
Ich bedanke mich bei den unten aufgeführten Kolleginnen und Kollegen für ihre wertvolle Mitarbeit, die sie im vergangenen Jahr geleistet haben.

Adrian Lussi
Discoloration of teeth from tetracyclines – even today?

Evaluation of a case series

Key words: acne vulgaris, tetracycline, third molar, tooth discoloration

Summary
The aim of this study was to examine whether brownish crown and root discoloration of wisdom teeth was related to treatment of acne with tetracyclines. For this purpose, 17 discolored third molars from nine patients were embedded without being decalcified, ground along the tooth axis, and examined using fluorescence microscopy. A thorough medical history served to determine the start and duration of any administration of tetracyclines. This confirmed the use of drugs against acne containing minocycline in all cases except one. The microscopic analyses of all teeth revealed intensely fluorescent bands in the dentin, which corresponded to the mineralization front at the time of tetracycline intake. More or less uniform discoloration of the entire crown was seen in association with treatment against acne prior to the completion of crown formation at the age of about 15 years. This uniform staining can be attributed to incorporation of minerals during ongoing maturation of the occlusal enamel, which is concomitant with the formation of the cervical crown regions. When acne was treated between 15 and 22 years of age, only the roots of the third molars displayed annular discolorations, which seemed to result from the incorporation of tetracyclines into dentin, while fine fluorescent incremental lines in root cementum were too thin to be apparent clinically. Three accidentally removed interradicular bony septa revealed that tetracyclines incorporated into alveolar bone remained there for about 2 years, but thereafter disappeared as a result of physiological remodeling.
Tooth discoloration from tetracyclines

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As acne is treated primarily at the ages of 14–24 years, these therapies potentially coincide with the formation of the third molars, which on average starts at about 9–10 years of age and lasts until the age of about 21–22 years (Moorrees et al. 1963, Mincer et al. 1993, Olze et al. 2003, De Salvia et al. 2004, Harris 2007, Meinl et al. 2007, Liversidge 2008, Martin-De La Heras et al. 2008, Kasper et al. 2009, Knell et al. 2009).

Using thorough medical histories and fluorescence microscopy, the aim of this study was to assess whether brownish discolorations of third molars could always be attributed to the intake of tetracyclines. Furthermore, it was evaluated whether the pattern of pigmentation was chronologically related to treatment of acne and allowed conclusions to be drawn about the mechanism of drug incorporation.

Materials and Methods

Patients and teeth – From 1996 to 2009, a total of 17 extracted third molars showing brownish discolorations of the crowns and/or roots were sent to the Institute of Oral Biology. The teeth had been obtained from five females and four males at ages of 18-9 to 25-4 (years-months; Tab. I). All patients were contacted and asked whether and at what time drugs against acne were used (Driscoll et al. 1993), and since the 1990s, tetracyclines have been the preferred drugs for these treatments (personal communications Swissmedic [www.swissmedic.ch]). All of the tetracycline preparations used against acne contain minocycline and are on the Swiss market under the proprietary names Aknoral®, Minac®50, Minocin® Akne, and Minocyclin-CIMEX®50. In comparison with the classical tetracyclines, minocycline is more lipophilic and chelates less calcium. As a result, it is better absorbed from the gastrointestinal tract and has a longer half-life (Good & Hussey 2003). Although minocycline preparations are not quite as effective against acne as the retinoid Roaccutan®, they are prescribed at similar frequencies, mainly because they have fewer side effects (Oprica et al. 2007). However, there are several reports indicating that minocycline, at least in certain individuals, causes

