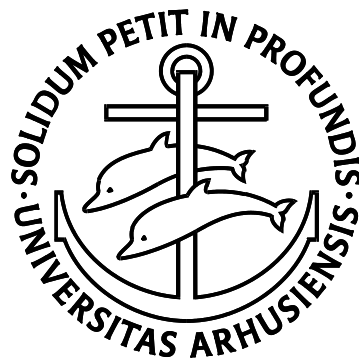


**The Effect of Tobacco Extract and Nicotine Alone and in Combination
on Fracture Healing in Rats**

Research Year Thesis

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Preface

This research year thesis is based on scientific work performed at Orthopaedic Biomechanics Laboratory, Hennipin County Medical Center, Minneapolis, MN, USA and Orthopaedic Research Laboratory, Aarhus University Hospital.

I am very grateful to have had the opportunity to gain an insight into the fascinating world of research. It has been exiting and challenging event, especially the 4 months in Minneapolis were the experiment took place.

This specific research project was initiated by my supervisors Prof. Kjeld Søballe, MD, DMSc. and Joan E. Bechtold, Ph.D. my deepest thanks go to them.

The result of my research year is a manuscript to an article, which has been submitted to the Journal of Orthopaedic Research. I would therefore like to thank my co-authors, which are besides my two supervisors, Troels T. Andreassen, Associate Professor MD, and Michael Ulrich-Vinther, MD, Ph.D. A signed statement concerning my work during this research project can be found at the end of this report.

The study was presented as a poster at the Annual meeting of the Orthopaedic Research Society, Washington DC in February 2005. Abstracts submitted to DOS 2005 and SICOT 2005 can also be found at the end of this report.

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The Effect of Tobacco Extract and Nicotine Alone and in Combination on Fracture Healing in Rats

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Abstract

The influence of nicotine and tobacco extract (without nicotine) alone and in combination on callus formation and mechanical strength of femoral fractures in rats was investigated after 21 days of healing. One hundred and four male Sprague-Dawley rats were divided into four groups receiving: saline, nicotine, tobacco extract, and tobacco extract plus nicotine. One week prior to fracture surgery Alzet miniosmotic pumps were implanted subcutaneously in all animals to administer saline or nicotine. The tobacco extract was administered orally. Over an intramedullary pin, a closed transverse femur fracture was performed and mechanically tested after 21 days of healing. Ultimate torque and torque at yield point of the tobacco extract group was decreased by 21 % ($p=0.010$) and 23 % ($p=0.056$), respectively, compared with the vehicle group and by 20 % ($p=0.023$) and 26 % ($p=0.004$), respectively, compared with the nicotine group. No difference was found between the tobacco extract and tobacco extract plus nicotine groups. An 18 % ($p=0.013$) reduction in torque at yield point was observed in the tobacco extract plus nicotine group compared with the nicotine group. No differences in ultimate stiffness, energy absorption, callus bone mineral content, and callus external dimensions at the fracture line were found between the groups. Serum levels of nicotine were between 40 – 50 ng/mL. Equivalent serum levels are observed in persons smoking one pack of cigarettes per day. In summary, tobacco extract decreased the mechanical properties of healing fractures. Nicotine given in a dose inducing serum levels equivalent to those observed in daily smokers, did not, however, affect mechanical properties of healing fractures.

Key words: tobacco extract, nicotine, fracture healing, mechanical strength, rat

Introduction

Fracture repair is slower in smokers compared to people who do not smoke, and the rate of non-union is augmented among smokers. This has been shown both in clinical trials concerning open/closed tibiae shaft fractures and spinal fusions (1-6).

Furthermore these findings have been supported by a number of fracture and spinal fusion studies in rodents exposed to different levels of nicotine (7-9). The negative impact might be explained by nicotine's inhibition of osteoblastic activity (8;10) and by its vasoconstrictive action on the microvasculature (11-13). The vasoconstrictive effect is seen during revascularization of cancellous bone graft during spinal fusion (14). It might also be that nicotine attenuate a wide range of cytokines which are normally expressed during bone formation (15).

