Original article

Effect of methotrexate on the mandibular development of arthritic rabbits

Thomas Michael Präger*, Philipp Meyer*, Smbat Rafayelyan*, Kirsten Minden** and Paul-Georg Jost-Brinkmann*

*Department of Orthodontics, Dentofacial Orthopedics and Pedodontics, Charité – Universitätsmedizin Berlin and **German Rheumatism Research Centre Berlin (DRFZ), Berlin, Germany

Correspondence to: Thomas Michael Präger, Department of Orthodontics, Dentofacial Orthopedics and Pedodontics, Charité – Universitätsmedizin Berlin, Assmannshauser Strasse 4–6, Berlin 14197, Germany. E-mail: thomas.praeger@gmx.de

Summary

Introduction: Juvenile idiopathic arthritis affecting the temporomandibular joint (TMJ) can cause severe disturbances of the mandibular development. Methotrexate (MTX) is often administered as a common used remission-inducing agent to treat this disease. The aim of this study was to investigate the effect of low dose MTX on the mandibular growth in arthritic rabbits.

Subjects and methods: Eighteen 10-week-old female New Zealand white rabbits were randomly assigned to three groups with six animals in each group. After being sensitized to ovalbumin (OA), the first and the second group received intra-articular injections with OA. The first group remained untreated, the second was treated by weekly injections of MTX. Cephalograms were taken from each animal at 10, 13, 16, 19, and 22 weeks of age and six mandibular distances measured.

Results: All distances showed an increase between 10 and 20 per cent, whereas growth was more accentuated in the sagittal dimension. Significant differences in the overall growth could be observed between the arthritic and the control animals and less accentuated between the arthritic and the MTX animals. In contrast, existing differences between the groups were not significant during the intervals, but time had the greatest influence on mandibular growth.

Conclusions: MTX seems to have a positive impact on growth in rabbits suffering from experimental arthritis of the TMJ.

Introduction

Juvenile idiopathic arthritis (JIA) is the most frequent chronic inflammatory rheumatic disease in children and adolescents (1).

Its onset is before the age of 16 with two major peaks: between 2 and 3 years and at the ages of 11 and 12. As there is no known cause for the inflammation of the synovia it is regarded as an autoimmune disorder (2).

It can be subdivided into different clinical forms, some are only oligoarticular and self-limited, others show a polyarticular and chronic course (3) and it is characterized by inactive and active stages (4).

In Europe an incidence of JIA of 7.8 in 100,000 and a prevalence of 32.6 in 100,000 in Caucasians have been reported and oligoarthritis is the most common subtype (incidence: 3.7/100,000, prevalence: 16.8/100,000, Thierry et al. (5)).

In previous studies, temporomandibular joint (TMJ) affection has been between 17 and 87 per cent (6). As TMJ involvement is not always accompanied by clinical signs, a delay of its diagnosis and hence of the therapy may result (7).

During the active phase chondral and subchondral bone lesions may generate severe mandibular growth impairment (8).

Cephalometric studies in children with JIA indicated morphological similarities with Class II/1 patients characterized by a steeper mandibular plane and mandibular retrognathia (9).

The mandible in JIA is distinguished by a reduced length of its body and the ramus which results in a restricted posterior face height (10) as well as a posterior rotation of the jaw (11).
The therapy consists functional treatment (12), medical treatment based on non-steroidal anti-inflammatory drugs, intra-articular glucocorticoid injections, and disease modifying anti-rheumatic drugs such as methotrexate (MTX) (13). In recent years, ‘biologics’ aiming at the inhibition of tumour necrosis factor (TNF) or interleukin (IL)-1 and -6 have complemented the therapy (14).