Tab. I Details of patients, drugs used against acne, collected teeth, and fluorescent labeling found microscopically.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Drug</th>
<th>Age at extraction</th>
<th>Tooth</th>
<th>Crown staining</th>
<th>Fluorescent labeling</th>
<th>Age range</th>
</tr>
</thead>
<tbody>
<tr>
<td>female</td>
<td>Minocin Akne</td>
<td>20-6</td>
<td>28</td>
<td>none</td>
<td>45–70%</td>
<td>17–9–19-10*</td>
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<td>38</td>
<td>56–87%</td>
<td>18–1–20-5*</td>
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<tr>
<td>female</td>
<td>Minocin Akne</td>
<td>18-10</td>
<td>38</td>
<td>questionable</td>
<td>0–57%</td>
<td>16–Extr*</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>8–58%</td>
<td>15–6–19-7*</td>
</tr>
<tr>
<td>male</td>
<td>Minocin Akne</td>
<td>18-11</td>
<td>18</td>
<td>questionable</td>
<td>1–23%</td>
<td>15-10–16-4*</td>
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<td></td>
<td></td>
<td></td>
<td>48</td>
<td>total</td>
<td>64–100%</td>
<td>15–1–15-6*</td>
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<tr>
<td>female</td>
<td>Minocin Akne</td>
<td>25-3</td>
<td>18</td>
<td>none</td>
<td>13–54%</td>
<td>17–0–17-7*</td>
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<td></td>
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<td>48</td>
<td>29–83%</td>
<td>16–10–19-7*</td>
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<td>43–72%</td>
<td>~15–17-*</td>
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<td></td>
<td>38</td>
<td>cervical</td>
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<tr>
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<td>Minac</td>
<td>24-5</td>
<td>28</td>
<td>none</td>
<td>15–40%</td>
<td>~15–17-*</td>
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<td>38</td>
<td>79–100%</td>
<td>15–3–18-0*</td>
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<tr>
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<td>Minocin Akne</td>
<td>21-7</td>
<td>28</td>
<td>total</td>
<td>58–100%</td>
<td>12–10–18-10*</td>
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<td></td>
<td></td>
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<td></td>
<td>22-0</td>
<td>73–100%</td>
<td>14–8–18-8*</td>
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<tr>
<td></td>
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<td></td>
<td>48</td>
<td>8–100%</td>
<td>15–10–18-8*</td>
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<tr>
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<td>Minocin</td>
<td>25-0</td>
<td>28</td>
<td>none</td>
<td>34–37%</td>
<td>16–9–17-11*</td>
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<td>38</td>
<td>35–38%</td>
<td>16–11–18-1*</td>
</tr>
<tr>
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<td>25-4</td>
<td>18</td>
<td>none</td>
<td>3–37%</td>
<td>15–2–16-4*</td>
</tr>
</tbody>
</table>

1 Labeled zone of crown in percent of total crown height along EDJ
2 Labeled zone of root in percent of total root length along CDJ
3 Age range (in years-months) estimated, based on medical history and/or average data regarding the chronology of third molar development
acne had been taken. As these consultations usually did not yield satisfactory information, informed consent was obtained from all patients to also question their dentists, family doctors and/or dermatologists. A few teeth had been split for surgical removal. In favorable cases, the fragments could be repositioned along the fracture surface and fixed with a cyanoacrylate glue. If this was not possible, they were processed separately. Some of the specimens were sent dry, some in 70% alcohol, and others in 10% formalin.

**Histological processing** – Teeth stored dry were rehydrated in 70% alcohol; those sent in formalin were washed and also transferred to 70% alcohol. After alcohol fixation for 1 week at room temperature, specimens were macrophotographed with a M420 macroscope (Leica Microsystems, Heerbrugg, Switzerland). The cameras used were either a DS-5M (Nikon, Egg, Switzerland) with a resolution of 2560 × 1920 px or a ProgRes C14+ (Jenoptik, Jena, Germany) with a resolution of 2720 × 2048 px. Using a diamond band saw (EXAKT, Norderstedt, Germany), all teeth were subsequently divided along a bucco-lingual or mesio-distal plane parallel to the tooth axis. The two halves were dehydrated in a graded series of alcohol and embedded in Technovit 7200 VLC (Heraeus Kulzer, Wehrheim, Germany). From the light-cured blocks, non-decalcified ground sections of about 50 μm in thickness were made with the EXAKT cutting/grinding system and examined unstained with light and fluorescence microscopes.

**Microscopic evaluation** – For the microscopic evaluation, the following devices were used: (1) a MZ-10 stereomicroscope (Leica Microsystems) equipped with darkfield transmittent illumination, a mercury incident light source, a GFP fluorescence filter block, and a DFC-290 FX camera (resolution 1392 × 1040 px); (2) a DM 6000B light microscope (Leica Microsystems) equipped with interference-contrast transmitted illumination, a mercury incident light source, a GFP fluorescence filter block, and a DFC-350FX camera (resolution 1392 × 1040 px); (3) a TCS SP2 confocal laser scanning microscope (CLSM; Leica Microsystems) equipped with interference-contrast transmitted illumination and a laser incident light source of 405 nm excitation wavelength. Fluorescent emission was recorded at 490–700 nm with a resolution of 1024 × 1024 px.