One common feature of the animal studies was that the experiments used only nicotine and that the nicotine usually was administered in doses causing higher serum levels than those observed in smokers consuming a pack of cigarettes daily (12;16;17). Besides nicotine there are about 3500 other compounds in the particulate phase of cigarette smoke, including the lung carcinogenic agents (11;12;18).

To the best of our knowledge, no experiment has been conducted to examine the effect of the tobacco extracts without nicotine on fracture healing. Therefore the aim of this study was to investigate the effects of tobacco extract and nicotine, when given separately and in combination, on rat fracture healing. The tobacco extract was obtained from nicotine free cigarettes and administered intermittently by the oral route. Nicotine was administered continuously in a dose by which we achieved serum levels equivalent to those observed in daily smokers (11;12;16;17;19).

Materials and Methods

Animals and design

The experimental protocol was approved by the institutional review board (Animal Care, Use & Research Committee, Minneapolis Medical Research Foundation).

One hundred and four male Sprague-Dawley rats with an initial weight of 250-275 gram were randomized according to weight into four groups, receiving saline, nicotine, tobacco extract, and tobacco extract plus nicotine. Sample size was based on the following equation: $n = 2(t_{2\alpha} + t_{\beta}) \times SD^2/D^2$. Error of the first kind (2α) was selected to 5% and error of the second kind (β), the risk of concluding that two effects are identical if in fact the difference is below the minimal clinically relevant difference (D), was chosen to be 20 % which means a power of 80%. Based on previous studies, a SD of 20 % and a D of 18% for all variables is justified. Based on these assumptions at least 20 animals in each group had to be included. In the study we included 26, to allow for the loss of 6 animals per group. The rats were housed individually and allowed feed and water ad libitum, except the two groups that received tobacco extract orally during a 16-hour period. The room was maintained on a twelve hour light/dark cycle (the light cycle began at 07.00 hr) at approximately 21°C. Cages, food, and bottles were changed once a week.

Treatment was administered for 4 weeks – corresponding to one week pre-fracture and 3 weeks post-fracture.

Preparation and administration of nicotine

Nicotine barbiturate was obtained from Sigma Chemical Co. (St. Louis, MO). Osmotic minipumps (Alzet 2ML4, Durect Cupertino, CA) were loaded with the nicotine preparation containing a concentration of 14.00 mg/ml. The 2ML4 pumps delivered 2.5 μ L/day for four weeks, which yielded a dosage of 3.0 mg/kg/day.

The pumps were implanted subcutaneously (under i.p. ketamine and xylazine anesthesia at a dose of 80-100 mg/kg and 2-3 mg/kg, respectively) in the intrascapular area using aseptic conditions. The incision sites were prepared by shaving followed by disinfection of the exposed skin with Povidone-iodine USP 10 %. One incision of approximately 5 mm was made in the skin of the dorsal mid-cervical region and the pump was implanted in the subcutaneous tissue of the lateral neck area. The surgical wound was closed by 9-mm staples and daubed with antiseptic crème (Betadine). All animals received a pump containing either saline (vehicle) or the nicotine solution.

Preparation and administration of tobacco extract

Tobacco extract was prepared from nicotine-free Quest Cigarettes (Vector Tobacco Inc, Timberlake, NC), as described by Demady et al. (26). Four cigarettes were cut open, the tobacco ground with a mortar and pestle and placed in a 50-ml plastic tube containing 40 ml water. The tubes were mixed overnight at room temperature using a tube tipper. The contents were filtered through gauze and the liquid was centrifuged at 5000 rpm for 20 min. The supernatant was vacuum filtered using a Buchner funnel and Whatman paper. The extract was filtered again using a 0.2- μ m sterile filter. Three batches were checked for uniformity by measuring anatabine on a GC-MS. The extract was diluted 1/20 for use to correspond to the amount of nicotine used and stored at 4°C. The solution was administered orally by 30 ml. drinking bottles and the daily amount consumed at the end of the 16-hour period was recorded.

