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ORIGINAL ARTICLE

Smoking Affects Bone Healing and Blood Perfusion

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ABSTRACT

Nicotine is the primary component in cigarettes that is responsible for the negative effects that tobacco has on the body. Nicotine is also the primary component responsible for the addiction that can develop from smoking. Tobacco addiction is also primarily caused by nicotine, which is another component of tobacco.

Aim: The effect that nicotine has on the rate at which bones can recover after being shattered is still a topic of ongoing debate. The study of bone repair and regeneration is made a great deal easier by the utilisation of a model that is known as distraction osteogenesis. The mandibular extension of a rabbit model is serving as the subject of the inquiry that is now being conducted.

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Method: Using the rabbit as a test subject, the purpose of this study is to evaluate the influence that nicotine has on blood perfusion, angiogenesis, and bone formation. There were a total of forty rabbits, and they were distributed among the control group, which received no nicotine, and the nicotine group, which received nicotine. The bunnies were distributed to the several groups in a haphazard manner. It was discovered that each rat had been exposed to nicotine or a placebo for a total of seven weeks during the course of the experiment. After a period of consolidation time ranging from two to four weeks post osteotomy, the animals were slaughtered and their mandibles were removed. After this, there was a lag time of three days, and then there was active distraction for eleven days. Utilizing the techniques of Laser Doppler monitoring and Collagen IV immunohistochemical labelling, respectively, allowed for the assessment of both vascularization and blood perfusion. Studies that were radiographic, histological, and immunohistochemical in nature were carried out in order to investigate the development of bone.

Result : According to the findings, being exposed to nicotine led to an increase in the density of microvessels, but it had a detrimental effect on the flow of blood and the development of bones. In addition to this, it was found that the expression of bone morphogenetic protein (BMP)-2 in osteoblasts was lower than it had been in the past. In the process of distraction regeneration, ischemia and a low oxygen tension were both present, and the creation of cartilage islands on a consistent basis was a sign of both of these circumstances.

Result: As a result of our investigation, we got to the realisation that nicotine consumption slows down the process of bone regeneration. We hypothesise that this takes place as a result of the fact that nicotine leads to ischemia and has an effect that is directly inhibitory on osteoblastic cells. It has been demonstrated that nicotine causes an increase in angiogenesis; however, this improvement is not adequate to compensate for the harmful effect of vasoconstriction caused by nicotine.

INTRODUCTION

Since the first clinical report of using distraction osteogenesis to extend human mandibles, there has been a growing interest among surgeons in employing this surgical approach for the treatment of craniofacial malformations. This interest has been spurred on by the success of using distraction osteogenesis to extend human mandibles. The very first clinical report that was released was what brought all of this attention to the topic. In addition, distraction osteogenesis is an excellent study model that may be utilised to investigate the processes of tissue healing and regeneration. There are some parallels to be seen between it and embryonic development, the production of long bones in neonates, and the healing process following a bone fracture. Mechanical stimulation, such as that which is delivered by regulated traction, is capable of triggering a cascade of biological responses, one of which is bone regeneration. This is because mechanical stimulation causes the bone to remodel itself. As long as the mechanical traction is being applied, the molecular signalling that occurs during distraction osteogenesis is enhanced and maintained for an extended period of time. This continues for as long as the traction is being applied mechanically.

Nicotine is the primary psychoactive ingredient found in tobacco, and it is also the primary component responsible for the addiction that is caused by tobacco usage. It was said to be of the utmost relevance among the potentially hazardous compounds present in cigarette products, but the influence that it has on the healing of bones is still up for discussion at this point in time. [Citation needed] Researchers found that nicotine did not have a significant effect on the process of bone repair and regeneration in a number of the papers and studies that they conducted. On the other hand, the researchers found that nicotine had a detrimental influence in some of the other reports and studies that they conducted.