All fluorescence micrographs were captured as gray-level images. The GFP filter blocks and the laser excitation at 405 nm were deliberately chosen after a trial demonstrating that with these settings, the fluorescent signal was stronger than with the UV excitation that is generally recommended for visualization of tetracycline labeling. In addition, background fluorescence and autofluorescence, particularly of tissues rich in collagen, were markedly reduced when using the GFP filter.

On the overviews taken with the stereomicroscope, the start and extension of labeling were determined quantitatively. Using the program SigmaScan Pro (SPSS, Chicago, IL, USA), the position of the occlusal margin and the width of the fluorescent bands were recorded on the enamel-dentin junction (EDJ) and/or the cemento-dentinal junction (CDJ). These distance measurements were then transformed into percentages of the total crown height from the cusp tip to the cemento-enamel junction (CEJ) and/or the total root length from the CEJ to the apex. Using these percentage values in combination with indications from the literature regarding average ages at the onset and completion of wisdom tooth formation (Fig. 2b), estimates of age at the start as well as of the duration of fluorescence labeling were derived and correlated with the anamnestic data on acne treatment (Tab. 1). In cases where root growth had not been completed yet, the size of the root fraction already formed was estimated based on morphological criteria. Measurements of total root length were then adjusted accordingly.

### Results

All 17 teeth revealed similar gray-brown annular pigmentations. In addition to the roots, the crowns of three specimens were also discolored, in part only in the cervical region and in part totally (Tab. 1). Medical histories, which in several cases included the treating family doctors and dermatologists, established that eight patients had taken minocycline, mostly Minocin®Akne, but also Minac®50. In addition, age periods of drug intake were determined with adequate accuracy. However, in one female, the cause of the tooth discoloration could not be established. She claimed that she had never been treated for acne, could not remember any intake of antibiotics, and did not have a family doctor.

From seven patients, more than one tooth was available. This allowed intra-individual comparisons of tooth formation rates. They showed that in four out of six pairs of maxillary and mandibular molars, the development of the maxillary molar was more advanced. In two pairs of left and right teeth, formation of the left one was always somewhat faster.

**Crown discolorations** – Three of the specimens examined demonstrated obvious crown discolorations. Among these was tooth 18, shown in Figures 1a–c, the entire crown of which exhibited a gray-brown hue that intensified in the cervical area to become a darker circular band.

In the overview micrographs (Fig. 2a, b), the whole enamel layer fluoresced: strongly along the EDJ and more faintly in the more superficial regions. In the cervical crown dentin corresponding to the more darkly stained enamel, a series of fluorescent bands lying close together and following the typical course of incremental lines (von Ebner’s lines) was observed (Fig. 2b).

The level at which the occlusal margin of dentin fluorescence met the EDJ (arrow in Fig. 2c), the CLSM revealed a change in intensity of enamel labeling. The border between the two intensities ran in a slight curve to the tooth surface and corresponded to the occlusal margin of the darker crown pigmentation. Enamel areas on the cervical side of this border fluoresced more intensely and revealed clearly labeled incremental lines (lines of Retzius; Fig. 2c). On the occlusal side of the border up to the cusp tip, the outer enamel fluoresced uniformly but faintly, whereas labeling was strong along the EDJ (Fig. 2c, d). In the latter region, fluorescing enamel prisms gave the impression of undulating tufts projecting from the EDJ (Fig. 2d).

**Root discolorations** Tooth 18 with the crown pigmentation (Fig. 1a–c) also exhibited several faintly brown rings of staining on the root, which spared only the apex. Tooth 48, shown in Figures 1d and e, was obtained from the same patient. Its root exhibited a broad grayish band extending from the CEJ over the entire cervical third. In the last example, tooth 38 (Fig. 1f, g), only two dark brown bands were seen in the apical half of the root.

