ANTI-INFECTIVE EFFECTIVENESS OF ASCORBIC ACID IN THE ORAL MUCOSA OF GUINEA PIGS

Dental Science

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ABSTRACT

Objective: To know the anti-infective effectiveness of ascorbic acid, after a linear incision in the mucosa of the mandibular antero-inferior segment of the oral cavity of the guinea pigs, housed in the vivarium of the Biology Center of the Central University of Ecuador.

Materials and methods: This study is experimental in-vivo and longitudinal, where an incision was made in the mucosa of the mandibular antero-inferior segment of the oral cavity in guinea pigs, three groups of 10 guinea pigs were formed, Group C, received mega doses (1.42 ml / 214.2 mg) which contained high amounts of ascorbic acid, Group B received normal doses (0.095 ml / 14.25 mg) and group A or control group did not receive ascorbic acid, this dose was applied one day before, during, and one day after the incision. The clinical evaluation was performed at 24 and 72 hours post incision, a sample was taken by swab from the wounded area at 72 hours for the planting of bacterial cultivation. Results: The clinical examination shows that at 24 hours in group C, 0% bleeding, edema, and erythema, and 100% epithelial junction, while in group B, 30% bleeding, 50% edema, 50% erythema and 100% epithelial junction and in group A 50% bleeding, 40% edema, 40% erythema and 20% epithelial junction; at 72 hours the results in group C and B showed better healing percentages in relation to group A, in this group some guinea pigs still showed bleeding edema and erythema in the wounded area.

Conclusion: It is determined that the guinea pigs who received mega doses of ascorbic acid presented a better result in the process of healing after clinical examination at 24 and 72 hours and the swab exam shows a lower amount of bacterial colonization, thus demonstrating the effectiveness of ascorbic acid in mega doses of infectious processes.

KEYWORDS

GUINEA PIGS / INFECTION / ASCORBIC ACID / MEGA DOSES

INTRODUCTION

Among the complications that a dental professional could find in the oral cavity is infection, in which the bacteria responsible are mostly the same that make up the normal oral bacterial flora, a combination of aerobics and anaerobics (1). According to Kasper D. et al. 2013 (2), this is a process of complicated interactions between bacteria and host, which can be classified in several stages: encounter of the microorganism with the host and incursion, multiplication, evasion of host defenses, invasion, tissue tropism, tissue damage. There are several conditioning factors that influence the spread of infections that depend on the conditions of patients and pathogenic organisms. In addition, it should be taken in mind, that if the general dental principles and the appropriate aseptic rules are not applied correctly, any intervention can cause an infectious condition (3).

According to Chiapasco M. (2004), (4) infectious processes in the oral cavity should not be underestimated, they can develop serious clinical conditions, which require hospitalization of the patient and can be life-threatening. This is why this research seeks to determine the contribution of Ascorbic Acid in the immune activity, trying to verify its anti-infective effectiveness. Ascorbic Acid is known to have a stimulating effect on the phagocytic activity of leukocytes, on the function of the reticulo-endothelial system, and on the formation of antibodies; it can also stimulate the production of inter-ferons, which are the proteins that protect the cells against viral attacks (5). The role in immunology is of vital importance, since it is directly involved in the production of immunoglobulins, complement factor C3, prostaglandins, it also destroys the neuraminidase of the viruses, preventing cell infection and reproduction (6).

The study in guinea pigs is considered, due to the fact that they are very versatile animals, who have served man kind in many different ways. They present unique features that distinguish them from other rodents used in the laboratory, some of the important characteristics are: early maturity, ascorbic acid requirement in the diet and sensitivity to many antibiotics (7), the same that allow the choice made to carry out this experimental work, mainly due to the similarity of the oral mucosa and the inability to metabolize Ascorbic Acid as well as human beings.

In guinea pigs, among the main means and routes of infection suggested include abrasions of the oral mucosa, which similar to

humans possess a varied bacterial flora, so they are prone to develop frequent infectious processes and periodontal disease, in guinea pigs due to the constant consumption of harsh foods and in humans due to the close encounter to several local and general factors and also when subjected to dental surgical procedures (8).

For this reason, the following hypothesis is proposed: The administration of Ascorbic Acid is effective in reducing infectious processes and it favors the healing procedure of the oral mucosa of the guinea pigs.

MATERIALS AND METHODS

The present study is experimental in vivo and longitudinal, performed on animals (guinea pigs), which was approved by the Ethics and Research Committee of the Central University of Ecuador (SEISH-UCE).