Frequently, biologicals are given together with MTX, resulting in a superior therapy compared to monotherapy with biologicals or MTX alone (15). One explanation is that biologicals are targeting different cells. MTX mainly inhibits the activation of B and T lymphocytes (16, 17), in contrast TNF inhibitors suppress monocytes and myeloid dendritic cells (18). In this respect, MTX, although established since decades in the therapy of rheumatic diseases, still plays an important role in the therapy of JIA. Improved growth rates of children suffering from JIA under MTX therapy have been reported (19), and particularly those with polyarticular and extended oligoarticular JIA gain significant benefit from early treatment with MTX. MTX decreases symptoms and may slow joint damage (13).

Despite a considerable number of papers dealing with the effect of anti-rheumatic drugs on growth in general, the information about MTX and mandibular development is sparse.

The aim of this study was to investigate the effect of a systemic administration of MTX on an experimentally induced arthritis in the TMJ of rabbits and its impact on mandibular growth by means of cephalometric measurements. The null hypothesis was that there is no difference in mandibular growth in rabbits suffering from arthritis of the TMJ compared to those with intramuscular injections of MTX. In this context mandibular growth should be considered as an increase in length of three vertical and three sagittal distances.

Subjects and methods

Animals

Eighteen female New Zealand white rabbits (Charles River Sulzfeld Germany, 8 weeks old at arrival, average weight: 1.8 kg) were housed at the animal facilities of the University. The animals were kept in wire cages in groups of three animals, daylight was kept up for 12 hours daily. All animals had access to food and water ad libitum. Animal nutrition was based on ssniff® Complete feeds for rabbits and guinea pigs (ssniff Spezialdiäten GmbH, Soest, Germany), a complete diet for all development stages. To ease feeding in case of arthrogenic pain, the animals could choose complete diet for all development stages. To ease feeding in case of arthrogenic pain, the animals could choose complete diet for all development stages. To ease feeding in case of arthrogenic pain, the animals could choose complete diet for all development stages.

The study was approved by the local Ethics Committee for animal welfare (LAGetSi No. G0049/05).

Induction of arthritis

At week 10 of age, the animals in groups ART (n = 6) and MTX (n = 6) were systemically pre-sensitized with Ovalbumin (OA) (Sigma-Aldrich, St. Louis, Missouri, USA), using 1 ml of OA solution with a concentration of 1 mg/ml dissolved in equal volumes of physiologic saline and Freund's complete adjuvant (Sigma) (20).

The procedure was repeated 2 weeks later, replacing Freund's complete adjuvant by Freund's incomplete adjuvant (Sigma) at the same concentration.

One week later, the sensitivity was tested by a subcutaneous injection of 1 ml (1 mg/ml) OA dissolved in physiologic saline on the back of the animals at an area of 3 × 3 cm after shaving.

Sensitization was considered successful if the erythema was at least 1 × 1 cm and the induration 0.5 × 0.5 cm.

At week 13, after successful sensitization had been confirmed, all animals in group ART and MTX had arthritis induced in both TMJs by an intra-articular injection of 0.1 ml of OA solution (5 mg/ml, dissolved in physiologic saline) under intra-peritoneal anaesthesia with ketamine hydrochloride (25 mg/kg body weight) and xylazine (4 mg/kg body weight) (IPA).

Cephalograms

In week 10 and then every 3 weeks lateral cephalograms were taken in IPA in accordance with Tavakkoli-Jou et al. (21).

Each animal was set into a custom-made restraining box and the head was positioned on an integrated head holding and chin-rest device which allowed a reproducible and consistent positioning of the head in relation to the radiation source and the film.

The box was then placed at a fixed distance such that the midsagittal plane of the head was 20 cm from the X-ray film (Kodak MM25; Kodak, Stuttgart, Germany) and a fixed focal point-to-film distance of 120 cm was achieved. The cephalograms were exposed using an X-ray unit (Siemens Mobilet Plus, Model no: 6215300 X 037 E) with 60 kV, 10 mA, and 0.5 seconds exposure time. All films were developed according to the manufacturer's instructions.

