“VITAMIN C MEGA DOSE vs. STANDARD DOSE IN SMOKERS WITH SUBCLINICAL HYPOVITAMINOSIS”

“A CONTROLLED, RANDOMIZED CLINICAL TRIAL”

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<table>
<thead>
<tr>
<th>TABLE OF CONTENT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> INTRODUCTION</td>
</tr>
<tr>
<td>DEFINITIONS</td>
</tr>
<tr>
<td><strong>2</strong> JUSTIFICATION</td>
</tr>
<tr>
<td><strong>3</strong> OBJECTIVES AND PURPOSES</td>
</tr>
<tr>
<td>3.1 PURPOSE</td>
</tr>
<tr>
<td>3.2 GENERAL OBJECTIVE</td>
</tr>
<tr>
<td>3.3 1.2 SPECIFIC OBJECTIVES</td>
</tr>
<tr>
<td><strong>4</strong> THEORETICAL BACKGROUND</td>
</tr>
<tr>
<td>4.1 CHRONOLOGICAL DESCRIPTION</td>
</tr>
<tr>
<td>4.2 EPIDEMIOLOGY OF SMOKING:</td>
</tr>
<tr>
<td>4.2.1 Generalities</td>
</tr>
<tr>
<td>4.2.2 Study of Observation of the Future (MTF, by its abbreviation by initials in English)</td>
</tr>
<tr>
<td>4.3 GENERALITIES OF VITAMIN C AND DOSE CONCEPT</td>
</tr>
<tr>
<td>4.3.1 Generalities</td>
</tr>
<tr>
<td>4.3.2 Concept on Standard Dose</td>
</tr>
<tr>
<td>4.3.3 Concept on Megadose</td>
</tr>
<tr>
<td>4.3.4 Definitions of DOSE - unified for this present study</td>
</tr>
<tr>
<td>4.4 BODY RESERVE AND VITAMIN C REQUIREMENTS</td>
</tr>
<tr>
<td>4.5 VITAMIN C METABOLISM AND URINARY SPILLOVER</td>
</tr>
<tr>
<td>4.5.1 Vitamin C Metabolism</td>
</tr>
<tr>
<td>4.5.2 Urinary Spillover</td>
</tr>
<tr>
<td>4.6 TOXICITY</td>
</tr>
<tr>
<td>4.7 SIDE EFFECTS AND PRECAUTIONS OF VITAMIN C USE</td>
</tr>
<tr>
<td>4.7.1 Side Effects</td>
</tr>
<tr>
<td>4.7.2 Precautions</td>
</tr>
<tr>
<td><strong>5</strong> METHODOLOGY</td>
</tr>
<tr>
<td>5.1 TYPE OF STUDY</td>
</tr>
</tbody>
</table>
5.2 CONCEPTUAL HYPOTHESIS
5.2.1 Alternate Hypothesis
5.2.2 Null Hypothesis

5.3 HYPOTHESIS OF THE VARIABLES OF SIDE RESULT
5.3.1 Side Effects

5.4 POPULATION AND SAMPLE
5.4.1 Reference Population
5.4.2 Study Population
5.4.3 Sample

5.5 INCLUSION AND EXCLUSION CRITERIA
5.5.1 Inclusion criteria
5.5.2 Exclusion criteria

5.6 DEFINITION OF THE INTERVENTION
5.6.1 Protocol of Intervention
5.6.2 Preparation and Application
5.6.3 Procedure for the venous catheterism
5.6.4 Study procedure

5.7 RANDOMIZATION AND MASKING
5.7.1 Procedure
5.7.2 Initial considerations for the intravenous managing of Vitamin C Megadose

5.8 VITAMIN C PRESENTATION
5.8.1 Intravenous Presentation
5.8.2 Handling of the product
5.8.3 GATHERING OF INFORMATION

5.9 VARIABLES

5.10 CONTROL OF BIASES
5.10.1 Bias of Selection
5.10.2 Bias of Information
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.11 STATISTICAL ANALYSIS</td>
<td>36</td>
</tr>
<tr>
<td>5.12 ETHICAL CONSIDERATIONS</td>
<td>37</td>
</tr>
<tr>
<td>5.12.1 SPECIAL CONSIDERATIONS</td>
<td>37</td>
</tr>
<tr>
<td>6 RESULTS</td>
<td>40</td>
</tr>
<tr>
<td>7 DISCUSSION</td>
<td>44</td>
</tr>
<tr>
<td>8 ACKNOWLEDGMENTS</td>
<td>46</td>
</tr>
<tr>
<td>9 REFERENCES</td>
<td>47</td>
</tr>
</tbody>
</table>
TABLES, GRAPHICS AND FIGURES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Classification of Variables</td>
<td>34</td>
</tr>
<tr>
<td>Table 2</td>
<td>Manual of Variables</td>
<td>35</td>
</tr>
<tr>
<td>Table 3</td>
<td>Characteristics of patients at the beginning of the study for the two groups</td>
<td>41</td>
</tr>
<tr>
<td>Table 4</td>
<td>Comparison before and after the GDE and GMD intervention</td>
<td>42</td>
</tr>
<tr>
<td>Graphic 1</td>
<td>Comparison of Vitamin C Levels in urine in the two groups at the end of the follow up, day 17</td>
<td>43</td>
</tr>
<tr>
<td>Figure 1</td>
<td>Recruitment and Follow up of Patients</td>
<td>40</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

There is enough literature which supports the relationship between Vitamin C intake and animal, cellular cultures, and human health. Aside from the functions in the forming of collagen, Vitamin C acts as a powerful antioxidant, it increases absorption of inorganic iron; it plays an essential role in the metabolism of folic acid, of some amino acids and of hormones. Vitamin C occupies a place in the history of nutritional epidemiology, because the discovery that scurvy was preventable by handling diet, was an outstanding demonstration of the relationship between a concrete deficiency in diet and a specific disease. No doubt, the medical study of the English Naval, James Lind in 1753, on the treatment of scurvy, has been considered one of the first intervening studies carried out, although, the size of the sample he used (2 patients by each treatment) was insufficient according to modern standards. Lind gave citric fruits to a group of sailors in addition to their standard diet, and he compared it with the other group that only took the standard diet.

Vitamin C was isolated in 1928 by Dr. Szent-Gyorgyi, work by which he received The Nobel Prize. Latter on, its chemical structure was discovered in 1930, and in 1933 it synthesized for the first time.

In 1954, Linus Carl Pauling, from US receives the Nobel Prize in Chemistry for his research on Chemical bonds of Vitamin C and the atomic structure of hemoglobin. Dr. Linus Pauling was an outstanding scientist in the medicine world; and he has been the only individual who has achieved two Nobel Prizes - not shared. What Albert Einstein described for Physics, Pauling contributed for Chemistry. Medicine and Nutrition would be two completely separated fields, if we would not count on Pauling’s discoveries. In his books, - rated as best sellers, and in his multiple articles published, Pauling proposed the intake of high dose of Vitamin C for the improvement of multiple pathologies, manly for infectious diseases, cancer and aging.