In the micrographs, fluorescent labeling was evident along incremental lines in the root dentin (Fig. 2c, 3e, 4d). At the CDJs, these markings formed small hooks bent toward the coronal (Fig. 3d). Originating from these hooks, fluorescent lines running coronally and following the incremental lines of root cementum were visible with the CLSM (Fig. 3d, 4c, d). In the acellular extrinsic fiber cementum (AEFC) of the coronal root areas, these lines were more or less parallel to the CDJ and...
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of acne with minocycline during crown formation at ages from 9 to 15 years. After completion of crown development, however, only annular pigmentations of the root seem to occur when minocyclines are taken until the age of about 22 years. All discolorations were associated with a reproducible histological pattern of fluorescent labeling, which in turn corresponded fairly well with the periods of tetracycline treatment revealed by the medical history. Crown pigmentation was combined with fluorescent incremental lines in the crown dentin, while root pigmentation was associated with fluorescent incremental lines in root dentin and cementum.

This pattern conclusively shows that minocycline had been incorporated during third molar development, rather than after termination of tooth formation, as suggested by several previous reports (Fendrich & Brooke 1984, Poljak et al. 1985, Cale et al. 1988, Rosen & Hoffmann 1989, Berger et al. 1989, Westbury & Najera 1997, Good & Hussey 2003). Our findings also show that minocycline incorporated into dental hard tissues does fluoresce, although upon excitation with green light (wavelengths around 500 nm) and not or only weakly upon

Bone labeling – Pieces of interradicular bone septa which were accidentally removed together with three lower wisdom teeth were also examined microscopically. Two of the three bone fragments revealed fluorescent labeling at inner and outer surfaces, i.e., along the periodontal space and around vascular canals and bone marrow spaces (Fig. 4c). The two patients had taken minocycline until 8 months and 2 years prior to tooth extraction, respectively. In the third case without bone labeling, acne treatment had been terminated about 3 years before the removal of the wisdom teeth.

Discussion

The findings obtained in this study show that brownish discolorations of third molar crowns can result from a treatment of acne with minocycline during crown formation at ages from 9 to 15 years. After completion of crown development, however, only annular pigmentations of the root seem to occur when minocyclines are taken until the age of about 22 years. All discolorations were associated with a reproducible histological pattern of fluorescent labeling, which in turn corresponded fairly well with the periods of tetracycline treatment revealed by the medical history. Crown pigmentation was combined with fluorescent incremental lines in the crown dentin, while root pigmentation was associated with fluorescent incremental lines in root dentin and cementum.

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Fig. 2  Microscopic findings associated with crown discoloration in tooth 18 shown in Fig. 1a–c. a, b: Overviews in darkfield (a) and widefield fluorescence (b) illumination of a bucco-lingually ground section. Arrows (b) point to the occlusal margins of fluorescent labeling at the EDJ and CDJ, the respective age indications (in years-months) were derived from a detailed medical history; the circles over the EDJ at the cusp tip, at the CEJ, and at the apex mark the start and end of crown and root formation, the corresponding age indications are average values from the literature; the two rectangles mark the location of the details shown in c and d; E = enamel, D = dentin, P = pulp. c, d: Details of the cervical margin of the crown (c) and the palatal cusp (d) obtained with fluorescence illumination in the CLSM. The arrow (c) points to the margin of the dentin fluorescence, a corresponding margin in the enamel follows a slightly curved course from this point outwards and to the lower edge of the image. Original magnifications a, b: 6.5×; c, d: 100×.
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In order to correlate the fluorescent labeling with the chronological progression of third molar development, we used the information on tooth formation stages given by Moorrees et al. (1963) and Demirjian et al. (1973), although among human

UV excitation with the classically used Wood’s lamp (Westbury & Najera 1997). This is in agreement with the result of Cale et al. (1988), who also observed fluorescence upon excitation with light of 495 nm wavelength.

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...
teeth, third molars exhibit the largest variability regarding inception and rate of formation (Mincer et al. 1993, Meinl et al. 2007). The duration of up to 10 years for their complete development is the longest (Harris 2007), and in contrast to other tooth types, formation of wisdom teeth starts earlier in females, but progresses more rapidly and terminates earlier in males (Engström et al. 1983, Mincer et al. 1993, Meinl et al. 2007). Nevertheless, the attainment of specific developmental stages is significantly correlated with the chronological and skeletal age of the individuals (Engström et al. 1983).