To assess the repeatability of the imaging, the radiographs of week 10 were repeated two times in all animals. Consequently, all 54 radiographs were assessed by one blinded orthodontist and all distances were measured without knowledge of the origin. The values of the first, the second, and the third measurement were compared and according to the approach recommended by Vieira and Corrente (22), a t-test was carried out and a control chart used. As the differences between the three measurements were very small and showed no significant differences, imaging was restricted to one single at the following time points.
Cephalometric analysis
All cephalograms were traced by one investigator (TMP), following the method by Tavakkoli-Jou et al. (21). After having identified the reference points (Tables 1 and 2, Figure 2) the sagittal and vertical measurements were performed directly by means of a set square with ruler.

Arthritis therapy with MTX
After arthritis induction, the animals in group C received weekly intramuscular injections of MTX (0.4 mg/kg body weight) from the 13th until the 22nd week of age. The MTX solution was prepared at the pharmacy of the university clinics in a watery solution (3.0 mg/ml).

Samples for histology
At the end of the trial all animals were euthanized by an over dosage of anaesthetics. All TMJs were retrieved en bloc and placed in 4 per cent paraformaldehyde (Sigma-Aldrich) in phosphate-buffered saline for 24 hours. They were decalcified with 15 per cent dipotassium ethylenediaminetetraacetic acid for 12 weeks. Then, the samples were dehydrated through graded alcohol washes, and embedded in paraffin with an orientation that provided for sectioning along the sagittal plane of the joint; 7 µm sections cut on a conventional microtome were stained by hematoxylin and eosin.

Histomorphometry
All samples were investigated at up to a 200-fold magnification with an Olympus BH2 microscope (Olympus Optical Co., Ltd, Tokyo, Japan).

Table 1. Reference points of the mandible.

<table>
<thead>
<tr>
<th>Cm</th>
<th>Condylar midpoint</th>
<th>Midpoint of the condylar head</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ar</td>
<td>Articulare</td>
<td>Intersection between the external cranial base and the posterior margin of the ramus</td>
</tr>
<tr>
<td>Go</td>
<td>Gonion</td>
<td>Most inferior and posterior point on the external angle of the mandible</td>
</tr>
<tr>
<td>Id</td>
<td>Infradentale</td>
<td>Most antero-superior point on the lingual side of the alveolar process</td>
</tr>
<tr>
<td>B</td>
<td>Mandibular lingual alveolar margin</td>
<td>Most antero-superior point on the labial side of the incisors</td>
</tr>
</tbody>
</table>

Table 2. Linear measurements performed on the mandibular cephalograms.

<table>
<thead>
<tr>
<th>Sagittal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cm-B</td>
</tr>
<tr>
<td>Go-Id</td>
</tr>
<tr>
<td>Ar-B</td>
</tr>
<tr>
<td>Vertical</td>
</tr>
<tr>
<td>Cm-Go</td>
</tr>
<tr>
<td>Cm-Ar</td>
</tr>
<tr>
<td>Ar-Go</td>
</tr>
</tbody>
</table>

Ar, articulare; Cm, condylar midpoint; Go, gonion; Id, infradentale.

Five randomly selected sections from each joint were evaluated for severity of arthritis by two blinded independent observers, using a scoring system first described by Kapila et al. (20).

The inter-examiner agreement was calculated with the weighted kappa (κ) test. The reliability of the inter-examiner agreement was found to be good to very good according to Altman (23), where the strength of agreement is considered good for κ = 0.61–0.80 and very good for κ = 0.81–1.00. The strengths of inter-examiner agreement were κ = 0.82 for the total histological score, κ = 0.83 for synovial hyperplasia, κ = 0.73 for villous hyperplasia, κ = 0.84 for inflammatory cell infiltration, κ = 0.90 and for pannus formation. Differences between the assessments by the two observers were discussed and a consensus was reached after the measurement of inter-observer error.