The imbalance generated by oxidative stress and the antioxidant defense has been implicated in the pathogenesis of several diseases. Even though the effect of antioxidant complements in the diet - in many aspects of human health - has been amply investigated, the various studies have rendered contradictory results. The estimates on the requirements of Vitamin C, are focused on protecting against scurvy, - as if it were the only pathology generated by the absence or deficiency of this vitamin. On the other hand, optimum requirements may depend on
homeostasis. The administering of Vitamin C megadose claimed by Cameron and Pauling in the United States in the 70’s as a possible treatment for cancer, and the suggestion that deficient intake of Vitamin C could predispose to cancer, has generated multiple controversies. The megadose of Vitamin C produces high plasmatic concentrations, if it is applied intravenously.

The difference between this way of administering and the oral one had not been identified until Cameron, Campbell, Klenner and Pauling’s publications in the United States. But this publications have been controversial; hundreds of patients, having terminal cancer, were treated with 10 gr. of intravenous Vitamin C during 10 days, and then with 10 gr. orally for several months. The results were compared in more than 1000 retrospective and prospective controls. The patients treated with Vitamin C survived from 150 to 300 days more than the controls.

Other researchers reported benefit, which consisted in survival increase, well-being and lessening of pain; but all these studies were not controlled, and factors, not related to the intervention, may have affected the result. Two randomized, double-blind studies, from the Mayo Clinic, controlled with placebo, did not find any benefit with the Vitamin C.

These studies included 200 patients who were treated daily with 10 gr. of Vitamin C. The studies at the Mayo Clinic were considered conclusive. Nevertheless, in these studies, the Vitamin C administered was orally and in low doses, in contrast with the studies carried out by Pauling and Cameron who used intravenous megadose. It is important to keep in mind that the studies that use low doses or orally, are not comparable with the one that use high doses intravenously. The studies of the Mayo Clinic, neither support, nor reject the possible effect of applying Vitamin C in megadose intravenously in some pathology, such as cancer.

Other negative studies, have taken Vitamin C measures in plasma - after the intake. Vitamin C in the diet is 90% absorbed in the small intestine. It does not join together with proteins; and, therefore, it circulates freely in plasma and in tissues. As Vitamin C intake increases, plasmatic levels, serum, in platelets, and in leukocytes also increase. Plasma saturation is achieved with intake of 1000 mg daily (Levine et al., 1996). When Vitamin C in the diet is eliminated, ascorbic acid becomes undetectable in plasma after 35 days, in total blood: after 90 days, and in leukocytes: after 120 days. This suggests that Vitamin C level in leukocytes is the preferable measure of long-time intake, while serum levels and plasmatic, reflect more recent intake (Jacob et al.,
1987); this affirmation will explain the biases of multiple studies. The fact that leukocytes saturate in the presence of low daily intake of 100 mg a day indicates to us that plasma is the most appropriate measure in spite of its high variability in each person. The best method for measuring the nutritional status of Vitamin C is the estimation of the total body reserve, by using dilution of isotopes or excretion techniques.

Relevance of smoking in this study
It is a well known fact that smokers present plasmatic and leukocyte concentrations of Vitamin C - substantially lower than those of the ones who do not smoke. The traditional explanation that used to be given to this phenomenon, was the alteration of eating habits of smokers, which reduces Vitamin C contribution. Nowadays it is known that smokers have a higher Vitamin C metabolism and, therefore, greater requirements of it than non-smokers. Another important result is that smokers have a somewhat reduced Vitamin C absorption index. These facts determine some plasmatic and leukocyte lower levels of Vitamin C, which means that smokers run a higher risk of suffering from a marginal deficiency of Vitamin C.

The metabolic turnover starts to saturate itself towards 40-50 mg of metabolites / day amongst nonsmokers, and towards 70-90 mg of metabolites amongst smokers. In order to achieve said leveling, a total turnover of 60 mg of Vitamin C / day is required amongst the nonsmokers, and of 90 mg of Vitamin C / day amongst smokers. It is important to mention that plasmatic concentration is the parameter easiest to be measured - of Vitamin C status; nevertheless, in Colombia it is not possible even to obtain these measures. On account of the aforesaid, - for the purpose of this study - the level of Vitamin C will be measured in urine by way of the stripes: C-Strips®

Smoking is a great risk factor for the development of arteriosclerosis associated with coronary disease and peripheral vascular disease. The abnormal endothelial function, the increase in adhesiveness of monocytes and the oxidative damage, are the three mechanisms that contribute to the development of arteriosclerosis. Some researches suggest that complement with antioxidant vitamins, mainly Vitamin C, can help modulate these reactions. In the past, it was
believed that the diseases associated to smoking, were caused by the oxidative damage to the lipoproteins. This is due to the fact that increased levels of products of lipid peroxide were found in the urine of smokers. Nevertheless, in latter studies 16, the evidence suggests that abnormal endothelial function - a condition usually associated to chronic smoking -, could be involved in the pathogenesis of arteriosclerosis. A study of smokers in 1996 17 reported improvement in endothelial function, with Vitamin C. It is also known that smoking increases adhesiveness of monocytes and decreases plasmatic levels of Vitamin C. The capacity of monocytes for adhering to the endothelium is a crucial step in the etiology of arteriosclerosis. The complementing - to smokers - of a megadose of 2 grams of Vitamin C a day during ten days restored the plasmatic levels of Vitamin C and diminished adhesion of monocytes to the values found in nonsmokers 18

DEFINITIONS

In order to be able to appreciate the difference between megadose and standard dose, as well as Vitamin C requirements amongst nonsmokers, it is important to clarify basic terms 9.

Vitamin C: ascorbic acid, sodium ascorbate, calcium ascorbate

Standard Dose: the standard dose for an adult weighing 70 kg is 70 mg of Vitamin C a day, higher values are considered megadose 231.

Prophylactic Megadose: Any dose over 70 mg of Vitamin C a day 23.

Collecting (pool): is the total amount of Vitamin C present in fluids and tissues in the organism. It all depends on the contribution 20.

Plasmatic concentration balanced: is the concentration of Vitamin C in plasma of a person with constant daily contributions of Vitamin C 220.

Renal turnover: is the retention of no metabolized Vitamin C 20.

Metabolic turnover: is the retention of metabolites derived from Vitamin C 20.

Total turnover: is the sum of renal turnover and metabolic turnover. It reflects the amount of Vitamin C absorbed in the gastrointestinal tract. 220.
2 JUSTIFICATION

The multiple antioxidant mechanism of ascorbate through the intracellular sweeping of free radicals, of the blocking of lipid peroxide and of the hemodynamic control, has demonstrated being effective in the managing of the smoking individual. This effectiveness is possible if an intravenous megadose is used - by contrast with the standard dose. On account of the aforesaid, the results of the study can generate valuable information that may allow to count on evidence proper for making the decision on the including of megadose of Vitamin C in treatment guides for the smoker in our environment.

Vitamin C has been used in Medicine since 1933 when Reichstein et al and Hirst, Haworth et al, achieved - almost simultaneously - to synthesize ascorbic acid. The range of dose used at that moment was: from 50 mg to 5 g, and in some special cases up to 20 gr. a day, thanks to ascorbic acid being considered nontoxic. The North American Medical Doctor, Frederick Klenner was - in 1940 - the pioneer in the use of megadose of ascorbic acid in human pathologies, and because of that he had to face multiple criticisms, and he generated great controversies which persist up to now.