In the studies that were conducted on the effects of nicotine, a wide variety of wound healing models were utilised. Each of these models required a different quantity of nicotine to be administered, and they did so in a different way. As a consequence of this, the conclusions have been called into question, and the likely reason for this is because nicotine was supplied in a wide variety of various ways during the study. In light of this, it is of the utmost importance to do research on methods of nicotine delivery that are reliable and can be repeated, as well as to establish a direct connection between the plasma concentration of nicotine and the rate at which bones repair themselves. Glowacki and colleagues employed time-release nicotine to validate their theory that erroneous distraction osteogenesis of the rat jaw occurs. The hypothesis states that faulty distraction osteogenesis of the rat jaw occurs. Recently, we developed a rabbit model of mandibular lengthening in order to investigate the impact that nicotine has on bone regeneration by employing nicotine pellets with a timed release of 60 days' worth of nicotine. The purpose of developing this model was to investigate whether or not nicotine aids in the process of bone repair or hinders it. This particular research model included rabbits as its subjects. Surgical implantation of time release nicotine pellets in the subcutaneous tissue of the patient can give a more dependable and convenient technique of maintaining a constant blood nicotine level over an extended length of time when compared to the other methods of nicotine delivery. According to the findings of a study that was carried out under the heading distraction osteogenesis, the impact that nicotine has on the process of bone regeneration is directly proportional to the amount that a person takes in. At low plasma levels, it was discovered that nicotine did not have a significant

detrimental effect on bone healing; nonetheless, the histological images showed that the newly produced bony trabeculae were not as developed as those in the control groups. This was because members of the control groups did not take part in any nicotine consumption during the study. Despite the fact that there were just minute levels of nicotine present in the plasma, this was nevertheless able to take place. A process known as distraction osteogenesis, which involves the healing of bone, was greatly slowed down when the test subject was given a high amount of nicotine. This process is responsible for the repair of bone. The utilisation of this trustworthy rabbit model provides a platform upon which additional research into the effect that nicotine has on the bone healing system may be carried out. [Here's a good example:] The current study utilised a rabbit model of mandibular distraction osteogenesis in order to evaluate the effects that nicotine has on blood perfusion, angiogenesis, and bone healing. This was done so as to better understand how nicotine affects these processes.

MATERIALS AND METHODS

A total of forty rabbits, each of which was nine months old and weighed between 3.6 and 4.0 kilograms, were randomly divided into two groups: a control group and a nicotine group, with twenty rabbits in each group. Either pellets containing 1.5 mg/kg of nicotine with a timed release of 60 days or pellets containing a placebo were placed beneath the subcutaneous tissue in the rabbits' necks. A total of seven weeks was spent exposing each rat to nicotine throughout the course of the experiment.

Following the placement of the implant, a standard treatment consisting of distraction and mandibular body osteotomy was carried out. In a nutshell, a pre-operative dose of an antibiotic and analgesic was given to the animals (long-acting oxytetracycline 30 mg/kg and buprenorphine 0.03 mg/kg), and then the animals were put under anaesthesia with an intramuscular injection of ketamine 35 mg/kg, xylazine 5 mg/kg, and acepromazine 1 mg/kg. The animals were then monitored throughout the procedure using an intrave An incision was created in the skin along the inferior border of one of the sides of the mandibular body. This was done in preparation for the implant placement. A straight body osteotomy cut was performed directly anterior to the root of the first premolar. Titanium screws with a diameter of 2 millimetres were used to secure a bone-borne distractor that had been custom-made and implanted along a plane that was perpendicular to the osteotomy cut. These screws were used to secure the distractor so that it would remain in place. Following the realignment of the periosteum, the muscle, and the skin, the wound was closed using sutures of the 3-0 variety.

After the surgical treatment, the patient received an antibiotic known as long-acting oxytetracycline. This medication was administered intramuscularly on a twice-weekly basis for a total of two weeks. The patient received subcutaneous injections of buprenorphine at a dose of 0.03 milligrammes per kilogramme of their body weight twice day for ten days in order to reduce their pain. After each animal had regained consciousness, a veterinary technician continued to carefully monitor its condition and then continued to keep a close check on it after it was placed under rigorous supervision. Observations were made of the clinical state of the animals, as well as their weight and the amount of food that they consumed. The distraction was activated at 0.9 mm once each day for a total of 11 days after there was a latency period of three days. Following a period of consolidation lasting between two and four weeks, every group had the option to slaughter five animals. All of the measures were conducted in complete privacy, including the collection of the mandibular samples for later analysis in the laboratory.

Laser Doppler monitoring

Before the animals were put to death, a laser Doppler perfusion monitor was utilised in order to get an accurate reading of the volume of blood that was passing through the distraction regenerate. In order to expose the distraction regenerate while the patient was under anaesthesia, the skin and the muscle had to be sliced and pulled. Both the laser probe with the number 307 and the PH 07-4 Miniholder were positioned at a total of six different locations, some of which were anterior to the distraction regenerate and some of which were posterior. The LDPM is able to function because it is predicated on the idea that light that is reflected from an object experiences a change in frequency. The quantity of energy that is altered in the light is proportional to the flow of red blood cells that is taking place in the area around the probe. This is the relationship that has been found. The blood flow in the front and posterior bounds of the distraction regeneration were each represented by the means that were specific to those boundaries. After then, the recording was carried out, and the perfusion was evaluated using arbitrary perfusion units (PU).

Histology: After undergoing the micro-CT examination, the specimens were decalcified in a solution that was composed of 14.5% ethylenediaminetetraacetic acid buffer (pH 7.2). Following the decalcification process, the specimens were processed, and then they were finally imbedded in paraffin wax. A light microscopic examination was performed by first preparing axial slices with a thickness of 5 micrometres using a microtome. These slices were then stained with haematoxylin and eosin.

RESULTS

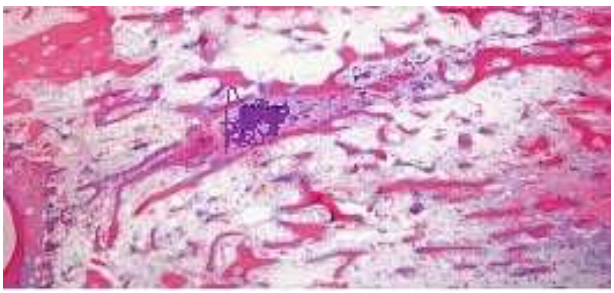
Clinical examination: Each of the forty rabbits carried out the experimental method accurately and to the best of their abilities. Following the surgery, each rabbit saw a temporary decrease in weight, but after a period of 14 days, they started to make improvements in their physique. All of the animals recovered from the surgery without any complications, and all of the distractor variables stayed constant right up to the moment that the animals were killed. In the end, every single one of the rabbits ended up acquiring a severe case of lateral cross bite, in addition to an overgrowth of their bottom incisors.

Table 1: Mean (SD) of blood perfusion (PU) in the rabbit mandibular distraction regenerates

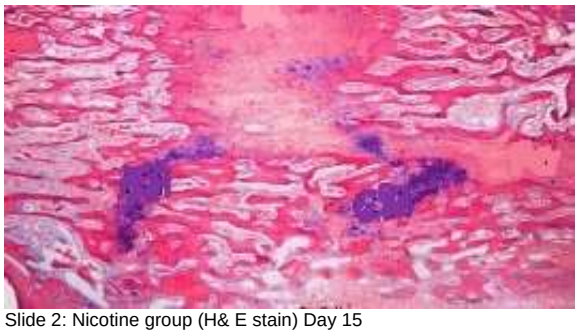
Group	Anterior		Posterior	
	Day 15	Day 30	Day 15	Day 30
Nicotine	7.89	4.98	7.97	7.88
Control	7.12	5.47	8.97	7.99
P	0.0049	0.0198	0.0040	0.0070