The study population is conformed by 30 guinea pigs, which were obtained at the Faculty of Veterinary and Zootechnics of the Central University of Ecuador, located in the Parish of Uyumbicho of Canton Mejía, with which 3 groups of 10 guinea pigs were established, in order to perform the experimentation; the sample is established according to the following inclusion criteria: Healthy, male, 3 months old guinea pigs (*Cavia porcellus*) weighing between 1000 - 1400 grams.

Procedure

It initiated with the conditioning of the vivarium of Biotechnology Center of the Central University of Ecuador 2 days prior to the transfer of the guinea pigs. Cages with certain requirements were used for care purposes and were properly disinfected with quaternary ammonium. The adaptation stage consisted of 8 days in which the guinea pigs received the same amount of food as in the hatchery and total control in the ambient temperature to avoid illness or death. To reduce the probability that guinea pig develops wounds in the oral mucosa and / or infectious processes due to contamination with the forage, the feed was changed to balanced, a process that took 15 days. The weight of each of the guinea pigs is obtained by a laboratory precision balance, after this a clinical examination of the oral cavity of the guinea pig was performed to rule out some type of infection or wounds in the mucosa, which did not present wounds with the change of diet.

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The expiriment phase was carried out in 5 days as described below:

Figure 1. Summary Of Activities Of The Experimental Phase In Guinea Pigs

Day	First Activity	Second Activity	Third Activity
1	Administration of Ascorbic Acid (first dose), with insulin needles and 1 ml syringes to the guinea pigs of Group C (Mega doses) 1.42 ml / 214.2 mg; of Group B (Normal Dose) 0.095 ml / 14.25 mg and Group A (Control) to which Ascorbic Acid was not administered.	15 minutes later a clinical examination of the oral cavity was performed to rule out mucosal lesions.	
2	Administration of Ascorbic Acid (second dose)	Sedation	Linear mucosal incision
3	Administration of Ascorbic Acid (third dose)	Clinical exam of the mucosa	
4	No activity		
5	Clinical examination of the mucosa of guinea pigs	Sampling using swab	

All of the guinea pigs under study, were submitted to clinical examination which was performed 24 hours after the incision. Photographs were taken as evidence and all the data obtained was recorded in files arranged in Excel, where the number designated to each guinea pig, weight, whether or not there is bleeding, edema, erythema, and epithelial union of the wound, is revealed.

At 72 hours after the incision, a sample of the wound site of the oral mucosa was taken with a single-use swab. The swabs were stored in sterile tubes with physiological serum and hermetically sealed to avoid contamination, subsequent to this the cultivation was being prepared. As a factor of cultivation blood agar was used, which was placed in Petri dishes, and therefore proceeded to the cultivation taking the swab sample with it, these boxes were autoclaved and cooled down to room temperature; once the cultivation factors were sterilized with agar, the work surface was cleaned with alcohol, an alcohol burner was lit and work was carried out near the flame, all this was done inside the laminar flow hood. The blood agar is poured into the Petri dishes, covered and was left to solidify, then a sample of liquid from the swab tube was taken with a sterile pipette; a sowing handle was taken, sterilized through the flame and was left to cool down for a few seconds; The sample is spread over the cultivation source with the help of the handle. The procedure is performed twice to have a triplicate of the same tube. Petri dishes are placed in an inverted position in the incubator at 35 degrees Celsius for 24 hours and the next day the growth was inspected.

When the Petri dishes were removed from the incubator, the colony forming units were counted, starting with the Control group, then the Normal Dose group and finally the group that received Mega doses. The observation was carried out in microscopes of the Laboratory of the Biology Center of the Central University of Ecuador, thus confirming the presence of bacteria of the genus Streptococci, typical of an infectious process. The data of Cologne Formation Units from the investigation were analyzed using the ANOVA statistics test.

RESULTS

In Table 1, at 24 and 72 hours, it can be observed that in group C (guinea pigs with megadoses of Ascorbic Ac) bleeding and edema (0%); group B (guinea pigs with normal dose of Ascorbic Ac), at 24 hours 30% show bleeding and 50% show edema, while at 72 hours 100% have no bleeding or edema; in the control group, 50% bleeding and 40% edema are observed within 24 hours, while at 72 hours 30% show bleeding and edema..