Just as there have been reported cases of improvement with the use of high doses of Vitamin C for various ailments in several regions in the world, there also exists a marked skepticism on its effectiveness due to lack of reliable evidence and/or negative results. Previous to this clinical essay, the authors carried out the “Systematic revision: effectiveness of medical use of megadose of Vitamin C in humans since 1950 until 2005”. The results from the above study motivated to the carrying out of this present clinical essay, which can generate new information that may allow to count on evidence proper for the making of a decision on the including of megadose of Vitamin C in treatment guides in Colombia.

RESEARCH QUESTION

Smoking habit has been associated to oxidative stress, and with the increase of risk of suffering from chronic diseases. Low concentrations of micronutrients such as: Vitamin C, beta carotene, also has been associated with the use of cigarette. Another aspect to be taken into consideration, is the increase of the cardiovascular risk, and the alteration of homeostasis of iron - when diminishing its absorption -, which can be evidenced in the blood cells test, in the
alterations of the hemoglobin levels and in the hematocrit 20. Although the molecular mechanisms that explain the above changes are not all together clear, they have been physiopathologically associated with the presence of oxygen free radicals, lipid peroxidation, and with the oxidation / antioxidation imbalance 18, 18. There exists scientific information on the benefit that may be obtained when correcting the basal levels of Vitamin C, which we can evidence when obtaining presence of ascorbate in urinary spillover 28.

In Colombia, there does not exist now a study that may evaluate the possible effects of administering of high doses of sodium ascorbate; therefore, our study is intended to develop it when responding to the following question:

*Is there any effectiveness in the use of Vitamin C megadose versus standard dose, for the treatment of subclinical hypovitaminosis in smokers?*

### 3 OBJECTIVES AND PURPOSES

#### 3.1 PURPOSE

1.3.1. To generate in Colombia a study that may contribute with knowledge on the effectiveness of Vitamin C megadose in smokers in our environment, and that it may serve as reference for similar studies in Colombia had in the world.

1.3.2. To justify the carrying out of subsequent studies on the use of Vitamin C intravenous megadose in other fields which have already been explored in various institutions outside of Colombia, rigorously respecting the ethical norms that this type of studies demand.

#### 3.2 GENERAL OBJECTIVE

To evaluate Vitamin C megadose in the treatment of subclinical hypovitaminosis in smokers, by contrast with standard dose.

#### 3.3. 1.2 SPECIFIC OBJECTIVES

- To determine the homogeneity of two study groups, GMD and GDE
- To compare vitamin C urinary excretion in GMD and GDE before and after the Intervention.
• To compare the levels of hemoglobin and hematocrit in groups GMD and GDE before and after the intervention.
• To compare the changes in the lipid profile in GMD and GDE.
• To identify the adverse effects of administering Vitamin C megadose in smokers in the GMD and GDE.
• To identify changes in cigarettes smoked before and after the intervention with GMD and GDE.

4 THEORETICAL BACKGROUND

4.1 CHRONOLOGICAL DESCRIPTION

1700, multiple deaths for scurvy in long travel sailors. The cause of deaths was unknown, as well as the substance that could avoid them.

1753, Dr. James Lind, Physician of the Scottish Navy discovers the antiscurvy properties of citric fruits. Lind carried out a famous experiment with 12 patients severely ill with scurvy. After several combinations, it was affirmed that the two that received 2 oranges and 1 lemon presented total recovery in 6 days.

1800, Naval Captains from the United Kingdom, ordered to include in all ships great stock of lemons, limes and oranges.

1928, Dr. Albert Szent-Gyorgyi, Hungarian Biochemist, Ph.D., discover Vitamin C; he called Hexuronic Acid.

1933, Reichstein and col. & Hirst Haworth and col, use Vitamin C for the first time in medicine. They were the first ones to successfully synthesize - in an experimental way - ascorbic acid. They tried doses from 50mg to 5 gr. per day in patients, and they concluded on the absence of toxicity. 3

1937, Dr. Albert Szent-Gyorgyi, receives the Nobel Prize in Physiology and Medicine in acknowledgment for his discoveries on “The Processes of Biological Oxidation and the role of Vitamin C and Fumaric Acid”.

At this moment, many efforts arose to investigate the value of Vitamin C and of other vitamins to prevent and treat diseases. Unfortunately, in this same period, antibiotics sulfa and penicillin
were developed with their quick curing answer to bacterial infections, generating as consequence that interest for the “slower” vitamins be manifestly diminished.

1938, Dr. Jungeblut demonstrated that ascorbic acid could inactivate poliomyelitis virus. He published a series of documents until 1939, in which he demonstrated that the administering of ascorbic acid to monkeys suffering from poliomyelitis produced a reduction in the severity of the disease.

Sabin, tried to reproduce Jungeblut’s work in monkeys. Disappointed with the negative findings, he searched for new methods for controlling this fatal disease, and his new successful discoveries literally “buried” the theory on the effectiveness of ascorbate against the polio virus during a whole decade.

1948, Dr. Frederick Robert Klenner, graduate Medical Doctor from the prestigious University of Duke, United States, published his first studies on the use of great doses of Vitamin C in the treatment of viral diseases. He inspired the Nobel, Linus Pauling - as well as - Dr. Irving Stone for his researches on the unquestionable benefits of ascorbate megadose in humans. Dr. Klenner died in 1984 being considered the pioneer of the Vitamin C megadose treatment in the world, with more than 3,000 cases treated during 30 years, in the United States. He published 27 scientific articles.

Klenner described - amongst others - his successful treatment in multiple cases of poliomyelitis by using adequate doses of ascorbic acid. Klenner discovered that the secret was in the massive doses which he used, and he tried to impart his knowledge to the skeptic medical profession.

In his article #56 in 1952, Klenner discussed Jungeblut’s plan and Sabin’s failure affirming the following:

“Jungeblut’s results were contradictory because the amount of Vitamin C used was - in some cases - inadequate for the degree of infection. Likewise, Sabin’s results were negative because he used very low doses of Vitamin C for high degrees of virus”. Klenner suggested that the measure of optimum dose for virus infections calculated on the basis of 70 - kilo adult was from 4.5 to 17.5 g of ascorbic acid given each two hours up to completing 200 g in one day. His multiple cases - described during three decades - demonstrated not only the effectiveness of
Vitamin C for viral diseases, but also, its lack of toxic effects. His legacy was that if it is continuously maintained high ascorbic acid in plasma and tissues, an environment extremely unfavorable is created for the growth and viral reproduction in the human body.

1950, Doctors McCormick (Canada), Irwin Stone (United States) and Kirchmair (Germany) start researches with Vitamin C megadoses. They publish successful results for the treatment of viral diseases, bacterial diseases, and intoxications with heavy metals. Irwin Stone presented in 1965 a number of arguments in order to support his thesis that the optimum intake of Vitamin C for humans - in order to achieve the best health condition, lies in the range from 1 to 5 gr. per day.

1954, Linus Carl Pauling, US, receives the Nobel Prize in Chemistry for his researches on chemical bonds and atomic structure of hemoglobin.