Statistics
After having analysed the cephalometric data for normal distribution by means of the Shapiro–Wilk test and for homoscedasticity by the Levene test, the values of the overall increase from the 13th to the 22nd week of age were tested by a one-way analysis of variance (ANOVA). If the difference between the three groups was significant, a post hoc test (Tukey honestly significant difference) followed. After Bonferroni correction, the level of significance was set at 0.0083.

The effect size of arthritis induction for the overall change was determined by setting the control animals and arthritis animals into relation and the effect size of the MTX therapy regarding the overall change was investigated by considering arthritic and MTX animals.

The values of the three weekly intervals were tested by a two-way mixed ANOVA, with time, group, and time and group interaction representing the fixed and the distances the dependant variable. After Bonferroni correction, the level of significance was set at 0.0083.

In addition, the means with standard deviation were calculated for all cephalometric distances.

As the histological data was not normally distributed, a non-parametric test was used. For each group the median together with the range of the histological score was determined. To compare the three groups, a pair-wise Mann–Whitney test was performed for the histological score. After Bonferroni correction, the level of significance was set at 0.016

Results
The general development in all three groups was uneventful and all animals showed comparable weight increase and growth.
The overall change of all distances is depicted in Table 3. All distances showed an average increase between 10 and 20 per cent, whereas growth was more accentuated in the sagittal direction. A clear order could be found when comparing the three groups, with control animals showing the greatest increase, followed by MTX and arthritic animals. The difference was significant for Ar-Go, Cm-Go, and Go-Id when comparing arthritic with control animals, whereas for the comparison between arthritic and MTX animals, only Go-Id and Ar-Go showed significant differences. No significant difference existed between healthy and treated animals. The effect size was most pronounced for Ar-Go.

The development of the distances in intervals of 3 weeks is presented in Table 4. All distances increased over time. Growth was most intense at the beginning of the trial and slowed down to its end. The increment was greatest in the control group followed by the MTX and arthritic group. The two-way mixed ANOVA (Table 5) confirmed the time effect as highly significant, in contrast neither group nor the combination of time and group supplied a significant difference.

The result of the histological examination can be seen in Table 6, the related Mann–Whitney test in Table 7. Signs of synovial inflammation were mainly present in arthritic animals and less accentuated in joints with MTX therapy. The joints of the healthy control animals were mainly inconspicuous. The difference between all three groups was significant. The picture of an arthritic joint is presented in Figure 3.

We could find no significant difference between the first and second measurement when determining the error of the method.

### Table 3. Percentage change of the six distances in the three experimental groups from the 13th to the 22nd week of age, means with standard deviation.

<table>
<thead>
<tr>
<th>Distance</th>
<th>CON</th>
<th>ART</th>
<th>MTX</th>
<th>Go-Id</th>
<th>Ar-Go</th>
<th>Cm-Go</th>
<th>Cm-Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cm-B</td>
<td>14.6±3.1</td>
<td>17.0±3.0</td>
<td>12.0±0.5</td>
<td>17.7±3.3</td>
<td>12.6±1.6</td>
<td>18.5±0.8</td>
<td></td>
</tr>
<tr>
<td>Go-Id</td>
<td>11.5±1.6</td>
<td>10.5±2.5</td>
<td>11.6±1.7</td>
<td>11.5±1.9</td>
<td>7.7±1.9</td>
<td>14.4±0.4</td>
<td></td>
</tr>
<tr>
<td>Ar-Go</td>
<td>13.9±2.0</td>
<td>16.1±1.5</td>
<td>12.0±1.5</td>
<td>15.7±0.8</td>
<td>10.1±3.5</td>
<td>17.9±0.8</td>
<td></td>
</tr>
<tr>
<td>Cm-Go</td>
<td>0.274</td>
<td>0.624</td>
<td>0.029</td>
<td>0.615</td>
<td>0.435</td>
<td>0.882</td>
<td></td>
</tr>
<tr>
<td>Cm-Ar</td>
<td>0.091</td>
<td>0.001</td>
<td>0.800</td>
<td>0.001</td>
<td>0.014</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Tukey</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.000</td>
<td>0.256</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

