

Facial symmetry evaluation after experimentally displaced condylar process fracture in methotrexate treated rats¹

Avaliação da simetria facial após fratura experimental com desvio do processo condilar em ratos tratados com metotrexato

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ABSTRACT

PURPOSE: To investigate the facial symmetry of high and low dose methotrexate (MTX) treated rats submitted to experimentally displaced mandibular condyle fracture through the recording of cephalometric measurements.

METHODS: One hundred male Wistar rats underwent surgery using an experimental model of right condylar fracture. Animals were divided into four groups: A – saline solution (1mL/week); B – dexamethasone (DEX) (0,15mg/Kg); C – MTX low dose (3 mg/Kg/week); D – MTX high dose (30 mg/Kg). Animals were sacrificed at 1, 7, 15, 30 and 90 days postoperatively (n=5). Body weight was recorded. Specimens were submitted to axial radiographic incidence, and cephalometric mensurations were made using a computer system. Linear measurements of skull and mandible, as well as angular measurements of mandibular deviation were taken. Data were subjected to statistical analyses among the groups, periods of sacrifice and between the sides in each group ($\alpha=0.05$).

RESULTS: Animals regained body weight over time, except in group D. There was reduction in the mandibular length and also changes in the maxilla as well as progressive deviation in the mandible in relation to the skull basis in group D.

CONCLUSION: Treatment with high dose methotrexate had deleterious effect on facial symmetry of rats submitted to experimentally displaced condylar process fracture.

Key words: Surgery, Oral. Mandibular Condyle. Methotrexate. Temporomandibular Joint. Maxillary Fractures. Rats.

RESUMO

OBJETIVO: Avaliar a simetria facial de ratos tratados com metotrexato (MTX), em dose alta e baixa, submetidos à fratura experimental do processo condilar com desvio por meio de mensurações cefalométricas.

MÉTODOS: Cem ratos Wistar machos foram submetidos a procedimento cirúrgico utilizando modelo experimental de fratura de côndilo do lado direito. Os animais foram distribuídos em quatro grupos: A – soro fisiológico (1mL/semana); B – dexametasona (DEX) (0,15mg/Kg); C – MTX baixa dose (3mg/Kg/semana); D – MTX alta dose (30mg/Kg). Os períodos de sacrifício foram de 1, 7, 15, 30 e 90 dias de pós-operatório (n=5). O peso dos animais foi documentado. Foram realizadas mensurações lineares da maxila e da mandíbula, bem como angulares do desvio mandibular. Os dados foram submetidos a análises estatísticas entre os grupos, períodos de sacrifício e entre os lados em cada grupo ($\alpha=0,05$).

RESULTADOS: Os animais recuperaram peso ao longo do tempo, exceto no grupo D. Houve redução no comprimento mandibular com alterações também na maxila e desvio progressivo da mandíbula em relação à base do crânio no grupo D.

CONCLUSÃO: O tratamento com metotrexato em alta dose teve efeito deletério na simetria facial de ratos submetidos à fratura do processo condilar.

Descritores: Cirurgia Bucal. Côndilo Mandibular. Metotrexato. Articulação Temporomandibular. Fraturas Maxilares. Ratos.

Introduction

Condylar process fractures are common^{1,2}. A favourable prognosis has been reported when these fractures are treated by closed methods³. The ability of the ascending ramus to repair and remodel following trauma has been tested experimentally^{4,5}. Callus formation, with simultaneous repositioning of the condyle, can lead to normal temporomandibular joints^{5,6}. However, there is some concern regarding skeletal changes, represented by mandibular asymmetry⁷⁻⁹.

The experimental model of condylar fracture, unilateral and medially displaced, allows the histological study of healing of fracture and articular sites^{3,6}, as well as allows cephalometric analyses of their effects on facial structures^{5,9}. This model follows the most frequent form of mandibular condyle fracture^{1,2,10}.

Since its introduction as an antifolate agent more than fifty years ago, Methotrexate (MTX) has been widely used drug in medical, surgical, dermatological and pediatric specialties. MTX and its active metabolites have made a significant impact on the treatment of a wide range of diseases such as neoplasms, rheumatoid arthritis and psoriasis as well as being used as immunosuppressive agents in organ transplantation¹¹⁻¹⁸. The administered dose of the drug is the differential in a given treatment. High doses are recommended for oncologic treatment^{19,20}, while therapy with low doses are recommended for treatment of chronic inflammatory diseases such as rheumatoid arthritis and psoriasis^{21,22}.