Dr. Linus Pauling was one of the scientists having greater fame in the world of medicine. His work is consists of three aspects: The first one, was a CHEMISTRY aspect when he discovered the laws that describe how molecules interact. What Albert Einstein described for Physics, Pauling contributed for Chemistry. Medicine and Nutrition would be two fields completely separated, if we were not to count on Pauling’s discoveries.

The second aspect was on account of his activity in the subject of Anti-radiation - the World peace. A natural progression for a scientist having great introspection and a great heart, was an inexhaustible campaign against atomic bombs. Although this generated his second Nobel Prize in 1962 (Peace), becoming the only human being in having obtained this reward in two opportunities (not shared), it also represented for him criticism on the part of the North American Government, that considered him as having Communist ideals. Due to these insinuations, his passport was confiscated. There existed the probability of obtaining his third Nobel Prize - when deciphering the DNA molecule; but he could not travel to England - on account of not having his passport - to complement his studies with the scientists, Watson and Crick, who - when receiving the Nobel Price - affirmed that it should have been shared with Pauling.

His third aspect began in 1968 at the age of 65 years, and it was the Vitamin C. He created the term: Orthomolecular Medicine - when referring to the science of giving the body the correct molecules in order to achieve an optimum nutrition. In spite of some criticism on the part of the medical profession, Pauling was able to demonstrate that Vitamin C in megadose is effective...
against some types of cancer.

1963, Dr. Archie Kalokerinos and Doctors Glen & Ian Dettman (Father and son) in Australia begin works with Vitamin C megadose. In 1974, Kalokerinos publishes the work entitled: “Every Second Child”, which is a compilation of findings on multiple sudden deaths of newborns amongst Australian aborigines due to lack of Vitamin C.

1978, they begin a 7-month study that ended in July, 1979 in which 900 patients were treated with high doses of sodium ascorbate - prophylactically, as well as therapeutically. The doses used fluctuated between 1000 mg per day for children, to 90,000 mg per day in adults. This study concluded that the pathologic entity which best responds to treatment with megadose of Vitamin C is the viral disease, followed by allergy, skin diseases, cardiovascular disease, swelling and prophylactics. During the last meeting of the author of this work with Doctors Dettman and Kalokerinos in Australia in 2003 in a postgraduation course on Chelation Therapy, it was proven that they still apply in this country Vitamin C megadose to patients with various acute diseases, accumulating inexhaustible source of reports on positive results.

1974, Doctors David Klasson, Edward Cameron and Allan Campbell carry out studies on cancer and Vitamin C; they were published in Chemo-Biological Interactions.

1975, Dr. Robert Cathcart, physician from California, USA, started a decade of treatments during which he registered more than nine thousand patients treated with Vitamin C. He is a prestigious Orthopedist who owes his fame to the discovery of the design error in the Austin Moore Prosthesis, by designing his own Cathcart Prosthesis which - presently - is used in hundreds of patients in USA, Canada and Australia. In what refers to Vitamin C, he discovered the principle of inverse proportionality of treatment with ascorbate and intestinal tolerance.

1976, Australian cinematographic industry creates the movie: “God Knows Why, But it Works”. It is a documentary on the life of Doctor Kalokerinos which narrates the life of this physician working at the remote Australian desert, and his discovery: The death rate amongst aborigine newborns is the highest one in the world due to acute lack of Vitamin C - when administering massive doses of ascorbate - this death rate was reduced to zero and it has been kept so, in spite of skepticism and medical opposition.

1984, Dr. John Marks, Professor and Director of Medical Studies in Cambridge, expresses in his article in the Toorak Times, Page 583, dated August 29, his findings, in a scientific study entitled:
“The overdose of vitamins and its effects”. His results are described ahead.

1992, World-wide Health Seminar in Sandiego, United States. Professor David Harrobin discussed the role of Vitamin C in the production of PGE1, and Professor Benjamin Siegal described how the production of Endogenous Interferon was stimulated by high quantities of ascorbate.

1993, Matsuda and Tanaka evaluate in Japan the effectiveness of Vitamin C megadose in burned patients.

2002, Korcok, Hammond and Wilson evaluate the influence of Vitamin C in sepsis.


2005, Rumbold A, Crowther CA Complementation of Vitamin C in pregnant woman

4.2 EPIDEMIOLOGY OF SMOKING:

4.2.1 Generalities 21

The use of cigarettes is the most important cause of preventable morbidity and early mortality in developed countries. In the United States, it is estimated that, yearly, smoking caused approximately 440,000 premature deaths with an approximate cost of $157 billions of losses in health. Nicotine is highly addictive; it increases the levels of dopamine. Cigarette is responsible for one of five deaths in the United States. The rates of prevalence of intake of cigarette in youngsters have increased. Tobacco intake could have a genetic component.

Smokers have twice more risk of suffering from fatal cardiac disease, 10 more times of suffering from pulmonary cancer, as well as more risk of suffering from mouth, pharynx, esophagus, pancreas, kidney, bladder, and cervix cancer, and they have a high incidence of vascular cerebral disease, and peptic ulcers compared to nonsmokers; as well as suffering hip joint fractures, wrist and vertebral fractures, have four times more risk of suffering pneumonia by pneumococcal. In the United States, more than 90% of the cases of Chronic Obstructive Pulmonary Disease, occur in smokers. Both, smokers and passive smokers are associated to deterioration of elasticity of the arteries progressing towards arteriosclerosis.
Due to cigarette intake, cigars and chewing tobacco, nicotine is one of the addictive drugs of greater use in the United States. In 2004, 29.2 percent of the population from the United States, over 12 years of age, that is: 70.3 million persons have used tobacco at least once a month previous to the interview. This figure includes 3.6 million youngsters between 12 and 17 years of age. During year 2004, young adults between 18 and 25 years of age reported the highest rate of present use of any type of tobacco product (44.6 per cent).

The statistics from the Center for the Control and Prevention of Diseases (CDC, by its abbreviation by initials in English)

Indicate that the use of tobacco continues being the main avoidable cause of mortality in the United States, causing more than 440,000 premature deaths yearly and resulting annually in more than $75 millions in direct medical costs. (See www.cdc.gov/tobacco/issue.htm). During the four last decades, it is calculated that the habit of smoking cigarettes has caused 12 million deaths, including 5.5 millions by cardiovascular diseases, 4.1 millions by cancer, 2.1 millions by respiratory diseases and 94,000 deaths related to the use of cigarette of the mother during pregnancy.


Passive inhalation of cigarette smoke that other persons smoke, also known as tobacco environmental smoke, is a mixture of smoke coming from the burning end of tobacco products (smoke from lateral current), and the smoke from the main current which is exhaled when smoking. This mixture contains many chemical substances (including formaldehyde, cyanide, carbon monoxide, ammonia and nicotine), many of which are known carcinogens. The persons who do not smoke but that are exposed to cigarette smoke, - either at home or at work -, have a risk of from 25 to 30 percent greater of developing cardiac diseases, and from 20 to 30 percent greater of developing pulmonary cancer; besides, inhalation of smoke from other smokers may cause respiratory problems in persons who do not smoke, including: cough, phlegm, and reduction in pulmonary function. Children exposed to cigarette smoke have greater risk of developing the sudden death syndrome in suckling, acute respiratory infections, ear problems and more severe asthma.
4.2.2 Study of Observation of the Future (MTF, by its abbreviation by initials in English)

In spite of the demonstrated risk to health associated to smoking, American youngsters continue smoking. Nevertheless, in relation to smoking habit, the rates of intake during the last 30 days *(years)* - amongst high school students has diminished from the maximum points reached in 1996 by 8th grade students (21.0 percent) and 10th grade (30.4 percent), and the one reached in 1976 by 12th grade students (38.8 percent). In 2005, the rate of use during the last 30 days had diminished to 9.3 percent for 8th grade students, to 14.9 percent for the ones in grades from 10th grade and 23.2 percent for the 12th grade ones.