CON: control group; ART: arthritis group, MTX: MTX group. The effect size is indicated as partial η squared. The result of the one-way ANOVA is demonstrated as significance. In case of significant differences, the inter-group comparison of the Tukey test is presented. Significant values are in bold letters. After Bonferroni correction, the level for significance was set at \( P < 0.0083 \). ANOVA, analysis of variance; Ar, articular; Cm, condylar midpoint; Go, gonion; Id, infradental; MTX, methotrexate.

### Table 4. Values for the six mandibular distances from the 10th to the 22nd week of age in (mm), presented as means with standard deviation.

<table>
<thead>
<tr>
<th>Distance</th>
<th>CON</th>
<th>ART</th>
<th>MTX</th>
<th>Go-Id</th>
<th>Ar-Go</th>
<th>Cm-Go</th>
<th>Cm-Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cm-B</td>
<td>10</td>
<td>13</td>
<td>16</td>
<td>19</td>
<td>22</td>
<td>64.1±2.2</td>
<td>67.9±1.8</td>
</tr>
<tr>
<td>Go-Id</td>
<td>67.9±1.8</td>
<td>73±1.3</td>
<td>75±1.3</td>
<td>77.7±2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ar-Go</td>
<td>63.5±1.9</td>
<td>67.3±1.9</td>
<td>71.3±1.7</td>
<td>72.8±1.7</td>
<td>75±2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cm-Go</td>
<td>63.9±2.1</td>
<td>67.7±2.1</td>
<td>74.5±2.2</td>
<td>74.5±2.2</td>
<td>77.1±1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cm-Ar</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.256</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Tukey</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Ar, articular; Cm, condylar midpoint; Go, gonion; Id, infradental; MTX, methotrexate.
Discussion

Results

In three out of six distances, a significant difference between the groups could be found. As a consequence the null hypothesis has to be rejected.

Mandibular growth seems to be greatest in the control animals and stronger in the MTX group than in the arthritic animals.

Growth inhibition in the arthritic group is consistent with observations in patients suffering from JIA, where an inflammation of the TMJ causes an inhibition of mandibular growth (24).

The difference of the overall growth was accentuated in distances related to the condylar process. In two of the three vertical and only in one sagittal distance this was significant. This observation is also backed by the greater effect size in the vertical distances, which indicates a more substantial impact of the inflammation on vertical growth.

An explanation might be a disturbance in the condylar region. The condyle is seen as a centre of growth or as a pacemaker for regional growth or at least as a growth site (25). Consequently, an inhibition of growth in this region may result in a restriction of the vertical increment in the whole mandible.

This is in accordance with Tavakkoli-Jou et al. (21) who found a vertically accentuated effect in growing rabbits.

Growth in animals receiving MTX was lesser inhibited. This is reflected by the fact that, despite weaker growth in the therapeutic group the differences were not significant compared with the healthy animals. In contrast, for two out of six distances a significant difference could be stated in comparison with the untreated animals. An improvement of general growth parameters as a consequence of MTX therapy has been reported in children suffering from JIA (19).

In a clinical study analysing the data of patients with polyarticular JIA, Ince et al. (26) found, that those receiving MTX, showed less severe TMJ involvement. They concluded that the treatment with MTX was effective in minimizing TMJ destruction and craniofacial dysmorphology in patients suffering from the polyarticular form of the disease.

There are different possible explanations for the protective role of MTX. Administered in lower doses, it may contain the inflammation in autoimmune diseases (27). In this study, signs of synovial inflammation were pronounced in the arthritic group and to a lesser extent in the MTX group but not in the control group.

MTX is known for suppressing the T-cell proliferation. By restraining the production of IL-1 and by blocking the binding to its target cells (13, 28) it may be protective for the cartilage.