Closed fracture surgery

The animals were anesthetized using intraperitoneal injection of ketamine/xylazine at a dose of 100 mg/kg and 2.0 mg/kg, respectively. When the animals reached the surgical stage of general anesthesia, preparation for surgery was initiated, including shaving and disinfection of the left leg.

A lateral parapatellar approach to the left stifle was performed by a 5-mm skin incision (without damaging the patellar ligament). The longitudinal fibers of the quadriceps mechanism were divided and the patella was dislocated medially. Internal rotation of the tibia and valgus of the knee resulted in exposing of the femoral condyles. The marrow canal was reamed with a 1.5-mm 18 gauge needle. A 1.4-mm K-wire (D. Trocar PT 9, 054 1.4 mm) of 33 mm in length was introduced in a retrograde fashion. The pin was countersunk and seated in the proximal femur. Muscles and fascia was reapposed using Ethilon 4-0 suture (Ethicon) and the skin was closed in standard fashion, also using Ethilon 4-0 suture.

Immediately after K-wire placement, a closed transverse fracture in the mid-diaphyseal region of the femur was performed using a fracture apparatus designed according to the original specifications by Bonnarens and Einhorn (20). Presence of the transversal fracture line was confirmed by palpation.

Serum Analysis

To determine nicotine and cotinine levels, blood samples were taken from a tail vein at: fracture, after two weeks of healing, and at sacrifice. The blood samples were centrifuged at 3000 rpm for 15 min. Serum was then stored at -70°C until the gas chromatography (Hewlett – Packed 5890 Series II) with nitrogen-phosphorus detection assay was performed (21).

In a separate study with a similar setup as in the main study the amount of tobacco extract was determined by measuring the urine concentration of the tobacco alkaloids anabasine and anatabine. These markers were determined by gas chromatography-mass spectrometry (22) on 24 hr urine samples collected from rats in metabolic chambers on days 1, 7 and 14.

Mechanical testing

The rats were euthanized with pentobarbital (100 mg/kg intraperitoneally; Beauthanasia-D) and X-rays of the femurs were taken to inspect fracture healing and placement of the intra-medullary K-wire. Femurs were stripped of soft tissue and the K-wire was carefully removed by gentle axial pull with rotational motions. In the fractured femur, the external medial-lateral and anterior-posterior diameters were measured at the fracture line using a sliding caliper. The proximal and distal ends were potted in aluminum fixtures (Wood's metal) with low melting temperature metal while maintaining the callus areas moist. The fracture was tested on an axial-torsion materials testing machine (model AT3045-2800, Bose EnduraTEC Systems Corp., Eden Prairie, MN) with a low capacity 50 in-lb torque cell (Transducer Techniques, Temecula, CA). The femur was aligned so that the longitudinal axis of the mid-femur was approximately collinear with the longitudinal axis of the proximal and distal blocks of potting material and torsional actuator of the materials test machine. The fractures were loaded to failure in torsion at a rate of 0.5 degree/sec. Thereafter, the femurs were stored in 70% ethanol at 10°C until further examination.

Torques were measured to within ± 0.002 Nm in 1.14 Nm range, and angles were measured to within 0.25 % of the full scale (60°), or 0.15°. Torque and rotation were continuously recorded over 3 consecutive cycles with a personal computer and WinTest software (Bose EnduraTEC Systems Corp.) at a rate of 20 Hz. The data were translated into a load-deformation curve where ultimate torque (maximum load) and ultimate stiffness (equal to the maximum slope of the load-deformation curve) were calculated. The energy absorptive capacity was measured as the area under the load-formation curve until ultimate torque. The yield point was defined as the point at which a 20 % reduction in maximum slope occurred.