Diminishment in smoking rates amongst American youngsters corresponds to several years in which the high percentage of teen-agers indicating to believe that there is a “great” risk for health associated with smoking cigarettes, and they expressed their disapproval about smoking one package or more a day. Disapproval of smoking amongst students has increased for several years. For example, the percentage of 12th grade students who would disapprove the smoking of one or more packages of cigarettes per day, significantly increased, adding up from 76.2 percent in 2004 to 79.8 percent in 2005.

The National Survey on the Use of Drugs, and the Health or NSDUH, by its abbreviation by initials in English (formerly known as National Survey of Homes on Drug Abuse), is an annual survey of Americans over 12 years of age, carried out by the Administration of Services for Abuse of Substances and Mental Health (SAMHSA). www.samhsa.gov

These data come from the Observation Study of Future, in 2005. The Institute of Social Investigation of the University of Michigan, carries out this survey under the sponsorship of the National Institute on Drug Abuse, for the National Health Institutes, Health Department and Human Services of the United States. The survey has followed the use of illicit drugs and related attitudes, amongst 12th grade students since 1975, adding to the study, 8th and 10th grade students, in 1991. www.drugabuse.gov

“Use in life” refers to the use of drug at least once in the patient’s life.

“Annual use” refers to the participant having used drug at least once during the year previous to the one survey was responded to. “Use during the last 30 days” refers to the participant having used drug at least once during the month previous to the one in which the survey was
4.3 GENERALITIES OF VITAMIN C AND DOSE CONCEPT

4.3.1 Generalities

VITAMIN C (ASCORBIC ACID)

CHEMICAL CONSIDERATIONS

USP 23

Chemical Formula of ascorbic acid: C₆H₈O₆ (L - Ascorbic Acid)

Chemical Formula of glucose (origin of the ascorbic acid): C₆H₁₂O₆

PH of injectable Vitamin C USP solution: 5.5 to 7.0

**Characteristics:** hydrocarbon with reactive molecules of enediol group which accepts or gives electrons actively participating in the oxidation and reduction mechanism of the body

**Stability of the ascorbic acid in a solution:** The element which degrades ascorbic acid in a solution, is the oxygen. This degradation can be accelerated in the presence of copper ion. It is important that the container of injectable presentation be glass because plastic allows passage of oxygen to the solution.

Cysteine and cystine inhibit the catalytic effect of copper. Therefore, if these amino acids are aggregated to an infusion, the rate of degradation is diminished.

**Synthesis of ascorbic acid:** Most mammals use - at hepatic level - a system of 4 enzymes for producing their own ascorbic acid from glucose, and for compensating the needs. Man lacks the fourth enzyme, **L-gulonolactone oxidase** and therefore, it depends on an exogenous source of Vitamin C - daily.

From glucose, we find the following substances:

D GLUCOSE --D GLUCURONIC ACID -- D GLUCURONIC LACTONE ACID--L GULONOLACTONE -- ASCORBIC ACID

Mammals production of Vitamin C varies in a significant way, mainly under stress situations. The female goat can produce 13 g. of ascorbic acid under situations of low levels of stress, and up to
100 g. in one day in the presence of highly stress generating situations. On its part, the female rat not stressed produces 5 g. and under stress, it produces 15 g. The daily production of Vitamin C, and the absence of renal calculus (stones) in the female goats demonstrate the absence in ascorbate-calculus relation (rate) that some used to propose. The body reserve of Vitamin C in adults, varies between 20 and 50 mg/kg of body weight and daily catabolism under normal conditions fluctuates in 3% of this body reserve. Based on the aforesaid, daily intake of Vitamin C required for keeping tissue saturation is 1mg/kg body weight for adults. According the aforesaid, the standard dose for a 70 kg - adult is 70 mg of Vitamin C a day; higher values are considered megadose. 22 (See annex 2)

4.3.2 Concept of Standard Dose 23
Review on the dosage for adults and children

- Vitamin C deficit: oral prophylactics 50-500 mg / 24 hours. IV: 100mg-1000 /24 hours during the period in which deficit persists
- Dietary need during pregnancy, lactation and in old age: 70-100 mg/24 hours
- Scurvy (oral, IV): 100mg /8 hours during a week; afterwards, 100mg / 24 hours during several weeks
- Severe burns (oral / IV): 200-500 mg /day, until scarring.

RDA (Recommended Daily Dose): RDA for ascorbic acid in Canada and the United States has established the figure: 75 mg per day for adults from 19 to 30 years old. 24

4.3.3 Concept of Megadose

- Colombia: In his book: Reconstructive and Aesthetic Plastic Surgery, Doctor Felipe Coiffeman recommends the following: “During the first days of treatment of burns, high doses of Vitamin C are recommended (6-8 gr. daily) which will be incorporated to the solutions indicated for liquid recovery, or else they will be injected by way of venoclisis.
- US: In 1970, Doctor Robert Cathcart discovered that at least 80% of adult patients
tolerate from 10 to 15 g. of ascorbic acid orally in 24 hours without having diarrhea; nonetheless, it was affirmed that sodium ascorbate is more effective intravenously and intramuscular than oral ascorbic acid. Intravenous preparation is carried out with Ringer lactate and **60 grams** sodium ascorbate by liter. 25

- **Japan:** Megadose Therapy with Ascorbic Acid (66 mg/kg. / h) attenuates lipidic peroxidation post-burning, the requirements for liquid volumes of reanimation and generation of edema in severely burnt patients. 26

- **Australia:** Therapy for avoiding lipidic peroxide post-burning varies from one hospital to the other. Some centers use hypertonic solutions. The most effective dose for stabilizing vasculature is reducing the need for endovenous liquids is 340 mg/kg/day.