The purpose of this study was to investigate the facial symmetry of high and low dose MTX treated rats submitted to experimentally displaced mandibular condyle fracture through the recording of cephalometric measurements.

Methods

The study animals (100) were 3-month-old Wistar rats, with a mean body weight of 339.8±47.8g. All animals were acclimatized for 1 week under standard laboratory conditions at a temperature of 25°C with a 12-hour light cycle. The rats had free access to tap water and an ordinary diet of rodent feed (Labina, Agribands Purina, Sao Paulo, Brazil), administered in powder form for 2 weeks postoperatively, and in regular form in the remaining period. The study was approved by the local committee on research ethics (Protocol 09/2009).

Study design

All drugs were administered intraperitoneally, according to the weight of the rats. They were divided into four groups of 25

animals. Group A: control, saline solution (1 mL/week), with the first dose administered two hours before surgery; Group B: DEX (0,15 mg/Kg) (Decadron®, Prodome, Campinas, Brazil), single dose, immediately after surgery²³; Group C: MTX low dose (3mg/Kg – adjusted with normal saline solution to a volume of 1 mL), weekly (Miantrex®, Pfizer, Guarulhos, Brazil), with the first dose administered two hours before surgery²¹; Group D: MTX high dose (30 mg/Kg – adjusted with normal saline solution to a volume of 1 mL), single dose administered two hours before surgery²⁴.

Surgical procedure

Each animal was pre-medicated with 80.000 IU/kg of benzatin penicillin (Benzetacil®, Eurofarma, Sao Paulo, Brazil). General anesthesia was induced by an injection of 50 mg/kg of ketamine 10% (Cetamin®, Rhobifarma, Hortolandia, Brazil), and 0.8 mg/kg of xylazine 2% (Xilazin®, Rhobifarma, Hortolandia, Brazil). A right side preauricular incision of 1 cm was made, followed by blunt dissection through the masseter muscle, just below the zygomatic arch, and exposure of the condylar process. Fracture was achieved by using a mosquito (Halstead) forceps, and the condylar fragment was deviated medially³. Care was taken so as not to damage the articular surfaces. The procedures were concluded by suturing in layers. Postoperative analgesia was induced by an injection of tramadol 0.3 mg/Kg (Tramal®, Pfizer, Guarulhos, Brazil) after the surgical procedure. Animals were sacrificed by CO₂ asphyxiation at 1, 7, 15, 30 and 90 days postoperatively (n=5), their heads were removed and fixed in 10% buffered formalin. Body weight was recorded on the first day of the experiment and at death.

Cephalometric evaluation

Following formalin fixation for 1 week, radiographs in axial (dorso-ventral) projection of the skull were obtained, and care was taken to maintain the horizontal and vertical planes. Radiographs were taken with a standard dental machine at 56 kV and 10 mA, with an exposure time of 0.4s. A constant 30 cm focus-to-film distance was maintained and periapical films were used (Kodak Ektaspeed Plus®, Rochester, NY, USA). Radiographs were subjected to computerized cephalometric evaluation. The radiographic images were digitized, and measurements obtained with Imagelab® software (Softium Informatica, Sao Paulo, Brazil).

From the axial radiographs, the following distances were measured bilaterally, in mandibles: AP- II – angular process to lower insertion of incisor (the most anterior limit of the lower bone insertion of this tooth); AP-IP' – angular process to lower incisal point (the intersection of the lingual face of lower incisors with the

midline)⁵; and in skull: TB-IF – tympanic bulla (the most anterior portion of this round, voluminous structure of the skull base) to infraorbital foramen (the vertex of the image of this foramen); and IF-IP – infraorbital foramen to upper incisal point (the intersection of the lingual face of upper incisors with the midline). Angular measurements were also registered. Angle α : deviation of the mandible, right angle with reference to a line between both tympanic bullae³; angle \hat{A} : deviation of the mandible relative to the maxilla, formed between lines from the midpoint between both TB, one to IP and another to IP'⁹ (Figure 1).

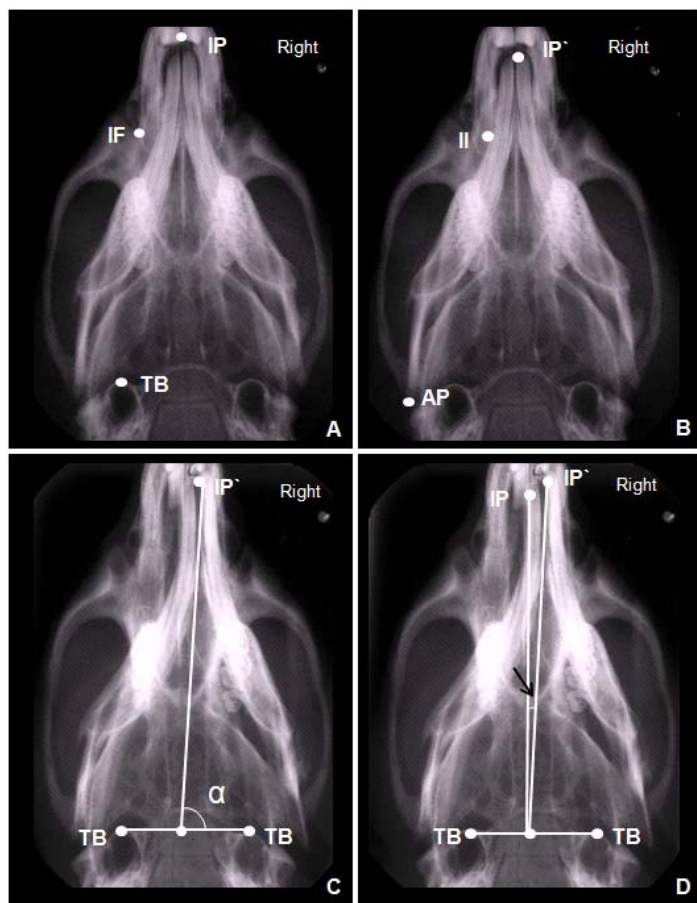


FIGURE 1 – Axial radiographs and cephalometric reference points. **A.** Skull; TB = tympanic bulla, IF = infraorbital foramen and IP = upper incisal point. **B.** Mandible; AP = angular process, II = insertion of incisor e IP' = lower incisal point. **C.** Measurement of angle α . **D.** Arrow = measurement of angle \hat{A} .

Statistical analysis

Results were analyzed using BioEstat 5.0 software (Sociedade Civil Mamiiraua / MCT – CNPq, Belem, Brazil). The criterion of significance was a probability of 0.05. Mean values of rate of gain or loss of body weight were obtained. A one-way analysis of variance (ANOVA) was performed to evaluate possible differences among the rate of gain or loss of body weight in the

periods of sacrifice within the same group. Tukey’s test was used to determine statistical significance between each period. Data obtained from the cephalometric evaluation were tested by one- and two-way ANOVA and Tukey’s test. The paired Student’s t-test was also used to compare the mean values from the right and left sides.

Results

Macroscopic features

After the surgical procedure, the animals continued with food and water intake. Signs of drug-related toxicity (piloerection and lethargy), diarrhea (soft feces) and mucositis were observed in animals undergoing chemotherapy with MTX high dose. The animals also continued to gain weight. The one-way ANOVA showed that there was a statistical difference in weight gain among the periods of sacrifice in control ($p<0.001$), DEX ($p<0.001$) and MTX low dose ($p<0.001$) groups. There was no statistical difference in weight regain among the different periods of sacrifice in MTX high dose group ($p=0.249$). Tukey’s test showed the statistical differences between periods of sacrifice and is represented by different letters in Table 1.

TABLE 1 - Mean values and standard deviations (SD) of the rate of gain or loss of weight (g) and significance of Tukey’s test.

Period	Groups			
	Control Mean ± SD	DEX Mean ± SD	MTX low dose Mean ± SD	MTX high dose Mean ± SD
1 day	-11.4±11.0 B	-27.8±3.0 C	-8.4±10.2 B	-21.4±8.8
7 days	-12±67.5 B	29.6±20.6 B	-8.8±15.9 B	-47.4±19.8
15 days	26.6±9.5 B	15±17.8 B	16±18.4 B	-33±70.7
30 days	27.8±40.8 B	33.6±32.2 B	23.6±98.1 B	40.2±46.2
90 days	139±73.4 A	174.8±20.0 A	161.6±40.9 A	37.4±144.7

Distinct letters mean statistical difference between periods.

Macroscopic examination of the specimens revealed facial asymmetry, with deviation of the mandible to the right (side of fracture), and asymmetric wear of the incisors. Some animals from MTX high dose group showed no incisal wear and the continuous tooth growth led to a large extraoral projection.