Several forensic studies indicated that on the average, maxillary wisdom teeth are formed earlier than mandibular (Mincer et al. 1993, Martin-De La Heras et al. 2008). Our intraindividual comparisons confirm this finding, although only four out of six maxillary molars were more advanced in development than their mandibular counterparts. No significant differences between left and right teeth have yet been found (Mincer et al. 1993, Meinl et al. 2007), but in our sample of two left/right pairs, the formation of the left specimen was always more advanced.

Crown discolorations – According to current knowledge, enamel mineralization proceeds in two steps (Schroeder 2000). At first, ameloblasts secrete a protein matrix in which hydroxyapatite crystals are deposited up to a concentration of about

Fig. 4  Microscopic findings associated with root and bone discoloration in tooth 38 shown in Fig. 1f, g. Overviews in darkfield (a) and widefield fluorescent (b) illumination of a mesio-distally ground section. Arrows (b) point to the occlusal and apical margins of fluorescent labels at the CDJ, the respective age indications (in years) were derived from the medical history; the two rectangles mark the location of the details shown in c and d; E = enamel, D = dentin, P = pulp, B = interradicular bone. c, d: Details of the interradicular bone (c) and CMFC (d) obtained with fluorescent illumination in the CLSM; arrow heads point to labeled endosteal and periosteal bone surfaces (c) as well as growth lines in cellular cementum (CC; c, d); note the intense auto-fluorescence of remainders of periodontal ligament (PL) rich in collagen (c). Original magnifications a, b: 6.5×; c, d: 100×.
25%. In a second step, the enamel maturation, enamel matrix proteins are degraded and resorbed almost completely, while hydroxyapatite crystals grow in thickness, until the enamel attains a mineral content of about 95%. Consequences of this formation in two stages are also evident in the wisdom teeth showing crown pigmentation. The darkly stained cervical enamel which fluoresced more intensely and corresponded to labeled crown dentin was apparently in the process of matrix secretion and initial mineralization when minocycline was taken. In contrast, the weaker, but uniform fluorescence of the enamel farther toward the occlusal can be attributed to minocycline incorporation during enamel maturation alone. This interpretation agrees with observations of Bevelander & Nakahara (1965), suggesting that tetracycline is not only built into forming enamel, but also into enamel that has already initially mineralized. At least in part, this could also explain the macroscopically visible discoloration of the entire enamel in tooth 18 shown in Figures 1a–c.

The question of whether tetracyclines in general (Hammarström 1967) and minocycline in particular (Bowles & Boxmeyer 1997) are bound to proteins or mineral is still a matter of debate. In regard to this controversy, it was noteworthy that in the teeth with crown discolorations in this study, enamel components such as lines of Retzius and prisms fluoresced, and that this labeling was still visible after termination of root formation, hence years after completion of enamel development and thus almost total removal of enamel matrix proteins. This finding strongly suggests that minocycline, similar to other tetracyclines (Bennet & Law 1967), is incorporated into mineralizing enamel, and that this incorporation is at least partly related to mineral deposition.

Rather surprising was the observation that crown pigmentation occurred only in patients who received acne treatment at 12–14 years of age, that is, at a relatively early stage of crown formation. If administration of minocycline starts only a little later, i.e., immediately following initiation of root formation, it seems to be complete or associated with such a low degree of mineral deposition that visible discolorations are unlikely.

Root discolorations – Labeling of radicular dentin also corresponded to the location of the mineralization front at the time of minocycline intake. Darker intervals between individual fluorescent lines can most likely be attributed to fluctuating serum levels of minocycline and confirm the observation that after administration, tetracycline is incorporated into teeth very rapidly (Bevelander & Nakahara 1965) and deposited primarily in intertubular dentin (Love & Chandler 1996). In teeth from patients with an accurate history, series of fluorescent lines could be assigned to periods of minocycline consumption, and various teeth from one individual demonstrated an identical pattern of fluorescent labeling, although sometimes shifted in an occluso-apical direction due to differing tooth formation periods of maxillary and mandibular molars.