Dual-energy X-ray absorptiometry (DEXA)

For all fractures only one fracture line and no loose fragments was observed. The two ends of bones tested were realigned at their original position. The femurs were placed in 70% ethanol with the anteromedial surface downward and scanned by DEXA using the regional high resolution analysis program for small animals (QRD-2000; Hologic, Inc. Waltham, MA). The amount of bone around the fracture line was measured in an 8.0-mm-high diaphysial segment, 4 mm proximal and 4 mm distal to the fracture line. BMC was measured in this segment.

Statistical Analysis

The data were tested for normality and homogeneity of variance and when these conditions were fulfilled, parametric analyses were applied. Otherwise, nonparametric analyses were used.

The effects of the different exposure were evaluated using the following groups: Vehicle, Nicotine, Tobacco extract, and Nicotine plus Tobacco extract. Differences between these groups were tested by one-way analysis of variance or Kruskal-Wallis test. In cases in which differences occurred, all pair wise multiple comparisons procedures were applied (unpaired t-test or Mann-Whitney Rank Sum Test). Changes in weight in the individual groups during the experiment were analyzed by using unpaired t-test as normality and homogeneity of variance were fulfilled.

The value of $p < 0.05$ (two-tailed) was considered statistically significant.

Results

Out of 104 animals operated on, 89 were included in the subsequent experiment. Seven animals died as a result of anesthesia, six as a consequence of insignificant fracture and two because of pump malfunction. The number of animals in each group was: vehicle (n=22), nicotine (n=22), tobacco extract (n=23) and nicotine plus tobacco extract (n=22).

The results of the mechanical testing of the fractured femurs are given in Table 1. After 21 days of healing, the ultimate torque of the fractures was decreased only in the group given tobacco extract alone, both in comparison with the nicotine group and the vehicle group (20 %, $p=0.023$ and 21 %, $p=0.010$, respectively). No differences were found between the tobacco extract group and the tobacco extract plus nicotine group (10%, $p = 0.239$). The ultimate stiffness and energy revealed no differences between any of the groups. Compared with the nicotine group and vehicle group, the torque at yield point in the tobacco extract group was decreased by 26 % ($p=0.004$) and 23 % ($p=0.056$), respectively. An 18 % ($p=0.013$) reduction in torque at yield point was observed in the tobacco extract plus nicotine group compared with the nicotine group, as was a 14% reduction when compared with vehicle group ($p=0.167$). Again, no difference was found between the tobacco extract group and tobacco extract plus nicotine group (10 %, $p=0.286$).

Serum nicotine levels at fracture, 2 weeks of healing, and sacrifice are given in Table 2. The levels of nicotine in the nicotine group and the tobacco extract plus nicotine group were in the range of 39 – 49 ng/mL during the observation period. Both in the vehicle group and the tobacco extract group no measurable nicotine levels were observed during the observation period. The levels of cotinine in the nicotine group and the tobacco extract plus nicotine group were in the range of 255 – 320 ng/mL during the observation period. No measurable cotinine levels were observed in either the vehicle group or the tobacco extract group during the observation period.

The daily amount of tobacco extract consumed in the tobacco extract group and tobacco extract plus nicotine group did not reveal any differences (data not shown).

Dimensions and BMC are given in Table 3. No significant differences in anterior-posterior or medial-lateral external callus dimensions were observed at sacrifice. Neither did the callus DEXA-BMC reveal any significant difference between the four groups.

The mean body weights in each group at the beginning and the end of the study are given in Table 4. No differences in body weight were found between the groups at the day of pump implantation. All animals gained weight during the study. In the nicotine group, the body weight at fracture was 4 % lower than in the vehicle group ($p=0.03$). However, at sacrifice no difference in body weight was found between the nicotine group and the vehicle group. At fracture, the tobacco extract group and the tobacco extract plus nicotine group had an increase in body weight compared with the vehicle group (7%, $p<0.001$ and 5%, $p<0.001$, respectively). At sacrifice, no differences in body weight were seen between the tobacco extract plus nicotine group and vehicle group, whereas the body weight of the tobacco extract group was increased by 5% compared with the vehicle group ($p=0.01$).