Cephalometric evaluation

Mandible

Angular process to lower insertion of incisor (AP- II)

The paired Student's t-test showed that there was no significant difference between the right and left sides. On the left side one-way ANOVA showed that there was statistical difference among periods of sacrifice in Control and DEX ($p<0.010$), with progressive growth according to Tukey's test, but not in MTX low dose ($p=0.087$) and MTX high dose ($p=0.683$) groups. On the right side there was also statistical difference among periods of sacrifice in Control and DEX ($p<0.013$), with progressive growth according to Tukey's test, but not in MTX low dose ($p=0.508$) and MTX high dose ($p=0.285$) groups (Figure 2). Two-way ANOVA showed that there was a statistical difference, on the left side, among the groups in the period of 7 days, but no significant difference in the periods of 1 day, 15, 30 and 90 days. Furthermore there was no significant difference among the groups on the right side.

Angular process to lower incisal point (AP-IP')

The paired Student's t-test showed that there was a significant difference between the right and left sides in group DEX, in the periods of 7 ($p=0.034$) and 15 ($p=0.015$) days and in MTX high dose group in the period of 7 days ($p=0.038$). On the left side one-way ANOVA showed that there was a statistical difference among periods of sacrifice in all groups ($p<0.010$), just as on the right side ($p<0.012$), with progressive growth according to Tukey's test (Figure 2). Two-way ANOVA showed that there was a statistical difference, on the left side, among the groups in the periods of 7 and 15 days, but no significant difference in the periods of 1 day, 30 and 90 days, while on the right side there was a significant difference among groups in the periods of 7 and 15 days, and no significant difference in the periods of 1 day, 30 and 90 days.

Tukey's test showed that on the left side, to the AP-II, in the period of 7 days there was statistical difference between DEX and MTX high dose; to the AP-IP', in the period of 7 days there was statistical difference between DEX and MTX high dose, in the period of 15 days there was statistical difference between Control and MTX high dose and on the right side in the period of 7 days there was statistical difference between Control and DEX; DEX and MTX high dose and MTX low dose and MTX high dose; in the period of 15 days there was statistical difference between Control and MTX high dose.

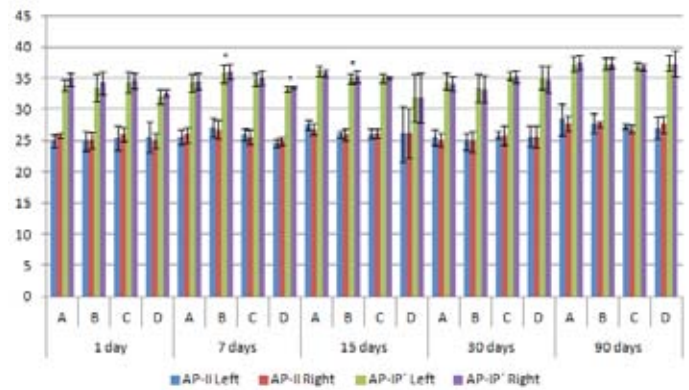


FIGURE 2 - Mean values and standard deviations (SD) of the cephalometric mensurations (mm) of the mandible of all the groups according to the experimental period. A=Control, B=DEX, C=MTX Low dose, D=MTX High dose. (*) Statistical difference between right and left sides.

Skull

Tympanic bulla to infraorbital foramen (TB-IF)

The paired Student's t-test showed that there was a significant difference between the right and left sides only in DEX group, in the period of 30 days ($p=0.042$). On the left side one-way ANOVA showed that there was a statistical difference among periods of sacrifice in all groups ($p<0.005$), with progressive growth according to Tukey's test, while on the right side there was a significant difference among periods in Control ($p<0.001$), MTX low dose ($p=0.015$) and MTX high dose ($p<0.001$) groups, with progressive growth according to Tukey's test, and no significant difference in DEX ($p=0.355$) group (Figure 3). Two-way ANOVA showed that there was a statistical difference, on the left side, among the groups in the periods of 1 day, 7 and 15 days, but no significant difference in the periods of 30 and 90 days, and on the right side there was a significant difference among groups in the period of 1 day, but no significant difference in the periods of 7, 15, 30 and 90 days.