### Table 2. Usual Dose of Vitamin C for Intestinal Tolerance

<table>
<thead>
<tr>
<th>PATIENT’S CONDITION</th>
<th>GRAMS 24 HOURS</th>
<th>NUMBER OF DOSES IN 24 HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5 – 15</td>
<td>4</td>
</tr>
<tr>
<td>Asthma</td>
<td>15 – 25</td>
<td>4 - 8</td>
</tr>
<tr>
<td><strong>Anxiety</strong></td>
<td><strong>15 – 25</strong></td>
<td><strong>4 - 6</strong></td>
</tr>
<tr>
<td>Exercise</td>
<td>15 – 25</td>
<td>4 - 6</td>
</tr>
<tr>
<td>Common cold</td>
<td>30 – 60</td>
<td>6 - 10</td>
</tr>
<tr>
<td>Cancer</td>
<td>15 – 100</td>
<td>4 - 15</td>
</tr>
<tr>
<td>Spondylitis</td>
<td>15 – 100</td>
<td>4 - 15</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>15 – 100</td>
<td>4 - 15</td>
</tr>
<tr>
<td>PATIENT’S CONDITION</td>
<td>GRAMS 24 HOURS</td>
<td>NUMBER OF DOSES IN 24 HOURS</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Burns</td>
<td>25 – 150</td>
<td>6 - 15</td>
</tr>
<tr>
<td>Influenza</td>
<td>100 – 150</td>
<td>8 - 15</td>
</tr>
<tr>
<td>Bacterial infections</td>
<td>30 – 200</td>
<td>10 - 18</td>
</tr>
<tr>
<td>Mononucleosis</td>
<td>150 – 200</td>
<td>12 - 18</td>
</tr>
<tr>
<td>Viral pneumonia</td>
<td>150 – 200</td>
<td>12 - 18</td>
</tr>
</tbody>
</table>

* Source: Dettman G., Kalokerinos A., Dettman I. Vitamin C Nature’s Miraculous Healing Missile, Edited by Frederick Todd Melbourne, Australia 1993 pg. 69 (By authorization from the author).

Table 2 illustrates maximum Vitamin C doses tolerated by patients at the moment of improvement of symptoms, and the appearing of diarrhea

4.3.4 Definitions of **DOSE**, unified for this present study

Considering the doses used in various studies according to the above point, the biosafety margin of Vitamin C, and the opinion of the experts in Australia, the “megadose” criteria has been unified for this study, to 30 gr. of Vitamin C (endovenously), and “standard dose” to 200 mg (endovenously).

- **Standard Dose**: 200 mg IV (GDE)
- **Megadose**: 30 gr. IV (GMD)

4.4 **BODY POOL AND VITAMIN C REQUIREMENTS**

Body reserves of ascorbic acid in healthy persons are normally: 1.5 gr. But there are greater reserves if intake is over 200 mg per day. Nevertheless, there are not organs having highly significant reserves - as the case of zinc - in the prostate, and of iodine in the thyroid.

Concentration of leukocytes and platelets is greater than in plasma or in erythrocytes. In conditions of deficiency, the concentration in leukocytes slowly declines; for this reason, it is best to determine the deficiency by evaluating the levels of ascorbic acid in leukocytes than in plasma.
The boy pool of Vitamin C in adults, varies between 20 and 50 mg / kilo of body weight. A 100-kilo adult will have a reserve of 5 g. of Vitamin C, and the daily catabolism in a condition of relaxation, is 3% of his body reserve. This means that in the total absence of Vitamin C in the diet, the body reserve would exhaust in 45 days.

It is considered that daily intake of Vitamin C required for keeping tissue saturation is 1mg/kg. of body weight, with a range of 1 to 1.7 mg/kg of body weight for adults. In children and teen-agers from 6.5 to 7 mg/kg. of body weight by day 22. We noticed that in children, the Vitamin C requirement is greater than in adults. This is due to a greater metabolic catabolism. This figure does not include the extra requirements of Vitamin C necessary to compensate the significant losses - such as in the case of acute stress, viral diseases or burns. The female rat, for example, produces - under stress conditions - up to 215 mg of Vitamin C by kilo of body weight in one day, probably due to an enzymatic induction.

It is worth noting, besides, that the above mentioned limit of human pool is identical to the laboratory mouse. The same as in men, gorilla (and all primates), and the laboratory mouse, lack endogenous synthesis of Vitamin C; therefore, they need to take it daily in the diet. This is due to the genetic absence of the L-Gulonolactone oxidase enzyme in the liver, which has been considered as an innate metabolism error.

4.5 VITAMIN C METABOLISM AND URINARY SPILLOVER

4.5.1 Vitamin C Metabolism

Ascorbic acid is quickly absorbed from the gastrointestinal tract, and it is broadly distributed in the tissues of the body. Plasmatic concentrations of ascorbic acid increase as the dose taken is increased up to achieving a peak with doses of 90 to 150 mg in one day.

After it has been observed, ascorbic acid is reversibly oxidized to dehydroascorbic acid. Then, it is metabolized to oxalic acid and to ascorbate-2- sulfate (inactive), substances which are excreted in the urine. When exceeding the body needs, ascorbic acid is quickly eliminated without modifying itself, through the urine. This, usually occurs when the intake exceeds 200 mg.
Ascorbic acid goes through the placenta, and it is distributed within maternal milk. It can be removed by hemodyalisis.

### 4.5.2 Urinary Spillover

In men, there exists a spillover excretion of Vitamin C in urine when the plasmatic level reaches 1 mg/100 ml. "It is a common error from dietitians, nutritionists and physicians, to think that this is due to tissue saturation. Ascorbate is a unique substance present in variable quantities in different parts of the organism".

The greatest reserve of ascorbate is found in the retina; likewise, there are considerable amounts in white corpuscles, erythrocytes and in plasma. Other sites of reserve are the suprarenal glands and semen in men (The seminal fluid has 70 mg/L - eight times greater than plasma).

When the intake of ascorbate is enough to cause this spillover, a small quantity of the molecule is eliminated through the glomerules, and by tubular absorption, a percentage of Vitamin C molecules is reabsorbed into the blood.

Nevertheless, as has already been explained, the lowest concentration is found in the plasma, so the urinary spillover does not necessarily mean that all body tissues are saturated. Many physicians consider unnecessary all level of ascorbate that produces a urinary spillover, because they think that there exists a total tissue saturation in the organism, and that - therefore - any additional intake will simply be excreted and wasted. Obviously, this is incorrect.

Even though ascorbate spillover is an indication that - at least - the plasma threshold has been exceeded, it has limitations; because when ascorbate is deficient, it is sent to the place where it can be best used; besides, when a renal failure occurs, or when the glomerular filtration mechanism is altered, great amounts of ascorbate can appear in the urine - even in the presence of low levels of plasma or of leukocytes.

Professor, Benjamin Siegal expressed in his conference in 1980 in the World-wide Health Seminar in San Diego, United States, that the endogenous production of interferon was stimulated by Vitamin C - in the presence of high doses. The aforesaid, is only one of many examples of the usefulness of administering humans, doses over the ones of RDA.
The test stripes for presence of urinary Vitamin C, are a useful tool for determining in each sample, the amount of Vitamin C which is being excreted.

What does the absence of ascorbate in this test imply? Initially, it has to be supposed that the plasmatic levels are under 1mg/100 ml. Secondly, when the intake of ascorbic acid is sufficient to cause this spillover, the concentration in the tubules of the micromolecules is reabsorbed by a process which requires energy.

4.6 TOXICITY

Demole et al. arrived to the conclusion that the laboratory mouse can tolerate, - without side effects, - a daily intake of Vitamin C of 500 to 1000 times greater than its daily requirements.

During the last ten years, the question on chronic tolerance of high doses of Vitamin C, has attracted - each time more - the attention of researchers and - therefore - toxicity experiments have been carried out in animals, on the part of various research groups.

Kieckebusch and col. carried out an experiment - during 6 weeks - on toxicity in rats with daily doses of 6.5 g of ascorbic acid by kilo of weight. The authors concluded that the limit daily dose in rats - which is not toxic - is 10gm / kg. of body weight which corresponds to 700 g a day for a 70 - kg. man, and the maximum tolerated dose a day by men - in the presence of side effects (diarrhea, gastralgia) is 140 g.