In a similar model, Habu et al. (29) found IL-1β being related to the destruction of cartilage. Their findings could explain the curative impact of MTX in our study.

This is in line with observations showing that in humans biologic agents targeting IL-1 and IL-6 have proven very effective in treating JIA (30).

Cartilage

The inflammatory process had a strong impact on the cartilage tissue. Kristensen et al. (31) could show that the reduction of condylar growth is mainly associated with the developmental inhibition of the condylar cartilage.

Thus, MTX might have exerted its positive effect on mandibular growth by protection of the cartilage.

In a comparable antigen induced arthritis model in the knee joint of adult rabbits the therapeutic effect of MTX in relation to the damage of cartilage was obvious (32).

Bone

MTX also seems to inhibit osteoclast activation (33). This is important because joint degeneration, especially in the later stages, is accompanied by the destruction of osseous structures.

The overall growth of the control animals was approximately 15 per cent from week 13–22, which is in accordance with Masoud et al. (34) and below that of humans in a comparable state of physical development.

Intervals

Mandibular growth in all groups decreased from the first to the fourth interval, confirming Tavakkoli-Jou et al. (21), who observed an asymptotic tendency of the speed of growth. In most distances and all groups a turning point was identifiable from the second to the third interval followed by a further deceleration.

Although the overall growth was inhibited more distinctly in arthritis animals, the general pattern of the growth curve was the same as in the control group.

There was almost no difference between the three groups during the first interval which is comprehensible, because the induction of arthritis was performed only at the end of the first interval.

Table 5. Results of the two-way mixed ANOVA with distance as dependent and time, group, and time and group interaction as fixed variables.

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
<th>Group</th>
<th>Time × group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cm-B</td>
<td>0.000</td>
<td>0.011</td>
<td>0.922</td>
</tr>
<tr>
<td>Go-Id</td>
<td>0.000</td>
<td>0.005</td>
<td>0.725</td>
</tr>
<tr>
<td>Ar-B</td>
<td>0.000</td>
<td>0.022</td>
<td>0.993</td>
</tr>
<tr>
<td>Cm-Go</td>
<td>0.000</td>
<td>0.009</td>
<td>0.610</td>
</tr>
<tr>
<td>Cm-Ar</td>
<td>0.000</td>
<td>0.377</td>
<td>0.997</td>
</tr>
<tr>
<td>Ar-Go</td>
<td>0.000</td>
<td>0.073</td>
<td>0.981</td>
</tr>
</tbody>
</table>

Significant values are in bold letters. After Bonferroni correction, the level of significance was set at $P < 0.0083$. Ar, articulare; Cm, condylar midpoint; Go, gonion; Id, infradentale.

Table 6. Results of the histological investigation.

<table>
<thead>
<tr>
<th></th>
<th>Synovial lining hyperplasia</th>
<th>Villous hyperplasia</th>
<th>Inflammatory cell infiltrate</th>
<th>Pannus</th>
<th>Score median</th>
<th>Score min</th>
<th>Score max</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>0.25</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
<td>0.50</td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>ART</td>
<td>1.00</td>
<td>1.00</td>
<td>2.75</td>
<td>1.50</td>
<td>6.00</td>
<td>4.00</td>
<td>8.50</td>
</tr>
<tr>
<td>MTX</td>
<td>0</td>
<td>0.25</td>
<td>0.50</td>
<td>0.50</td>
<td>1.50</td>
<td>0.50</td>
<td>3.50</td>
</tr>
</tbody>
</table>

The score is demonstrated as median, the range is marked by the minima and maxima of the score. MTX, methotrexate.
ART versus MTX

CON versus MTX

The animal model creates the opportunity to assess the efficacy and the adverse effects of one single drug. Therefore, the animal model creates the opportunity to assess the particular effect of MTX on the disease in question. In contrast, patients receiving MTX are most often treated by a combination of different drugs, which makes it impossible to assess the particular effect of MTX on the disease in question. Therefore, the human model creates the opportunity to assess the efficacy of MTX not excluded.