Infraorbital foramen to upper incisal point (IF-IP)

The paired Student's t-test showed that there was significant difference between the right and left sides only in Control group, in the period of 90 days ($p=0.025$). On the left side one-way ANOVA showed that there was a statistical difference among periods of sacrifice in Control ($p=0.014$) group, with progressive growth according to Tukey's test, and no significant difference in DEX ($p=0.190$), MTX low dose ($p=0.337$) and MTX high dose ($p=0.371$) groups. On the right side there was a statistical difference between periods of sacrifice only in MTX

high dose ($p=0.013$) group, with progressive growth according to Tukey's test, and no significant difference in Control ($p=0.527$), DEX ($p=0.129$) and MTX low dose ($p=0.521$) groups (Figure 3). Two-way ANOVA showed that there was a statistical difference, on the left side, among the groups in the period of 15 days, and no significant difference in the periods of 1 day, 7, 30 and 90 days. On the right side there was a significant difference among groups in the periods of 15 and 90 days, but no significant difference in the periods of 1, 7 and 30 days.

Tukey's test showed that on the left side, to the TB-IF, in the period of 1 day there was statistical difference between Control and MTX low dose, Control and MTX high dose and MTX low dose and MTX high dose; in the periods of 7 and 15 days there was statistical difference between MTX high dose and the remaining groups; on the right side in the period of 1 day there was statistical difference between Control and MTX high dose and MTX low dose and MTX high dose; to the IF-IP, on the left and right sides in the period of 15 days there was statistical difference between Control and MTX high dose.

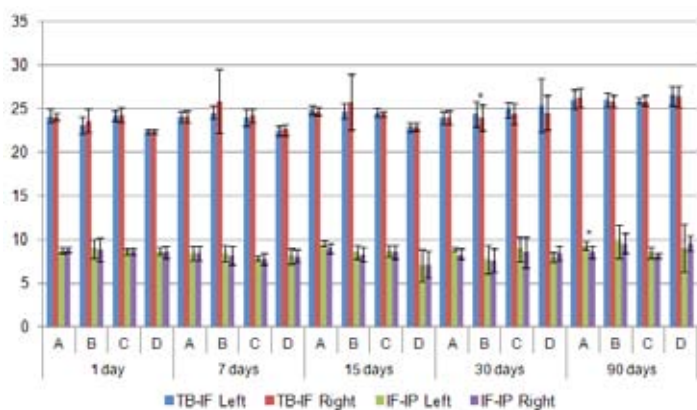


FIGURE 3 - Mean values and standard deviations (SD) of the cephalometric mensurations (mm) of the skull of all the groups according to the experimental period. A=Control, B=DEX, C=MTX Low dose, D=MTX High dose. (*) Statistical difference between right and left sides.

Angles α and \hat{A}

Mensuration of angle α revealed an increase in deviation over time in MTX high dose group. One-way ANOVA showed that there was a statistical difference among periods of sacrifice in MTX high dose ($p=0.010$) group, and no significant difference in Control ($p=0.560$), DEX ($p=0.923$) and MTX low dose ($p=0.654$) groups. Two-way ANOVA showed that there was not statistical difference among the groups ($p>0.050$). One-way ANOVA showed that there was not statistical difference among the periods of sacrifice in the measurements of angle \hat{A} . Two-way ANOVA

showed that there was a statistical difference among groups in the periods of 1 day ($p=0.015$), and no significant difference in the periods of 7($p=0.784$), 15 ($p=0.411$), 30 ($p=0.523$) and 90 ($p=0.313$) days (Figure 4).

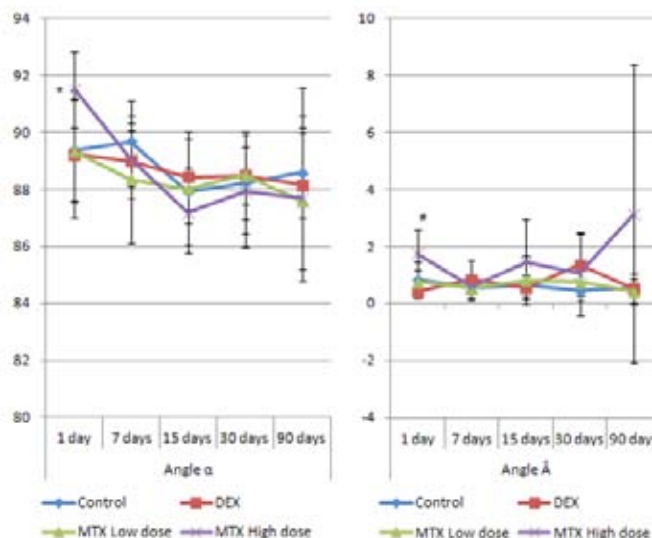


FIGURE 4 - Mean values and standard deviations (SD) of the cephalometric mensurations (degrees) of the angles α e \hat{A} of all the groups according to the experimental period. (*) Statistical difference between periods in MTX High dose group. (#) Statistical difference between groups.