There exist numerous publications on the systemic application of Vitamin C megadose in children, adults and elders. There are studies on daily oral doses up to 80 g during 10 days without side effects. Nevertheless, in some persons a laxative effect and diarrhea appears, which might be accompanied by gastralgia. These symptoms are more frequent in oral administering of free ascorbic acid, - than in the administering of sodium ascorbate. Some authors have reported cases having an hypotensor effect and of allergies with manifestations of erythema and urticaria.

It has been suggested that Vitamin C excess produces renal calculus (stones), due to oxalate being the main metabolite of ascorbic acid, and - therefore - it could lead to the forming of oxalate calculus in the kidney. Recent studies have demonstrated that the conversion of ascorbic acid into oxalate is quite limited, and it does not reach critical levels even after doses...
over 10 grams a day 33.

4.7 SIDE EFFECTS AND PRECAUTIONS IN THE USE OF VITAMIN C

It has not yet being demonstrated that ascorbic acid causes renal calculus. On the contrary, some authors affirm that it prevents them. Urinary tract infections can also be controlled with ascorbic acid. One patient out of one thousand might suffer pain when urinating. In a much less percentage, some patients can show light rash. There is not evidence of the Herbet and Jacob’s suspicion (1974) about ascorbic acid destroying vitamin B12.

The greatest problem that has been noticed, - if we can call it that way -, is a certain dependence on ascorbic acid that a patient acquires after a long period of time of being taking high maintenance doses.

Likewise, it is worth noting that false positives have been reported in some laboratory exams in blood chemistry. This factor ought to be present in the mind of every medical doctor who might work with Vitamin C megadose.

4.7.1 Side Effects
- The most frequent side effect is softening of fecal matter.
- Diarrhea or some gastrointestinal discomfort may appear when taking high doses - orally - very quickly.
- There is one report on erosion of tooth enamel when using chewing tablets of ascorbic acid during a 3 - year period. It was attributed to changes in saliva pH originating loss of calcium of the dental enamel.
- In laboratory, some methods to determine glycemia may produce false negatives
- Hemolysis may occur when applying injectable or oral Vitamin C megadose in patients having congenital deficiency of the glucose-6-phosphate dehydrogenase enzyme.

4.7.2 Precautions
- Before taking laboratory samples, the patient has to inform whether he is receiving
• In patients retaining liquids, it is preferable to administer ascorbic acid than sodium ascorbate.
• The woman on oral contraceptive has to inform her physician before treatment with ascorbate because there are studies which suggest increase of estrogens.
• The patient under the effect of alcohol, may experience - during treatment with megadose of ascorbate - somnolence and miosis. These symptoms disappear when diminishing the dose.
• Vitamin C can interact with some medicines. Special precaution must be taken when the patient is taking aspirin, flufenazina and warfarin.
• It is important that the diabetic patient know that taking Vitamin C before a urine sample for a test, may produce false positive for the reading of glucose. Therefore, to take Vitamin C only after the urine test in the morning.

5 METHODOLOGY

5.1 TYPE OF STUDY
A randomized, controlled, double-blind, clinical trial

5.2 CONCEPTUAL HYPOTHESIS
Vitamin C administered to patients who are smokers, diminishes the harmful effects of Vitamin C marginal deficiency, only if megadose is applied.

5.2.1 Alternate Hypothesis
In the GMD, smokers significantly diminish the harmful effects of Vitamin C marginal deficit with respect to the GDE

5.2.2 Null Hypothesis
In the GMD group, there are no modifications in the Vitamin C marginal deficit with respect to the GDE
5.3 HYPOTHESIS OF THE VARIABLES OF SIDE RESULT.

5.3.1 Side Effects
Megadose of Vitamin C administered to patients with smoking diminishes the side effects.

• Alternate Hypothesis
  The proportion of smoking side effects in patients in the experimental group, is significantly minor than the one in the control group.

• Null Hypothesis
  The proportion of the smoking side effects in patients in the experimental group is the same as the one in the control group.

5.4 POPULATION AND SAMPLE

5.4.1 Reference Population
Patients who smoke more than 10 cigarettes a day during ten years (heavy).

5.4.2 Study Population
Adult patients, smokers, in the city of Bogotá, who fulfill the inclusion and exclusion criteria defined in the research.

5.4.3 Sample
In order to calculate the size of the sample, 2 procedures were made. Initially, the software for the size of the sample of the Universidad Javeriana - University - 35, was used, and latter on, the formula of Studies for the evaluation of differences was used.

On the other hand, a size of sample put forward in the relevant literature and in the systemic review “effectiveness of the use of Vitamin C megadose in humans” 9; where 2 articles - that used intravenous Vitamin C megadose, and one that used it orally - were chosen as example of contrast in the Vitamin C megadose intravenously and orally; besides an article was added about smokers which reported these results - as far as the effectiveness of megadose in these
patients.

- **Calculation of Sample Size.**


- Error a: 0.05
- Error B: 0.2
- Proportion control group (standard Vitamin C dose): 0.06
- Proportion experimental Group (Vitamin C mega): 0.5
- Reason (Ratio) of assignation: 1:1
- Hypothesis 1 tail
- Formula: Method of arc sine
- Size of sample n= 11 in each / group
- Total individuals: 22

**Size of sample according to software Sample Size, Universidad Javeriana 35 - University** -

A sample of 46 random assigned patients was taken - assigned to groups: GMD 27 patients and GDE 19 patients.

### 5.5 INCLUSION AND EXCLUSION CRITERIA

#### 5.5.1 Inclusion criteria
- Signing written agreement
- Not being in hospital
- Being over 18 years old
- Being smoker of more than 10 cigarettes a day
- Having smoked in a continuous way for at least 1 year
- Both genders
- Having subclinical C hypovitaminosis

#### 5.5.2 Exclusion criteria
• Having personal record of anemia of any type (In order to rule out these participants, Hto and Hb will be taken before the intervention)
• Having personal record of urolithiasis and / or hyperuricemia (calculi)
• Having renal alterations of any kind
• Pregnancy and lactation
• Woman at reproductive age, having active sexual life, who is not planning contraception with a reliable contraceptive during the period of implementation, and observation of this study and / or that may be positive for pregnancy test (in order to rule out a possible pregnancy, a test will be carried out before the intervention of this present study)
• Suffering from pathologies acute (diseases) of any type
• Use of any Vitamin C complement 24 hours before the application.
• Not before breakfast

5.6 DEFINITION OF THE INTERVENTION

50 individuals (participants were chosen random. Group A, Group of Megadose (GMD), with 30 individuals received the treatment with intravenous Vitamin C “megadose” (high) (15 gr. during two consecutive days = 30 gr.) Group B, Group of Standard Dose (GDE) with 20 individuals received the treatment with Vitamin C intravenous “standard dose” (100 mg for two days = 200mg). The Vitamin C used in both cases is sodium Ascorbate. Latter on, the GMD received an oral dose of (1) gram of Vitamin C daily for 15 days. The GME received a placebo, having physical characteristics identical to the (1) gram of Vitamin C, for 15 days. Once concluded the application of the second intravenous dose, the auxiliary 1, in charge of keeping the blind, handed over a bottle with 12 capsules to each patient. She was the only one who knew if the bottle handed over contained the (1) gram Vitamin C capsules or the placebo.