Discussion

The present study shows that tobacco extract hampers fracture healing, whereas administration of nicotine does not seem to influence fracture healing, when administered in a dose inducing serum nicotine levels similar to those observed in daily smokers. Our nicotine data correspond with Abulencia et al. (8), who showed that rats treated with the same amount of nicotine as used in our experiment (3 mg/kg/day) had normal mechanical properties of healing fractures after 3 and 6 weeks. Abulencia et al. also used a 3 fold higher dose of nicotine and this treatment induced a decrease in fracture strength after 3 weeks of healing. However, this higher dose of nicotine did not influence mechanical strength after 6 weeks of healing.

Raikin et al. (7) treated rabbits with nicotine in a dose of 9 mg/kg/day and found that the mechanical strength of a mid-shaft tibial osteotomy was decreased by 34% after 8 weeks of healing. Spine fusion in rabbits treated with nicotine (6 mg/kg/day) was investigated after 5 weeks of

healing (9). Nicotine did not influence the mechanical strength of the fused spine. In both studies the administration of nicotine began at the initial surgery.

Daftari et al. (14) investigated the effect of nicotine (9 mg/kg/day) on revascularization of rabbit autologous cancellous bone implanted in the anterior eye chamber for 2 and 4 weeks. Nicotine treatment delayed the revascularization and augmented necrosis within the graft.

The animal experiments show that nicotine would need to be given in a dose higher than 3-6 mg/kg/day to impair mechanical properties of healing bone. However, on gene expression levels, nicotine in a dose of 3 mg/kg/day has been shown to decrease mRNA levels in rabbits with spine fusion (15). Nicotine hampered the gene expression of a wide range of cytokines associated with neovascularization and osteoblast differentiation (VEGF, BMP-2, BMP-4, BMP-6, bFGF and collagen I).

The effects of tobacco extract on bone formation have also been investigated in vitro. Galvin et al. (23) cultured chick embryonic tibiae in tobacco extract (with nicotine) and nicotine alone. They found that tobacco extract markedly depressed bone collagen synthesis. Nicotine alone also hampered collagen synthesis. However, the nicotine had to be present in a 12 fold higher concentration to induce the same depression in collagen synthesis as the one observed when using tobacco extract. Lenz et al. (24) cultured osteoblast-like cells obtained from chick embryo calvarias in a medium containing increasing concentrations of tobacco extracts (with nicotine). They found that collagen synthesis decreased with increasing concentrations of tobacco extract. They compared the hampering effects of tobacco extract with their previously published data on nicotine alone (25) and concluded that nicotine had to be present in at least a 3 fold higher concentration to induce the same depression in collagen synthesis. Fang et al. (10) cultured UMR osteoblast-like cells in varying concentrations of nicotine, and found that nicotine produced a decrease in DNA synthesis and an increase in alkaline phosphatase activity. In contrast to the results reported by Fang et al., the

experiment by Lenz et al. (25) showed that nicotine decreased alkaline phosphatase activity and augmented DNA synthesis.

These data are to be interpreted within the confines of the experimental model, and the nicotine and tobacco extract administration schedule and route. The closed fracture in the rat is a well-used animal model for studying modalities affecting fracture healing. However, it bears emphasizing bone healing in the rat is different than bone healing in humans.

The effect of pre-fracture exposure time and post-fracture healing time were not evaluated in this study. Due to the ability of the pump to administer a total four week interval, we exposed the rats to the nicotine and/or tobacco extract only for one week, followed the fracture healing for 3 weeks. It is unknown what effect a longer pre-fracture administration time would have. Also unknown is whether the decreased mechanical properties at 3 weeks with tobacco extract but not nicotine would persist at later time points.