Discussion

When considering the use of MTX treatment for patients, it is important to know its effect on facial symmetry after experimentally displaced condylar process fracture. In this study mandibular condyle fractures were induced in high and low doses methotrexate (MTX) treated rats, and cephalometric mensurations focused on facial symmetry were taken. The final result was an asymmetry of the mandible, also with asymmetry of the maxilla in MTX high dose treated rats.

A series of published studies showed different doses of MTX and administration routes used in experimental models^{21, 23-27}. An intraperitoneal administration route was selected since it is frequently used in animals and allows rapid absorption^{21, 24, 25}. A comparative study evaluated serum levels of different concentrations of MTX and observed that a 3 mg/Kg dose gave serum levels similar to the levels attained in humans following a standard "rheumatoid arthritis dose"²¹. Another comparative study evaluated a single-dose injection of 30 mg/Kg of MTX in tumor-bearing rats and observed that it exhibited antitumor activity²⁴. In addition, a 0.15 mg/kg DEX dose in animals resembles anti-

inflammatory treatment in humans²³. It is difficult to directly compare these doses with those used clinically, because of the relatively elevated rat metabolism. The presence of a characteristic response to each drug suggests the need for further study to describe the effects of polychemotherapy²⁴.

Our results show that after the surgical procedure, the animals continued with food and water intake. The treatment with high dose MTX showed signs of drug-related toxicity in animals as shown in a study that evaluated the impact of cancer chemotherapy on protein metabolism in healthy rats²⁴. The animals also continued to gain weight but the cumulative body weight gain over the study was not significant in MTX high dose treated rats^{9,24}.

An experimental model of condylar fracture, unilateral and medially displaced, which follows the most frequent form of mandibular condyle fracture and allows cephalometric evaluation, was used in this study⁶. Cephalometric evaluations from radiographs of specimens using a computer system, as in this study, lead to reliable measurements, reducing technical difficulties^{5,9,28}. Distances found were similar to those noted in other experiments^{5,28,29}. In the cephalometric evaluation it was possible to measure linear distances, and also the mandibular deviation, by measuring the angles α and \hat{A} ^{3,5,9,30,31}. From an experimental model, only cautious conclusions can be drawn concerning human subjects. There are differences in the temporomandibular joint form and muscular system between rodents and omnivores⁵.

Cephalometric evaluations identified significant progressive growth of the animals. The distance angular process to lower insertion of incisor, in mandible, of DEX and MTX high dose groups, shared significant differences between the sides in the periods of seven and 15 days. The distance tympanic bulla to infraorbital foramen, in maxilla, of DEX group also shared significant difference between the sides in the period of 30 days. The asymmetry between sides may be attributable to the drug treatment but there was probably a recovery over time because the only distance that showed significant difference between the sides in the period of 90 days was infraorbital foramen to upper incisal point, in maxilla, of Control group, and similar findings were reported before⁵.

Based on the results of the severe mandibular shortening, as well as maxillary shortening on MTX High dose group, it can be seen that the consequences on facial symmetry after condylar fractures in MTX high dose treated rats were worse than the ones in DEX, MTX low dose treated and healthy rats. Facial asymmetry is a consequence of the unfavorable healing of condylar fractures^{3,5,32,33} and it is important to know the role of drug treatments in this outcome. Studies are being conducted

in order to understand the effect of these drugs on the healing of condylar fractures.

Measurements of angle α were used to evaluate the mandibular deviation, with reference to the base of the skull, while angle \hat{A} measurements were used to evaluate the deviation of the median line of the mandible in relation to the maxillary median line. There was a tendency towards deviation of the mandible to the fracture side⁹. However, there was significance only in MTX high dose group, indicating negative effects on the fracture repair. Some animals from MTX high dose group showed no incisal wear, and the continuous tooth growth led to a large extraoral projection which may have contributed to the progressive mandibular deviation found. Thus, the effects of the condylar fracture were significant in the MTX high dose group, demonstrated by asymmetry of the maxilla and mandible, especially in the final period of the experiment. The fact that the results of angle \hat{A} have not followed angle α can be explained by a possible maxillary growth influencing mandibular growth and vice versa by occlusal intercuspation^{5,9,34}.

Conclusion

The treatment with high dose methotrexate had a deleterious effect on facial symmetry of rats submitted to experimentally displaced condylar process fracture.

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