5.6.1 Protocol of Intervention

• Keeping in mind that it was counted on the approval from the committee of ethics of the Universidad del Rosario - University -, the authorization for the use of liquid Sodium
Ascorbate in the research, on the part of the INVIMA, and the approval from the scientific committee from the laboratory donor of Biological Therapies of Australia for the initial clinical essay proposed to the committee of ethics of the Universidad del Rosario, on **August 29, 2005**, in hospitalized burned patients; but in view of not having achieved the size of the sample during years 2005 and 2006, in 2007 it was decided to adapt the clinical essay to individuals smokers, not hospitalized, and to present the corresponding modification to the committee of ethics to the Universidad del Rosario, as well as to the INVIMA. After the approval to the modifications to the clinical essay presented, - on the part of this organizations and involved entities, it was proceeded to presenting the project in health centers where smoking programs are dealt with, and to private individuals identified as heavy smokers, with the purpose of trying to achieve the size of the sample established for the project.

- The project was presented at the **Dispensario Médico Fuerza Aérea** - Air Force Medical Dispensary - the **IPS of the Caja de Compensación Cafam** - Cafam Compensation Fund -, and to private individuals, in order to procure voluntary participants who would fulfill the criteria for being included, and that would wish to participate in the study.

- The researchers’ support personnel who participated in the intervention of the researching physicians, carried out the procurement in order to assure the fulfillment of the methodology established for the study; for this, a pilot test was carried out before the study of intervention, with 2 patients to clarify possible doubts and explain the process. These two patients were, afterwards, included in the size of the sample.

### 5.6.2 Preparation and Application

- Materials, Catheters numbers: 18, 20 and 22
- Cotton swabs
- Catheter plug of intermittent access, saline or heparinized.
- 1, 2, 3, 4, 5, or 10 c.c. syringe.
- Isopropyl alcohol at 70%
- Gauze and applicators
Procedure for venous catheterism

- Calling the patient, introducing oneself, greeting him / her, have them come into the office or room for the procedure.
- Informing the patient and the family on the proceeding which is going to be carried out, objectives and risk, and confirming the confirmed written agreement.
- Calling the patient, introducing oneself, greeting him / her, have them come into the office or room for the procedure.
- Getting the equipment ready
- Telling the patient to sit on the stretcher.
- Choosing the site for puncturing according to: Therapeutic needs; availability of blood vessels to be canalized; caliber of the vessel; anatomic location and condition of the skin.
- Helping the patient to get comfortable according to the site of puncturing. If necessary, immobilizing the patient.
- Evaluating the need for help and requesting it.
- The operator, as well as the helper has to wear eye-protection y and mask.
- Washing the hands.
- Putting the gloves on.
- Referencing the puncturing site, with respect to anatomic structures; verifying the
trajectory of the vein by way of palpation from distal to proximal to identify its characteristics, differentiating between the vein and the artery and detecting abnormalities.

• Carrying out antisepsis of the skin in the following way: washing the chosen area with water and antiseptic soap, using gauze, drying the skin quite well. Applying the antiseptic in a circular way from the site chosen for puncturing towards the periphery, using applicators soaked in the antiseptic. Allowing the antiseptic to dry by evaporation. Applying isopropyl alcohol with applicators, also, and letting dry by evaporation. These three steps have to include, at least, a 10 cm. area around the site to be punctured.

• Placing tourniquet.

• Fixing the limb, firmly holding it with one hand by the subjacent part to the site of puncturing, and anchoring the vein slightly tensing the skin.

• With the other hand, taking the catheter by the mandrel pavilion, and with bevel upwards, puncturing - initially - the skin in a 30 degree angle and then lowering the angle 5 degrees, approaching the vessel in the anterior-posterior sense of the vessel. Holding the mandarin, advancing only the catheter until introducing it completely inside the vein.

• Withdrawing the mandrin and the tourniquet, while pressing with moderate digital pressure over the skin at the tip of the catheter, in order to avoid loss of blood and contamination. If it be necessary to take a blood sample, this is the appropriate time to do it, removing the pressure at the tip of the catheter and allowing blood to flow freely towards the collecting tube. It is not convenient to connect syringe, because contamination is facilitated and collapse of the catheter may be caused or of the vein.

• Connecting the venoclysis equipment or the obstructing plug, holding the handle of the catheter.

• Verifying permeability. Whether by the good flow of dripping or by infusion, with syringe, of normal saline solution or by tests of negative pressure.

• Immobilization: Drying the area and applying tincture of benzoin, letting it dry well by evaporation.

• Placing adhesive tape strips as follows: The first strip, 3 cm long by 2 cm wide covering the site of insertion; the second one, 7 mm wide by 6 to 10 cm long with the adherent
surface upwards so that the mid part holds the joining of the catheter underneath; then it is folded from side to side of the catheter - without crossing the two segments of the strip, in such a way that it adheres to the skin; and the third one, 4 cm long by 3 cm wide over the 2 previous ones for completing the immobilization of the catheter.

- Following, immobilizing the equipment or the plug with two strips of adhesive tape, 6 cm long by 1 cm wide one as butterfly shape and the other one, over the previous one and of the joining of the venoclysis equipment or of the plug, in transversal way.
- Placing a label on the site of the insertion with the following data: date, hour, type of caliber and length of the catheter, name of the person who canalized.
- Conveniently getting rid of all the residues, according to the biosafety protocol.
- Washing, again, the hands.
- Carry out the corresponding registers.

5.6.4 Procedure of the study

**DAY 1 (auxiliary 2)**

- **Group A (experimental), or MEGADOSE:** taking of samples (urine and blood). Then, to 250 cc of SSN 0,9% 15 gr. of Vitamin C in 50 cc of solution from the bottle are added. Giving the patient 1 or 2 glasses of water. Observing him / her all the time. At the end, the bottle of Vitamin C, marked and numbered, is put away in the refrigerator.
- **Group B (control), or Low Dose:** to 250 cc of SSN 0,9%, 100 mg in 1 cc of solution from the ampoule are added. Total mixture 251 cc. Returning in 20 minutes. Giving the patient 1 or 2 glasses of water. Observing him / her all the time. At the end, the ampoule of Vitamin C has to be gotten rid of.

**DAY 2 (auxiliary 2)**

- **Group A (experimental), or MEGADOSE:** In 24 hours from the first dose, to the same patient, to 250 cc of SSN 0,9% are added 15 gr. of Vitamin C in 50 cc of solution from the bottle. Total mixture 300 cc. When finishing the liquids, the patient will have received 30 gr. of Vitamin C. On this day, no laboratories are taken. The bottle of Vitamin C ought to
have the name of the patient and the number of his clinical record. Then, auxiliary 1, hands over the bottle with 15 capsules of Vitamin C, which contain 1 gr. of Vitamin C per capsule. He / she has to take 1 a day, during 15 days. The patient should not be informed on the amount of grams he / she is taking.

- **Group B (control), or Low Dose:** At 24 hours from the first dose, to the same patient, to 250 cc of SSN 0,9% are added 100 mg in 1 cc of solution from the ampoule. Total mixture 251 cc. