four cavities of 5 mm diameter and 3 mm depth were formed in the frontal bone of five rabbits (Fig. 1) One ml of minocycline at each concentration was injected into the Teruplug M-type, cut into appropriate sizes, implanted in the cavities, and the wounds were closed. Preoperative carprofen 4 mg/kg and enfloxacin 1 mg/kg were administered intramuscularly for analgesia and infection control. After the surgery, the animals were given normal food and water. Two rabbits were euthanized after 24 h and three after 48 h were euthanized with a lethal dose of pentobarbital, sutured wounds were opened, each specimen was removed and frozen at −81 °C, and local antimicrobial concentrations were measured at a later date.

The concentration of antibiotics was determined by mixing the excised samples with 0.2 ml of phosphate-buffered saline (PBS) and using clinical isolates of *Staphylococcus aureus* by the bioassay method, according to common practice (Fig. 2).

Clinical trial

Patients with cancer aged 20 years or older receiving high-dose zoledronic acid (4 mg intravenous infusion every 4 weeks) or denosumab (120 mg subcutaneous injection every 4 weeks) who visited Nagasaki University Hospital, Kansai Medical University Hospital, or Tokushima University Hospital between July 2020 and March 2023, and were scheduled to undergo tooth extraction, was included in this trial following their informed consent after receiving full explanation about this study. Patients with a history of allergy to tetracyclines or animal proteins, or those receiving warfarin potassium, sulfonylurea hypoglycemic agents, methotrexate, porphymer sodium, digoxin, progestin/follicle hormone combinations, oral contraceptives, vitamin A, and retinoids were excluded from the study.

Figure 1 Four bony cavities were made and each of them were implanted with a concentration of minocycline/atelocollagen mixture.

Figure 2 The concentration of antibiotics was measured by the sized of the inhibition circle around each sample using clinical isolates of *Staphylococcus aureus*.

Patients who were enrolled were randomly assigned to two groups, the Col Group and the Col + Mino Group, using a computer software by a data manager at an institution different from that of the investigator. Because tooth extraction procedures may differ slightly at each hospital, we used institution as an allocation factor so that patients could be randomly assigned to two groups at each hospital. Extraction was performed under local anesthesia with 2% xylocaine by oral and maxillofacial surgeons. Drug holiday was not performed before and after tooth extraction. In the Col Group, the above-mentioned atelocollagen was placed and the mucoperiosteal flap was sutured tightly (Fig. 3. The Col + Mino Group was administered intravenous minocycline hydrochloride (Minocycline, Nichi-Iko Pharmaceutical Co., Ltd., Toyama-city, Japan) at 10 mg/ml in saline solution, and 1 ml of the solution was injected into the Teruplug M-type. The Teruplug was cut according to the size of the extraction socket and placed inside, following which the mucoperiosteal flap was sutured tightly. Stitches were removed one week later. Thereafter, the patient was followed up every 1—2 weeks until 8 weeks later. Furthermore, as a historical control, we used a control group in which teeth were extracted from high-dose ARA patients and sutured without filling the extraction socket. Procedures for this group was performed at the Nagasaki University Hospital between April 2015 and April 2017. The development of MRONJ was evaluated by the oral and maxillofacial surgeon who performed the extraction.

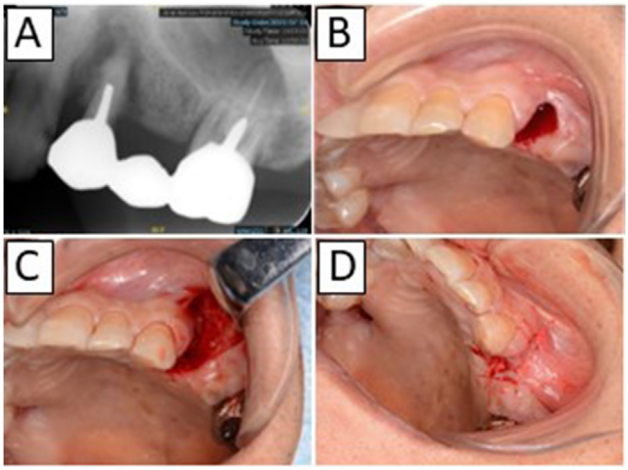

Figure 3 The extraction socket was tightly closed with a mucoperiosteal flap.

MRONJ was defined as absence of epithelialization of the extraction socket and exposure of bone or a fistula reaching the bone with a probe 2 months following tooth extraction. The incidence of MRONJ in the Col, Col + Min, and control groups was determined, and the difference in the incidence of MRONJ in each group was tested using Fisher's exact test. In addition, age, sex, primary disease, smoking history, diabetes, use of corticosteroids or immunosuppressive drugs, type of ARAs, duration of administration of ARAs, drug holiday, white blood cell count, lymphocyte count, serum albumin, serum creatinine, cause of tooth extraction, extraction site, number of teeth, apical lesion, alveolar bone loss of more than half, bone removal during extraction, local infection symptoms (swelling, pain, pus discharge, redness) and extraction method (Col group, Col + Mino group, and control group) were examined as independent variables, and univariate analysis was performed using Fisher's exact test or Student's T-test for their association with MRONJ development. Because of the small number of cases, multivariate analysis was not performed in this study.

All statistical analyses were performed using SPSS software (version 26.0; Japan IBM Co., Ltd., Tokyo, Japan). Two-tailed *P*-values smaller than 0.05 were considered statistically significant.

This study was performed in accordance with the 2013 Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Ministry of Health, Labor, and Welfare of Japan. Ethical approval was obtained by the Clinical Research Review Board of the university (#CRB7180001). This randomized, open-label trial was conducted as a specific clinical study in accordance with the Clinical Research Law enacted in April 2018 in Japan. Written informed consent was obtained from all the participants. The study protocol was registered in the Japan Registry of Clinical Trials (jRCT) on April 20, 2020 (jRCTs071200006). For historical control subjects in clinical research, the research plan was published on the homepages of the participating hospitals according to the instructions of the CRB, in accordance with the guaranteed opt-out opportunity.

Results

Animal experiment

The mean concentrations of minocycline at 24 and 48 h were 123.7 μg/ml and 10.2 μg/ml in the 10 mg/ml transplant cases and 2.35 μg/ml and 0.94 μg/ml in the 1 mg/ml transplant cases, respectively (Fig. 4). Concentrations were lower in the 0.1 mg/ml and 0.01 ml/mg transplanted cases at both 24 and 48 h. Considering that the minimum inhibitory concentration 80 for normal oral isolates to minocycline is less than 1 μg/ml and around 32 μg/ml for methicillin-resistant *S. aureus*,[24] 10 mg/ml injected into collagen was shown to maintain sufficient antimicrobial concentration even after 48 h. Therefore, 10 mg/ml minocycline injected into the atelocollagen was used in subsequent clinical studies.

Clinical trial

The patients included 19 males and 20 females, with a mean age of 64.6 years. Breast cancer was the most common primary disease (21 cases), followed by prostate cancer, lung cancer, and multiple myeloma. Nine patients received bisphosphonate (BP), twenty-seven received DMB, and three received both. In the Col and Col + Mino groups, where patient enrollment was relatively recent, the majority of patients received DMB, whereas in the control group, where patients were enrolled long back, BP and DMB were equally distributed (Table 1).

Maxillary teeth were extracted in 25 patients, and mandibular teeth were extracted in 14 patients. Apical lesions were the most common cause of tooth extraction (22 patients), followed by severe periodontitis, root

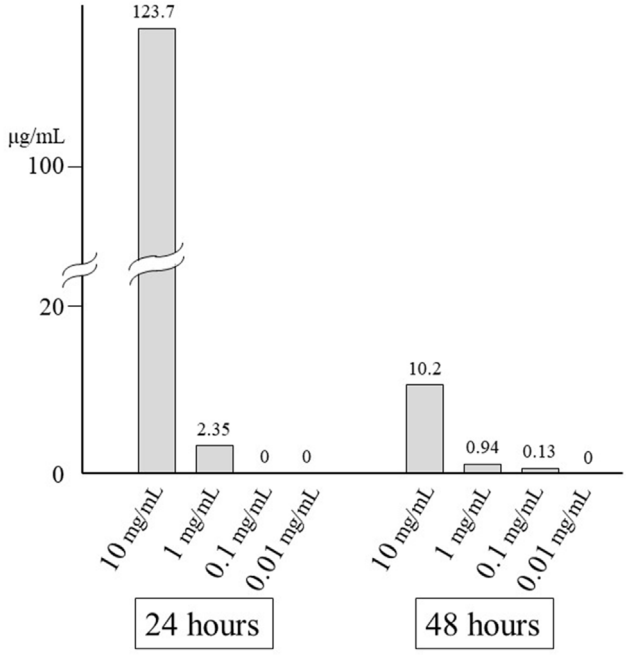

Figure 4 Results of animal experiments. 10 mg/ml mixture implantation showed high antibicrobial concentration up to 48 h.

Table 1 Summary of patients in the clinical research.

Variable		Number of patients/mean ± SD[b]		
		Col group	Col + Mino group	Control group
i) Background factors				
Age (years)	mean ± SD	65.8 ± 14.7	64.7 ± 12.0	63.5 ± 10.8
Sex	male	9	4	6
	female	4	9	7
Primary disease	breast cancer	6	8	7
	prostate cancer	0	2	4
	lung cancer	2	2	0
	multiple myeloma	2	1	0
Smoking habit	(−)	6	12	11
	(+)	7	1	2
Diabetes	(−)	10	12	11
	(+)	3	1	2
Corticosteroid	(−)	10	11	13
	(+)	3	2	0
Immunosuppressant	(−)	13	13	13
	(+)	0	0	0
Sort of ARA[a]	BP[c]	1	2	6
	DMB[d]	11	10	6
	BP → DMB	1	1	1
Duration of ARA adinistration (months)	mean ± SD	34.2 ± 31.5	26.5 ± 28.3	34.4 ± 24.2
Leukocytes (/μL)	mean ± SD	6677 ± 3884	6300 ± 2503	7410 ± 4.31
Lymphcytes (/μL)	mean ± SD	1735 ± 673	1349 ± 844	1562 ± 440
Serum albumin (g/dL)	mean ± SD	4.01 ± 0.429	4.01 ± 0.416	3.83 ± 0.501
Serum creatinie (mg/dL)	mean ± SD	0.925 ± 0.576	1.01 ± 0.877	0.936 ± 0.464
ii) Dental findings				
Site of tooth extraction	upper jaw	9	8	8
	lower jaw	4	5	5
Cause of tooth extraction	apical lesion	5	9	8
	periodontal disease	3	2	1
	root fracture	3	1	1
	stump	2	1	1
	pericoronitis	0	0	2
Number of extracted teeth	mean ± SD	1.4	1.5	2.0
Apical lesion > 3 mm	(−)	10	6	9
	(+)	3	7	4
Alveolar bone loss >1/2	(−)	11	8	8
Local infection symptom	(−)	6	4	7
	(+)	7	9	6
Bone removal during tooth extraction	(−)	7	2	12
	(+)	6	11	1
Root separation during tooth extraction	(−)	13	13	13
	(+)	0	0	0
iii) Development of MRONJ				
Development of MRONJ after tooth extraction	(−)	10	12	10

[a] ARA: antiresorptive agent.
[b] SD: standard deviation.
[c] BP: bisphosphonate.
[d] DMB: denosumab.

fractures, root stumps, and pericoronitis. All extractions were completed uneventfully, and no root fracture or root remnant was observed at the time of extraction. Post-extraction MRONJ occurred in 3 of the 13 control group patients (23.1%), 3 of the 13 Col group patients (23.1%), and 1 of the 13 Col + Mino group patients (7.7%) (Table 1). No specific adverse events occurred during the extraction and after the local administration of atelocollagen or minocycline in the extraction socket.

A univariate analysis of factors associated with the incidence of MRONJ in all 39 cases was performed (Table 2). The incidence was slightly higher in patients with diabetes mellitus, but not significantly $P = 0.059$). MRONJ occurs in patients with inflammatory disease teeth such as apical

Table 2 Factors related to the development of MRONJ after tooth extraction.

Variable		MRONJ (−)	MRONJ (+)	p-value
Age (years)	mean±SD[b]	64.0 ± 13.0	67.6 ± 8.46	0.495
Sex	male	15	4	0.695
	female	17	3	
Primary disease	breast cancer	12	3	1.000
	prostate cancer	8	4	
	others	12	0	
Smoking habit	(−)	25	4	0.344
	(+)	7	3	
Diabetes	(−)	29	4	0.059
	(+)	3	3	
Corticosteroid	(−)	29	5	0.213
	(+)	3	2	
Immunosuppressant	(−)			
	(+)			
Sort of ARA[a]	BP[c]	9	0	0.248
	DMB[d]	21	6	
	BP→DMB	2	1	
Duration of ARA adinistration (months)	mean ± SD	29.4 ± 28.5	38.0 ± 23.8	0.462
Leukocytes (/μL)	mean ± SD	6260 ± 3145	9170 ± 4509	0.063
Lymphcytes (/μL)	mean ± SD	1530 ± 743	1580 ± 386	0.886
Serum albumin (g/dL)	mean ± SD	3.96 ± 0.459	4.10 ± 0.308	0.507
Serum creatinie (mg/dL)	mean ± SD	0.950 ± 0.705	1.00 ± 0.435	0.861
Treatment group	Control group	10	3	0.498
	Col group	10	3	
	Col + Mino group	12	1	
Site of tooth extraction	upper jaw	21	4	0.686
	lower jaw	11	3	
Cause of tooth extraction	apical lesion	18	4	0.025
	periodontal disease	5	1	
	stump	4	0	
	root fracture	5	0	
	pericoronitis	0	2	
Number of extracted teeth	mean ± SD	1.69 ± 1.31	1.43 ± 0.535	0.612
Apical lesion > 3 mm	(−)	21	4	0.686
	(+)	11	3	
Alveolar bone loss >1/2	(−)	22	5	1.000
	(+)	10	2	
Local infection symptom	(−)	17	0	0.012
	(+)	15	7	
Bone removal during tooth extraction	(−)	16	5	0.418
	(+)	16	2	

[a] ARA: antiresorptive agent.
[b] SD: standard deviation.
[c] BP: bisphosphonate.
[d] DMB: denosumab.

lesions, periodontal disease, and pericoronitis while it did not occur from stump or root fracture, but there was no significant association with dental disease. On the other hand, the incidence of MRONJ was significantly higher in patients with local infection symptoms than in those without symptoms ($P = 0.012$).

Discussion

This study showed that atelocollagen functions as a carrier to retain antibiotics for a certain period and that the insertion of atelocollagen into the extraction socket did not interfere with healing. Although this was a study with a small number of cases, it suggested that the local administration of antibiotics in the extraction socket may reduce the risk of MRONJ after tooth extraction.

In Japan, the number of cancer patients with bone metastases is increasing due to the aging of the population and advances in cancer drug therapy.[25,26] Although ARAs have brought great benefits to patients with bone metastases, the occurrence of MRONJ, a side effect, has contributed to a decline in the quality of life of patients. Many reports indicate that MRONJ develops after tooth

extraction.[6–9] However, teeth that require extraction already have local infections, such as periodontal disease or apical lesions, and even if conservative measures are taken without extraction, MRONJ caused by dental infection may still occur. Many clinical and basic studies have reported a causal relationship between bacterial infections and MRONJ development.[27–35]

There have been some reports that tooth with severe periodontal disease, apical lesion, periapical bone sclerosis, or local infection symptoms should be extracted to prevent MRONJ occurrence.[12,36,37] On the other hand, the incidence of post-extraction MRONJ is not low in ARA-treated patients. In a Japanese multicenter study, MRONJ occurred after tooth extraction in 41 of 163 teeth (25.2%) in patients receiving high-dose ARA, and in 39 of 136 teeth (28.7%) in patients receiving high-dose DMB.[14,15] Although it is preferable to remove the source of oral infection before administering ARA, we often experience referrals to dentistry after these drugs have been started because treatment for cancer must take priority. Therefore, it is necessary to establish a new extraction method that does not cause MRONJ.

Some procedures, such as close primary suturing of the extraction wound[16] and placement of plasma rich in growth factors (PRGF) in the extraction socket[16–19] have been reported when performing tooth extractions in ARA-treated patients. However, both are case series with a small number of cases, and the level of evidence is low.[21] The disadvantages are that close primary suturing of the extraction socket requires a gingival periosteal flap and tension reduction incisions, which are more surgically invasive, and that PRGF is not widely used in clinical practice because it does not have antibacterial properties and its preparation is complicated. Because local infection and surgical invasion are important factors in the development of MRONJ after tooth extraction, we believe that extraction methods should be designed with infection control in mind.

Atelocollagen, a biomaterial with excellent biocompatibility, is believed to promote wound healing, and has been clinically applied to various sites, including extraction sockets.[38–40] Minocycline has been widely administered topically to periodontal tissue[41] and open wounds in the oral cavity.[42] We investigated whether a mixture of atelocollagen and minocycline could be placed in the extraction socket to reduce the risk of developing MRONJ after tooth extraction. Minocycline can be administered in the form of a paste or an ointment, but considering the possibility that these base agents may become foreign substances and delay wound healing, we decided to use injectable minocycline in this study. To investigate whether atelocollagen is an effective carrier to hold antibiotics locally, we first conducted the animal experiments. Rabbits have a more active metabolism than humans, and healing of extraction sockets is more rapid. Therefore, if the effective concentration of the antimicrobial agent can be maintained in the extraction socket for 48 h or longer in animal experiments, it is considered that the effective concentration of the antimicrobial agent is also maintained in the extraction socket in humans. These results confirmed that local administration of minocycline together with atelocollagen maintained good drug concentrations after 48 h. Therefore, we conducted this preliminary study with a small number of patients before conducting a large controlled study in a clinical setting. Although statistical analysis was difficult owing to the small number of cases, the present study suggests that minocycline-containing atelocollagen loaded into the extraction socket to achieve antimicrobial action may reduce the incidence of MRONJ, and we plan to conduct an RCT in the future.

This study had several limitations. It is true that the premise of this study is weak in terms of causality because the pathophysiology of MRONJ is still unclear, i.e., it is not yet certain whether inflammation or infection after tooth extraction is the true cause of MRONJ. In addition, since this is a preliminary study and the number of cases is small and there may be bias among the three groups, it is not possible to determine in this study whether insertion of minocycline plus atelocollagen in the extraction fossa reduces the incidence of MRONJ. Future large-scale randomized clinical trials are needed to verify these findings.

Declaration of competing interest

The authors declare no conflict of interest. This research received no external funding.

Acknowledgments

The authors declare no funds, grants, or other support were received during the preparation of this manuscript. Atelocollagen was provided free of charge by the Olympus Terumo Biomaterials Corporation.

We would like to thank Editage (www.editage.com) for the English language editing.

References

1. Hayashida S, Soutome S, Yanamoto S, et al. Evaluation of the treatment strategies for medication-related osteonecrosis of the jaws (MRONJ) and the factors affecting treatment outcome: a multicenter retrospective study with propensity score matching analysis. *J Bone Miner Res* 2017;32:2022–9.
2. Graziani F, Vescovi P, Campisi G, et al. Resective surgical approach shows a high performance in the management of advanced cases of bisphosphonate-related osteonecrosis of the jaws: a retrospective survey of 347 cases. *J Oral Maxillofac Surg* 2012;70:2501–7.
3. Ruggiero SL, Kohn N. Disease stage and mode of therapy are important determinants of treatment outcome for medication-related osteonecrosis of the jaw. *J Oral Maxillofac Surg* 2015; 73:S94–100.
4. Kim HY, Lee SJ, Kim SM, et al. Extensive surgical procedures result in better treatment outcomes for bisphosphonate-related osteonecrosis of the jaw in patients with osteoporosis. *J Oral Maxillofac Surg* 2017;75:1404–13.
5. Mücke T, Koschinski J, Deppe H, et al. Outcome of treatment and parameters influencing recurrence in patients with bisphosphonate-related osteonecrosis of the jaws. *J Cancer Res Clin Oncol* 2011;137:907–13.