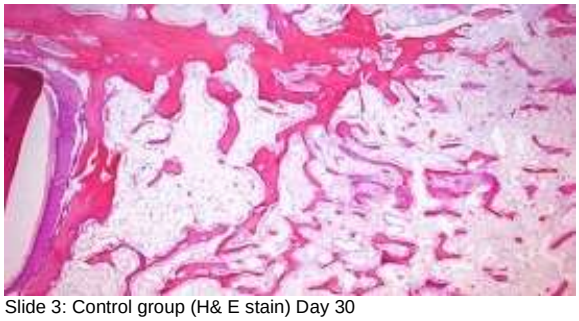
Histology: At the end of the second week, it was observed that bone union had taken place in the core region of the distraction regeneration in the control group, along with the visibility of a localised patch of fibrous tissue. This discovery was made in conjunction with the realisation that bone union had taken place in the core region of the distraction regeneration. It was frequently noticed that the group that had been subjected to nicotine had significant fibrous patches as well as cartilage islands.

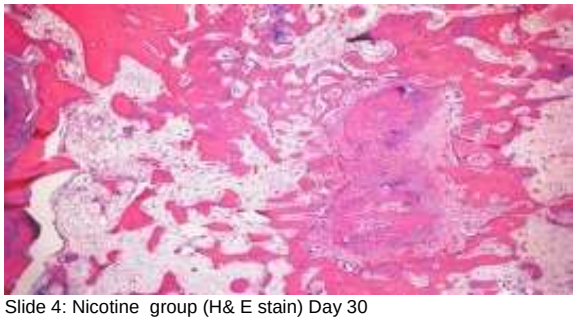
At week 4, full bone union was observed in the centre area of the distraction regeneration in the control group, along with partial corticalization. This was observed in the distraction regeneration. On the other hand, those in the nicotine group frequently observed numerous loci of chondrocytes that were surrounded by a mineralized matrix.



Slide 1: Control group (H& E stain) Day 15


Slide 2: Nicotine group (H& E stain) Day 15


Slide 3: Control group (H& E stain) Day 30


Slide 4: Nicotine group (H& E stain) Day 30

DISCUSSION

The current study utilised 1.5 grammes of nicotine pellets with a temporal release of 60 days in order to imitate the blood nicotine level of heavy smokers. This was done by simulating the blood nicotine level of heavy smokers. On the rabbits that were sacrificed after 2 weeks of consolidation, the osteotomy was performed 3 weeks after the nicotine implantation; on the rabbits that were sacrificed after 4 weeks of consolidation, the osteotomy was performed 1 week after the nicotine implantation. As a consequence, the nicotine exposure time for all of the animals was 7 weeks.

The results of the plain X-ray, the micro-CT, and the histology all pointed to the same conclusion, which was that the use of nicotine inhibited the process of distraction osteogenesis, which is the process by which bones are repaired.

The distracting regeneration in a normal instance of craniofacial distraction osteogenesis generally proceeds through considerable mineralization by intramembranous ossification without a cartilage intermediary. This occurs in a normal case of craniofacial distraction osteogenesis. This procedure is carried done in the absence of cartilage. Even though the production of endochondral bone is extremely uncommon, it has been known to happen on occasion during the early stage of the distraction regenerate process, on the periphery of the wound, at the point where the callus has outgrown its blood supply. This can happen at any time during the distraction regenerate process. Cartilage is an ideal medium because it has reduced oxygen requirements, and it also serves as a temporary bridge until the vascularization catches up with the demand for oxygen in the medium. If any of the circumstances that cause ischemia or low oxygen tension in the distracted regenerate occur, there is a possibility that the predominant bone formation pathway would switch from trabecular to endochondral ossification. This would change the major bone formation pathway. During week 2 of the consolidation phase, the existence of evident cartilage development in the nicotine group showed a tone of ischemia and reduced oxygen tension in the distraction regenerate. Even after an additional four weeks of consolidation, the bone formation in the group that had been exposed to nicotine was not comparable to that of the normal group. This was the case despite the fact that the normal group had been exposed to nicotine for the same amount of time. Even at this late stage, it is possible to demonstrate that the mineralized matrix completely encircles the chondrocytes. In addition, we looked into how the presence of nicotine influenced the process of angiogenesis and blood flow in the distracted regenerate and came to the following conclusions: In comparison to the nicotine group, which exhibited a noticeably larger density of neovessels, the control group had a much lower density of these new blood vessels. According to the findings of a number of studies, nicotine is able to directly affect the small blood arteries, where it can cause vasoconstriction and systemic venoconstriction, both of which can lead to hypoxia. This ability of nicotine to directly affect the small blood arteries was found to be true. A recent study also reported that nicotine stimulated the accumulation of hypoxiainducible factor-1 (HIF-1), whose function is tightly regulated by cellular oxygen concentration, and activated downstream hypoxiaresponsive genes such as vascular epithelial growth factor. Both of these findings were based on the findings of the same study. The activation of the buildup of HIF-1 by nicotine was responsible for both of these effects, which were detected as a result (VEGF).