Table 1 Post-incision Bleeding And Edema In The Guinea Pig Mucosa At 24 And 72 Hours

Time	Guinea pig group	Bleeding		Edema		Total
		yes	no	yes	no	
24 hours	A, Group Control	5(50,0)	5(50,0)	4(40,0)	6(60,0)	10(100,0)
	B. Normal dose of Ac. Ascorbic	3(30,0)	7(70,0)	5(50,0)	5(50,0)	10(100,0)

	C. Mega doses of Ac. Asorbic	0	10(100,0)	0	10(100,0)	10(100,0)
72 hours	A, Group Control	3(30,0)	7(70,0)	3(30,0)	7(70,0)	10(100,0)
	B. Normal dose of Ac. Asorbic	0	10(100,0)	0	10(100,0)	10(100,0)
	C. Mega doses of Ac. Asorbic	0	10(100,0)	0	10(100,0)	10(100,0)

In table 2, it can be observed in group C (guinea pigs with mega doses of Ascorbic Ac), that at 24 and 72 hours 100% present no symptoms of erythema but does present epithelial union; group B (guinea pigs with normal dose of Ascorbic Ac), at 24 hours 50% have erythema and 100% have epithelial junction, while at 72 hours 100% do not have erythema but do have epithelial junction; in the control group it is observed at 24 hours 40% presents erythema 20% presents with epithelial union, while at 72 hours 40% presents erythema and 80% presents epithelial union.

Table 2 Presence Of Epithelial Junction And Post-incision Erythema In The Guinea Pig Mucosa

Time	Guinea pig group	Bleeding		Edema		Total %
		yes	no	yes	no	
24 hours	A, Group Control	4(40,0)	6(60,0)	2(20,0)	8(80,0)	10(100,0)
	B. Normal dose of Ac. Asorbic	5(50,0)	5(50,0)	10(100,0)	0	10(100,0)
	C. Mega doses of Ac. Asorbic	0	10(100,0)	10(100,0)	0	10(100,0)
72 hours	A, Group Control	4(40,0)	6(60,0)	10(100,0)	0	10(100,0)
	B. Normal dose of Ac. Asorbic	0	10(100,0)	10(100,0)	0	10(100,0)
	C. Mega doses of Ac. Asorbic	0	10(100,0)	10(100,0)	0	10(100,0)

It can be verified according to the data obtained in the tables, that at 24 and 72 hours Group C presents values with better results, that is, less bleeding, without the presence of edema, erythema and wound closure in less time than the groups (A and B).

Table 4 shows the Colony Forming Units at 72 hours after the guinea pig incision, descriptive data that allows identifying the mean and the respective ranges of the UFC, the standard deviation that allows identifying the variability of data, group A being superior. The F test allows comparing the variation of the means in groups (A, B and C).

Table 4. Descriptive Forming Units Of The Colony

Guinea Pig groups	Mean $\pm$ D.E
A, Group Control	14,6 $\pm$ 2,06
B. Normal dose of Ac. Asorbic	9,1 $\pm$ 1,79
C. Mega doses of Ac. Asorbic	7,5 $\pm$ 1,84

Table 5. Association of ANOVA UFC groups

Analysis	Quadratic mean	P
Between groups	138,7	0,00
In groups	3,62	

When subjected to different doses there exists variation, statistically speaking the value of p (significance) <0.05. The confidence level of the test is over 95%. For comparison between groups it is to come to the Tukey test.

Table 6. Multiple comparisons HSD Tukey

(I) Groups	(J) Groups	Mean difference (I-J)	P
Group Control	Dosis normal	5,50	0,00
	Megadosis	7,10	0,00
Normal Dosis	Megadosis	1,60	0,16

With this test the variation between control group (A), normal dose (B) and megadoses (C) is evidenced, when presenting the difference between means, the significance value $p(0.00) < 0.05$.

Image 1. Observation Of Guinea Pigs Of The Control Group. The Wound Is Observed With Hemorrhage, Edema And Mild Erythema

Image 2. Observation Of Guinea Pigs Of The Normal Dose Group. Fibrous Tissue Is Observed In The Area Of The Incision. It Has No Edema Or Erythema

Image 3. Observation Of Guinea Pigs Of The Megadoses Group

DISCUSSION

Ascorbic acid is considered a potent antioxidant, which humans cannot yet synthesize, so he must obtain this from exogenous sources such as vegetables and fruits, the lack of this vitamin in humans can cause bleeding, as well as delays in healing and the predisposition to infectious processes (9).

In this study, the normal dose of Ascorbic Acid was administered in 10 guinea pigs, megadoses in 10 guinea pigs, to demonstrate the effectiveness it has in healing and in infectious processes, clinical,

macroscopic and microbiological changes of the mucosa of the guinea pigs were analyzed after one incision made, the two groups of guinea pigs were compared with the control group of 10 guinea pigs that did not receive ascorbic acid.