The effect of route of administration is not known. In this study, the nicotine was given with a mini osmotic pump, using technique and equipment with which our group has years of familiarity. The serum nicotine levels achieved with this route of administration were similar to those seen in patients who smoke a pack of cigarettes a day. The tobacco extract was given to the rats in their drinking water. The rats did consume their allotment of tobacco in this manner, and we did not see appetite changes (data not shown).

In conclusion, our study shows that tobacco extract administration decreases mechanical strength of healing rat femur fractures. However, nicotine treatment does not seem to depress the mechanical strength of healing fractures, when given in a dose inducing serum levels equivalent to those observed in daily smokers, for a one week period pre-fracture, and allowed for 3 weeks of healing.

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Appendix

Table 1 MECHANICAL PROPERTIES OF THE FRACTURED FEMORA

	Vehicle	Nicotine	Tobacco Extract	Tobacco Extract plus Nicotine	p value
Ultimate Torque (10 ⁻² N)	16.04 ± 0.9	15.89 ± 1.1 ^c	12.70 ± 0.7 ^{ab}	14.19 ± 1.0	0.050
Ultimate Stiffness (10 ⁻⁴ N/degree)	232 ± 23	257 ± 32	199 ± 23	207 ± 22	0.366
Energy (10 ⁻² N×degree)	122 ± 21	94 ± 10	102 ± 22	150 ± 37	0.403
Yield point (10 ⁻² N)	12.32 ± 1.2	12.94 ± 0.8	9.53 ± 0.8 ^b	10.56 ± 0.5 ^b	0.021

Mean values ± SEM

^a Significantly different from Vehicle, p < 0.05

^b Significantly different from Nicotine, p < 0.05

^c Significantly different from Tobacco Extract, p < 0.05

Table 2 SERUM CONCENTRATIONS OF NICOTINE AND COTININE DURING THE EXPERIMENT (ng/ml)

	Vehicle	Nicotine	Tobacco Extract	Tobacco Extract plus Nicotine	Between nicotine groups
Nicotine (ng/mL)					
Fracture	0 ± 0	43.36 ± 1.60	0 ± 0	41.95 ± 1.87	0.570
2 weeks of fracture	0 ± 0	46.18 ± 1.31	0 ± 0	38.73 ^b ± 1.95	0.003
Sacrifice	0 ± 0	49.14 ± 2.48	0 ± 0	47.64 ± 3.01	0.702
Cotinine (ng/mL)					
Fracture	0 ± 0	320.82 ± 10.51	0 ± 0	310.55 ± 8.74	0.456
2 weeks of fracture	0 ± 0	264.50 ± 4.67	0 ± 0	255.32 ± 9.91	0.407
Sacrifice	0 ± 0	274.14 ± 8.25	0 ± 0	285.55 ± 12.18	0.442

Mean values ± SEM

^a Significantly different from Vehicle, p < 0.05

^b Significantly different from Nicotine, p < 0.05

^c Significantly different from Tobacco Extract, p < 0.05

Table 3 CALLUS PROPERTIES OF THE FRACTURED FEMORAL

	Vehicle	Nicotine	Tobacco Extract	Tobacco Extract plus Nicotine	p value
BMC(mg)	109.16 ± 4.97	119.78 ± 4.49	114.30 ± 3.12	114.96 ± 2.85	0.314
Anterior - Posterior diameter (mm)	6.72 ± 0.17	6.59 ± 0.14	6.45 ± 0.13	6.65 ± 0.11	0.584
Medial - Lateral diameter (mm)	6.94 ± 0.16	7.02 ± 0.12	7.21 ± 0.15	7.13 ± 0.12	0.544