6. Aljohani S, Fliefel R, Ihbe J, Kühnisch J, Ehrenfeld M, Otto S. What is the effect of anti-resorptive drugs (ARDs) on the development of medication-related osteonecrosis of the jaw (MRONJ) in osteoporosis patients: a systematic review. *J Cranio-Maxillo-Fac Surg* 2017;45:1493—502.
7. Hallmer F, Andersson G, Götrick B, Warfvinge G, Anderud J, Bjørnland T. Prevalence, initiating factor, and treatment outcome of medication-related osteonecrosis of the jaw -a 4-year prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2018;126:477—85.
8. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American association of oral and maxillofacial surgeons' position paper on medication-related osteonecrosis of the jaws-2022 update. *J Oral Maxillofac Surg* 2022;80:920—43.
9. Yoneda T, Hagino H, Sugimoto T, et al. Bisphosphonate-related osteonecrosis of the jaw: position paper from the allied task force committee of Japanese society for bone and mineral research, Japan osteoporosis society, Japanese society of periodontology, Japanese society for oral and maxillofacial radiology, and Japanese society of oral and maxillofacial surgeons. *J Bone Miner Metabol* 2010;28:365—83.
10. Otto S, Tröltzsch M, Jambrovic V, et al. Tooth extraction in patients receiving oral or intravenous bisphosphonate administration: a trigger for BRONJ development? *J Cranio-Maxillo-Fac Surg* 2015;43:847—54.
11. Soutome S, Hayashida S, Funahara M, et al. Factors affecting development of medication-related osteonecrosis of the jaw in cancer patients receiving high-dose bisphosphonate or denosumab therapy: is tooth extraction a risk factor? *PLoS One* 2018;13:e0201343.
12. Soutome S, Otsuru M, Hayashida S, et al. Relationship between tooth extraction and development of medication-related osteonecrosis of the jaw in cancer patients. *Sci Rep* 2021;11:17226.
13. Hasegawa T, Hayashida S, Kondo E, et al. Medication-related osteonecrosis of the jaw after tooth extraction in cancer patients: a multicenter retrospective study. *Osteoporos Int* 2019;30:231—9.
14. Hasegawa T, Ueda N, Yamada SI, et al. (JCDM). Denosumab-related osteonecrosis of the jaw after tooth extraction and the effects of a short drug holiday in cancer patients: a multicenter retrospective study. *Osteoporos Int* 2021;32:2323—33.
15. Ristow O, Rückschloß T, Moratin J, et al. Wound closure and alveoplasty after preventive tooth extractions in patients with antiresorptive intake -A randomized pilot trial. *Oral Dis* 2021;27:532—46.
16. Scoletta M, Arduino PG, Pol R, et al. Initial experience on the outcome of teeth extractions in intravenous bisphosphonate-treated patients: a cautionary report. *J Oral Maxillofac Surg* 2011;69:456—62.
17. Scoletta M, Arata V, Arduino PG, et al. Tooth extractions in intravenous bisphosphonate-treated patients: a refined protocol. *J Oral Maxillofac Surg* 2013;71:994—9.
18. Mozzati M, Arata V, Gallesio G, Carossa S. A dental extraction protocol with plasma rich in growth factors (PRGF) in patients on intravenous bisphosphonate therapy: a case-control study. *Joint Bone Spine* 2011;78:648—9.
19. Mozzati M, Arata V, Gallesio G. Tooth extraction in patients on zoledronic acid therapy. *Oral Oncol* 2012;48:817—21.
20. Beth-Tasdogan NH, Mayer B, Hussein H, Zolk O. Interventions for managing medication-related osteonecrosis of the jaw. *Cochrane Database Syst Rev* 2017;10:CD012432.
21. Igarashi H, Nishizawa S, Miyamoto T, Hikita A, Hoshi K. Involvement of impaired angiogenesis and myelosuppression in antiresorptive-agent related osteonecrosis of the jaw mouse model. *Tokai J Exp Clin Med* 2023;48:22—31.
22. Ministry of the Environment. *Act on Welfare and management of animals*. https://www.env.go.jp/nature/dobutsu/aigo/1_law/files/aigo_kanri_1973_105_en.pdf. [Accessed 30 March 2024].
23. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol* 2020;18:e3000410.
24. Minocycline [MINO]. *Antibiotic-book*. http://www.antibiotic-books.jp/drugs/73. [Accessed 21 May 2023].
25. Nagashima F, Furuse J. Treatments for elderly cancer patients and reforms to social security systems in Japan. *Int J Clin Oncol* 2022;27:310—5.
26. Tsukamoto S, Kido A, Tanaka Y, et al. Current overview of treatment for metastatic bone disease. *Curr Oncol* 2021;28:3347—72.
27. McGowan K, McGowan T, Ivanovski S. Risk factors for medication-related osteonecrosis of the jaws: a systematic review. *Oral Dis* 2018;24:527—36.
28. AlRowis R, Aldawood A, AlOtaibi M, et al. Medication-related osteonecrosis of the jaw (MRONJ): a review of pathophysiology, risk factors, preventive measures and treatment strategies. *Saudi Dent J* 2022;34:202—10.
29. Kim HY. Review and update of the risk factors and prevention of antiresorptive-related osteonecrosis of the jaw. *Endocrinol Metab* 2021;36:917—27.
30. Hallmer F, Andersson G, Götrick B, Warfvinge G, Anderud J, Bjørnland T. Prevalence, initiating factor, and treatment outcome of medication-related osteonecrosis of the jaw- a 4-year prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2018;126:477—85.
31. Ishimaru M, Ono S, Morita K, Matsui H, Hagiwara Y, Yasunaga H. Prevalence, incidence rate, and risk factors of medication-related osteonecrosis of the jaw in patients with osteoporosis and cancer: a nationwide population-based study in Japan. *J Oral Maxillofac Surg* 2022;80:714—27.
32. Yang G, Singh S, Chen Y, et al. Pharmacogenomic of osteonecrosis of the jaw. *Bone* 2019;124:75—82.
33. Yang G, Collins JM, Rafiee R, et al. SIRT 1 gene SNP rs932658 is associated with medication-related osteonecrosis of the jaw. *J Bone Miner Res* 2021;36:347—56.
34. Park S, Kanayama K, Kaur K, et al. Osteonecrosis of the Jaw Developed in Mice: disease variants regulated by $\gamma\delta$ T cells in oral mucosal barrier immunity. *J Biol Chem* 2015;290:17349—66.
35. Soundia A, Hadaya D, Esfandi N, et al. Osteonecrosis of the jaws (ONJ) in mice after extraction of teeth with periradicular disease. *Bone* 2016;90:133—41.
36. Soutome S, Otsuru M, Murata M, et al. Risk factors for developing medication-related osteonecrosis of the jaw when preserving the tooth that can be a source of infection in cancer patients receiving high-dose antiresorptive agents: a retrospective study. *Support Care Cancer* 2022;30:7241—8.
37. Otsuru M, Fujiki Y, Soutome S, et al. Risk factors for dental findings of the development of medication-related osteonecrosis of the jaw: investigation of 3,734 teeth in cancer patients receiving high dose antiresorptive agents. *J Dent Sci* 2024;19:203—10.
38. Kim DM, Lim HC, Hong JY, et al. Validity of collagen plugs for ridge preservation in a canine model. *Implant Dent* 2017;26:892—8.
39. Oghli AA, Steveling H. Ridge preservation following tooth extraction: a comparison between atraumatic extraction and socket seal surgery. *Quintessence Int* 2010;41:605—9.
40. Tsai SJ, Chen MH, Lin HY, Lin CP, Chang HH. Pure type-1 collagen application to third molar extraction socket reduces

postoperative pain score and duration and promotes socket bone healing. *J Formos Med Assoc* 2019;118:481—7.

41. Javed S, Kohli K. Local delivery of minocycline hydrochloride: a therapeutic paradigm in periodontal diseases. *Curr Drug Deliv* 2010;7:398—406.

42. Yanamoto S, Soutome S, Tsuda S, et al. Inhibitory effect of topical antibiotics/antiseptics administration on bacterial growth in the open wound of the jawbone surgery: randomized controlled, preliminary study. *J Dent Sci* 2021;16:154—9.