This study enabled us to assess the effect of MTX on TMJ arthritis without the bias of other drugs. In contrast, patients receiving MTX are most often treated by a combination of different drugs, which makes it impossible to assess the particular effect of MTX on the arthritis or on growth. Therefore, the animal model creates the opportunity to assess the efficacy and the adverse effects of one single drug.

Dose and MTX

The dosage of MTX was based on a low dose therapy for adolescents suffering from JIA. As the main information about the clinical efficacy of this medication derived from humans, the administered dosage has to be scrutinized. In a review Ramanan et al. found that the human dosage is mainly between 5 and 10 mg/m². Taking into consideration the surface of the animals, our dosage is in this range. The dose of MTX in rabbits being treated for experimental arthritis in the knee joint ranges from between 0.1 mg/kg/week (36) and 30 mg/kg/week (32). As there are only few studies dealing with MTX therapy of arthritis in the rabbit, an under-dosing seems to be possible and thus a higher efficacy of MTX not excluded.

This study enabled us to assess the effect of MTX on TMJ arthritis without the bias of other drugs. In contrast, patients receiving MTX are most often treated by a combination of different drugs, which makes it impossible to assess the particular effect of MTX on the arthritis or on growth. Therefore, the animal model creates the opportunity to assess the efficacy and the adverse effects of one single drug.

Animal model

The New Zealand white rabbit is a common model for the investigation of craniofacial development. Bang and Enlow (25) described a centre of growth in the mandibular condyle and a general backward and upward growing of the jaw. Similarities between the anatomy of the TMJ of humans and rabbits have also been described by several authors (37, 38).

Losken et al. (39), however, objected that despite structural similarity, during the 14th and 24th week of age, which is comparable to that of 6–18 years old humans, the growth of the rabbit’s mandible is falling behind and it achieves only about one-third of the human one’s.

This study followed the methodology of Tavakkoli-Jou et al. (21). But, as they stated that only the mandible and especially the condylar region had been affected by AIA, we concentrated exclusively on the mandibular parameters.

Other growth impeding effects proved by cephalometric studies in the rabbit have been disk displacement (41), the repositioning of the masseter muscle (46), and the paralysis of the masseter muscle caused by Botulinum toxin (47).

In more recent studies growth was investigated by computed tomography, allowing for a three-dimensional assessment of the jaw (45, 48).

Although three-dimensional diagnostics can supply more information by adding a third dimension two-dimensional cephaldontometry is still established and has been used to detect sagittal and vertical growth inhibition (21, 41).

Weaknesses

Fundamental differences exist between the model and JIA.
In the model the inflammation begins by an immune reaction of the synovia which resembles the pathological image of JIA. However, despite its morphological similarities, it is limited to the affected joint and to its synovia in particular. In contrast, the clinical course of JIA, is generally not restricted to one joint, but it is caused by systemic processes including all connective tissues and the masticatory muscles. These systemic processes start much time before alterations in the joints are detectable and therefore an involvement of the joints is a result of this immunologic process and not its origin.

Consequently, the present study only allows us to draw very limited parallels with the systemic impact of the disease and to assess the effect of MTX on the synovia and the adjacent articular tissue, but not on the whole organism.

Another weakness of our study is the small number of animals involved. For animal welfare and legal reasons and as a result of a carefully reflected study design, the number of permitted animals in an experiment is limited.

But, according to the law of large numbers, results of an experiment are more reliable when it is done repeatedly and the number of observation units is large. Therefore, a larger number of animals and multiple imaging would have been desirable.

Conclusion
In this study with a very limited number of growing rabbits, mandibular involvement of the joints is large. Therefore, a larger number of animals and multiple imaging would have been desirable.

Funding
This study was supported by intramural funding (start-up support for P.M., 836/2004).

References