The expression of VEGF, which is a mediator in the process of angiogenesis, has a significant and positive correlation with the phenomenon of angiogenesis. It is probable that this is what is responsible for the higher microvessel density seen in the nicotine group. It is interesting to note that the increased vessel density did not independently lead to a significant increase in the blood flow in the distraction regenerate. This is something that needs to be taken into consideration. According to these findings, nicotine is responsible for vasoconstriction and also promotes angiogenesis. Despite the fact that angiogenesis is increased, it is not possible for this to compensate for the ischemia that is caused by vasoconstriction in the distracted regenerate.

The majority of the time, the modulation of the processes that are engaged in the healing of tissues can be attributed to the activities of local cells and signalling molecules. One of the markers that may be used to evaluate the biological environment in distraction regeneration is the expression of endogenous BMPs, which is believed to be one of the markers that can be employed. This is due to the fact that the body's own endogenous BMPs are the most potent inducers of osteogenesis. The findings of various research indicate that the expression of BMPs normally maintains a high level for a length of time equal to two weeks after the termination of the distraction. Osteoblasts are the cells that are responsible for healing shattered bones once they have been fractured. They direct the following mineralization of bone matrix and are responsible for the synthesis of bone morphogenetic proteins (BMPs), as well as almost all of the constituents of bone matrix. Additionally, they are important for the formation of bone matrix. It was discovered that exposure to nicotine lowered the gene expression of BMPs in the autogenous bone graft used in the rabbit posterolateal spine fusion model. The model was conducted using rabbits. Researchers Kim et al. demonstrated that nicotine was a factor in the degenerative process of discs. They hypothesised that this was because nicotine inhibited the effect of BMP-2. On the other hand, there was no data indicating that nicotine has any direct effects on BMP in osteoblasts. According to the findings of the current research, it was discovered that the

intensity of the BMP-2 signal was significantly lower in the osteoblastic cells of the nicotine group during the active bone regeneration period at week 2 of consolidation. This was the case during the period in which the bone was actively regenerating. During the period in which active bone regeneration was taking place, this was seen. The process of active bone production was completed by the conclusion of the fourth week of the consolidation phase. The BMP-2 signal was no longer detectable in the osteoblastic cells of either the control group or the nicotine group after this point. This suggested that nicotine has an immediate effect on osteoblastic cells and their capacity to reduce BMP-2 expression.

CONCLUSION

In conclusion, continuous exposure to nicotine may create ischemia and hypoxia, which in turn stimulates the formation of new blood vessels (angiogenesis). However, the increased angiogenesis cannot fully compensate for the unfavourable effect that nicotine's vasoconstriction has on the body since it is not enough to counteract the vasoconstriction that nicotine causes. There is a possibility that chronic exposure to nicotine decreases BMP expression in osteoblasts, which also leads to impaired bone healing. This would be the case if the nicotine was present for a long enough period of time. An investigation into the longitudinal expression of angiogenesis and bone healing-related variables is required in order to acquire a more in-depth comprehension of the part that nicotine plays in the process of bone regeneration. This is necessary in order to gain a deeper understanding of the role that nicotine plays in bone regeneration.

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