The irregular margins of the dentin labeling, which were evident in the CLSM, are probably related to the mechanism of dentin mineralization. In intertubular dentin, hydroxyapatite is first deposited as globules, which subsequently fuse at a short distance from the mineralization front (Schroeder 2000). These globules seem to be evident along the margin between fluorescent and non-fluorescent dentin, which is a further indication that incorporation of minocyclin is associated with mineral deposition. The hook-shaped course of dentin labeling along the CDJ might result from the delayed mineralization of the outer radicular dentin. During root formation, this peripheral zone initially remains uncalcified, and thus allows periodontal cells to intermingle with collagen fibrils of the innermost cementum layer and outermost dentin. Only when this newly created fiber fringe is completed about 200–300 μm coronally of the growing root tip, is it mineralized together with the peripheral dentin (Bosshardt & Schroeder 1991).

The thin fluorescent lines in root cementum also run along growth lines, in AEFC approximately parallel to the CDJ. As AEFC is formed very slowly, fluorescent lines are much thinner and weaker than those in dentin. Even with the CLSM, they are resolved as separate lines only when periods of minocycline administration are widely separated. In the CMFC of root areas located farther apically, which is formed much faster, individual fluorescent lines running a course not exactly parallel to the CDJ can be identified. As a result, the entire root cementum coronal to the dentin marking contains fluorescent growth lines, but root pigmentation was always annular. Obviously, cementum labeling is too thin and weak to be recognized macroscopically. Therefore, root staining visible to the naked eye most likely results from incorporation of minocycline into dentin.

Bone labeling – Interradicular bone septa are built only during eruption of multi-rooted teeth and, therefore, can also incorporate tetracyclines administered against acne during third molar development. Indeed, two out of three accidentally removed bone fragments did reveal fluorescent labeling. The intervals between the completion of acne treatment and the tooth extraction in the three cases suggest that minocycline incorporated into mineralizing surfaces of alveolar bone can remain there and cause visible discoloration for about 2 years. After longer intervals of time, they seem to disappear, because the fluorescent labeling is eliminated through physiological bone remodeling.

In summary, we conclude from the obtained findings that minocycline, similar to other tetracyclines, is incorporated into mineralizing dental tissues during tooth formation, and thus causes brownish pigmentation of the crowns and roots. In the majority of cases, the differential diagnosis of these discolorations appears to be possible with a thorough medical history.

Acknowledgments

The authors are grateful to Jacqueline Hofmann-Lobiger and Jinan Fierz for skillful laboratory assistance and preparation of the microscopic slides. Many thanks are also due to the Center of Microscopy and Image Analysis, University of Zurich for providing the CLSM and support with its use. Last but not least, we thank the referring colleagues, in particular Dr. E. Schmid-Meier, who initiated this study, and Dr. G. Neugebauer, who provided the detailed information on the first two specimens described in this report.

Résumé

L’objectif de cette étude était de vérifier s’il existait une relation entre la décoloration brunâtre de la couronne et de la racine des dents de sagesse et le traitement d’acné avec des préparations à base de tétacyclines. 17 troisièmes molaires décolorées de neuf patients ont été encastrées non-décalcifiées, coupées en direction axiale et examinées à l’aide de la microscope fluorescente. Le début et la durée d’une administration de tétacyclines ont été déterminées par anamnèse. Tous les cas sauf un ont confirmé l’utilisation d’une préparation contre l’acné contenant des minocyclines.
Les analyses au microscope ont montré des bandes d’une fluorescence intensive dans la dentine de toutes les dents. Ces bandes correspondaient au front de minéralisation lors de l’administration de la tétracycline. Si la thérapie contre l’acné a eu lieu avant que la formation des couronnes soit terminée, c’est-à-dire avant l’âge de 15 ans, la couronne des troisièmes molaires présentait une coloration brunâtre plus ou moins uniforme. Ceci parce que pendant la formation de la partie cervicale de la couronne, l’émale de la surface occlusale mûrit en absorbant des minéraux.

Si la thérapie de l’acné a eu lieu entre l’âge de 15 et 22 ans, des rayures annulaires brunes ont été constatées uniquement sur les racines des troisièmes molaires. Il paraît évident que ces rayures sont le résultat de l’incorporation de la tétracycline dans la dentine, étant donné que les lignes fluorescents de croissance dans le cément sont trop fines pour être cliniquement visibles. Trois septa d’os accidentellement extraits avec les dents, ont montré que la tétracycline reste incorporée dans l’os pendant environ deux ans. Elle est ensuite éliminée par le remodelage physiologique.

**References**