In the study conducted by López Hernández et al. (2006) (10), Ascorbic Acid and / or tocopherol in post-surgical soft tissue healing (study in guinea pigs), mention that because of the characteristics that Acid Ascorbic has, it improves not only healing but also decreases the probability of the development of infectious processes. In the bibliographic review conducted by Logroño et al. in 2017 (11),

Use of mega doses of Vitamin C in the healing and disinflammation of surgical wounds, demonstrate the therapeutic efficacy of the intravenous dose of vitamin C, regard to the reduction of the inflammatory response and the increase in scar capacity before a mainly post-surgical wound. Studies and revisions that agree with the results of the present investigation where it was verified, observing at 24 hours, in the guinea pigs to which Ascorbic Acid was administered in mega doses, 100% epithelial union, in relation to the control group that presented 20%. Favorable postsurgical percentages were also obtained in relation to edema and erythema with 0% in the mega doses group, while 40% in edema and 40% in erythema were observed in the control group.

Authors such as Pinto F. 2013 (5), have applied Ascorbic Acid in mega doses for different diseases. He ensures that the use of Ac. Ascorbic in high doses acts effectively to counteract the progress and symptomatology of these diseases, due to the antioxidant property it has, and also shows several cases where favorable clinical changes are evident; in the present study to the guinea pigs who administered mega doses of Ac. Ascorbic 1.42 ml / 214.2 mg, clinical evolution data was obtained, after 24 hours there is no bleeding, edema or erythema which is supported by images 1, 2 and 3 of the previous page, facts for which the administration of mega doses is recommended when observing a better clinical evolution in a certain group of guinea pigs.

In order to analyze the bacterial load, the sample was taken at 72 hours by means of a swab from the wound site in the mucosa of the guinea pigs of each group, obtaining the following means 14.6 CFU for the control group; 9.1 CFU for normal dose; and 7.5 CFU for mega doses. The standard deviation allows identifying the variability of data, the control group being superior, so it is concluded that the administration of Ascorbic Acid in mega doses is effective in reducing infectious processes, since this group has a lower number of CFUs in the area of the wound, supporting what Oudemana-van H et al. 2014 (6), through clinical studies shows that a high dose of vitamin C can increase antibacterial defense, obtaining higher than normal percentages in the analysis of phagocytic cells. In addition, the protective effects against oxidative stress seem to mitigate the lesions and promote recovery in most tissues.

Wilson M et al. 2014 (12), states that there are very polarized views on the use of high doses of Ascorbic Acid, with passionate advocates, balanced by energetic critics such as Vicari T et al. 2014 (13), who mentions that there is no effective substance against infectious processes in addition to the use of antibiotics, which is contrasted with this investigation where a lower percentage of bacterial load is clearly obtained in the group to which mega doses of ascorbic acid was administered. This is a key reason why clinical trials should be carefully controlled, rather than being a review of the literature, these are necessary to get a clear view of this field. Thus, this research was carried out with the greatest possible objectivity and thus can be a support for the great benefits of Ascorbic Acid that could be observed during the experimental part of it.

In the study conducted by Hurtado M. in 2017 (14), effects of intravenous vitamin "C", perceived by cancer patients in a District of Lima, mention that the administration of ascorbic acid in high doses is toxic to cancerous cells, likewise, increases the antioxidant effect and decreases the levels of oxidative stress, concluding that the majority of patients experienced lower physical effects of cancer treatment, decreasing fatigue levels, increased level of well-being and appetite, increased physical performance and decreased anxiety.

According to studies carried out by several authors, it is shown that Ascorbic acid is effective in normal doses and much more if the administration is in mega doses, improving and speeding up healing;

therefore, this research supports the results obtained through direct observation with bacterial cultures and counting of Colony- forming Units (UFC).

CONCLUSIONS

Based on the results obtained in this investigation, it is concluded that the application of ascorbic acid in mega doses can be effective in healing and in the prevention of infectious processes in the surgical procedures of the oral mucosa, since this group of guinea pigs obtained better results in relation to bleeding, edema, erythema and epithelial union compared to the normal dose applied and even more in relation to the control group that did not receive any dose. It is concluded that group C (mega doses) has a lower bacterial load so that the possibility of developing infection decreases and with this the anti-infective effectiveness of ascorbic acid is confirmed by means of the counting of colony forming bacterial units.

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