Mean values ± SEM

^a Significantly different from Vehicle, p < 0.05

^b Significantly different from Nicotine, p < 0.05

^c Significantly different from Tobacco Extract, p < 0.05

Table 4 CALLUS PROPERTIES OF THE FRACTURED FEMORAL

	Vehicle	Nicotine	Tobacco Extract	Tobacco Extract plus Nicotine	p value
Body weight at (gram)					
pump implantation	253.0 ± 1.3	252.5 ± 1.7	252.8 ± 1.3	254.7 ± 1.6	0.698
fracture surgery	307.0 ± 2.7	296.2 ^{ac} ± 2.1	328.2 ^{ab} ± 2.5	321.7 ^{ab} ± 2.4	< 0.001
sacrifice	362.1 ± 4.7	365.0 ^c ± 5.2	379.0 ^{ab} ± 3.8	370.9 ± 3.7	0.038

Mean values ± SEM

^a Significantly different from Vehicle, p < 0.05

^b Significantly different from Nicotine, p < 0.05

^c Significantly different from Tobacco Extract, p < 0.05

Tobacco Extract But Not Nicotine Impair Fracture Repair in Rats



ORTHOPAEDIC BIOMECHANICS LABORATORY

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Introduction

Fracture repair is slower and less complete in smokers than in non-smokers. Animals studies have shown that nicotine can be a suppressing agent during fracture repair. However, the dose of nicotine used in these animal studies caused higher serum levels of nicotine than those observed in daily smokers¹. Besides nicotine, there are about 3500 other compounds in the particle phase of a smoked cigarette (the tobacco extracts)². To the best of our knowledge, no experiment has been conducted to examine the effect of the tobacco extracts without nicotine on fracture healing.

Aim of this study

To investigate the effects of nicotine and tobacco extracts, alone and in combination, on fracture healing using a rat closed fracture model.

Materials & Methods

Study design

89 Sprague-Dawley rats were randomized according to body weight, into four groups, receiving: nicotine, tobacco extract, nicotine plus tobacco extract, and vehicle.

Treatment

Nicotine bitartrate or saline was administered for 28 days through miniosmotic pumps (delivered 3.0 mg/kg/day).

Tobacco extract was prepared from nicotine-free Quest Cigarettes in an aqueous solution. Administered orally from 4 p.m. to 8 a.m.

Fracture surgery

After one week, a mid-diaphyseal fracture was performed³.

Statistics

One-way analysis of variance and multiple comparisons procedures were applied (Students t-test). Data are present as mean ± SEM.



Fig. 1 Fracture Stabilized by K-Wire

Results

Mechanical torsion test

Femurs were loaded to failure at 0.5 deg/sec in an axial-torsion testing machine. Ultimate torque, energy absorption and ultimate stiffness were derived. A yield point was defined as the load at which a 20 % reduction in slope occurred.

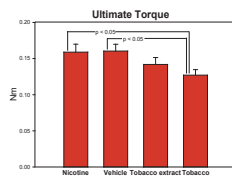


Fig. 2. Comparison of ultimate torque values shows a lower mean value for tobacco extract alone compared to vehicle and nicotine.

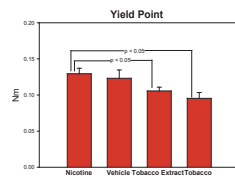


Fig. 3. Comparison of yield point values shows a lower mean value for tobacco extract and tobacco extract plus nicotine compared to nicotine.

Table 1. Ultimate Stiffness and Energy absorption

	Vehicle	Nicotine	Tobacco Extract	Tobacco Extract and Nicotine	p value
Ultimate Stiffness (10 ³ N/degree)	232 ± 23	257 ± 32	199 ± 23	207 ± 22	0.366
Energy (10 ³ N-degree)	122 ± 21	94 ± 10	102 ± 22	150 ± 37	0.403

One-way analysis of variance revealed no differences between the groups.

Serum analysis

Nicotine and cotinine (main metabolite of nicotine) levels were detected by gas chromatography with nitrogen-phosphorus detection.

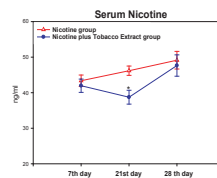


Fig. 4. Mean serum levels of nicotine after 7, 21 and 28 days of exposure. Bars indicate (s) SEM. * Significant difference (p=0.003)

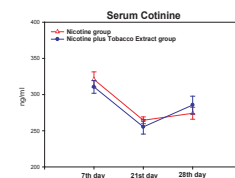


Fig. 5. Mean serum levels of cotinine after 7, 21 and 28 days of exposure. Bars indicate (s) SEM.

Human Serum levels: Nicotine 40 - 50 ng/ml for a person who smokes 20 cigarettes per day⁴.
Cotinine 250 - 350 ng/ml for a person who smokes 20 cigarettes per day⁵.

Conclusion

- I. Tobacco extract without nicotine decreases mechanical strength of the healing fracture compared with vehicle.
- II. Nicotine does not depress the mechanical strength of the healing fracture, when given in a dose inducing serum levels equivalent to those observed in daily smokers.

References:

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Abstract to DOS, August 2005

Introduction

Fracture repair is slower and less complete in smokers compared to people who do not smoke. The influence of nicotine and tobacco extract (without nicotine) alone and in combination on callus formation and mechanical strength of femoral fracture in rats was investigated after 21 days of healing.

Material and Methods

104 Sprague-Dawley rats were divided into four groups receiving; nicotine, tobacco extract, nicotine plus tobacco extract, and vehicle. One week prior to fracture surgery Alzet miniosmotic pumps were implanted subcutaneously to administer saline or nicotine, while tobacco extract were administered orally. A closed transversal fracture was performed as described by Bonnarens and Einhorn.

Results

After 21 days of healing ultimate torque and yield point of the tobacco extract group was decreased by 26 % ($p=0.010$) and 29 % ($p=0.056$), respectively compared to the vehicle and 25 % ($p=0.023$) and 36 % ($p=0.004$), respectively compared to the nicotine group, whereas no difference was found between the tobacco extract and tobacco extract plus nicotine group ($p = 0.239$). A 22 % ($p=0.013$) reduction in yield point were observed between the tobacco extract plus nicotine group and the nicotine group. No differences in ultimate stiffness and energy absorption were found between the groups.

Serum levels of nicotine between 40 – 50 ng/ml were achieved.

Conclusion

Tobacco extract without nicotine decreases mechanical strength of the healing fracture compared with vehicle.

Nicotine does not depress the mechanical strength of the healing fracture, when given in a dose inducing serum levels equivalent to those observed in daily smokers.

Abstract to SICOT, September 2005

Introduction

This study investigated the effects of nicotine and tobacco extract in combination and alone on fracture healing in rats. We hypothesized that mechanical parameters and callus properties of the fractured bone would be impaired by exposure to tobacco extracts and nicotine.

Materials

89 rats were included in the study, divided into 4 groups; 1) nicotine, 2) tobacco extract, 3) nicotine and tobacco extract and 4) saline (control). Nicotine bitartrate or saline was administered for 28 days through miniosmotic pumps (delivered 3.0 mg/kg/day). Tobacco extract orally in a 16 hour daily period.

Methods

A transverse, mid-diaphyseal fracture was induced and stabilized. 4 weeks observation period. By torsion to failure, ultimate torque, linear stiffness and energy absorption were derived. DEXA scans were performed using an 8 mm region of interest for BMC measurements. Callus volume and dimensions were obtained.

Results

ANOVA were performed on transformed data. Tobacco extract significantly lowered ultimate torque ($P=0.006$) but none of the other variables were impaired. Nicotine had no significant effect on any of the variables. Serum levels of nicotine and cotinine were between 40-50 ng/ml resp. 250-350 ng/ml, which are normal levels seen in smokers who smoke 1 to 1½ pack of cigarettes per day.

Discussion

Tobacco extract without nicotine impaired the mechanical properties of the fracture site. Nicotine alone had no adverse impact on fracture healing. Data suggests that nicotine may not be the only component impairing fracture healing in smokers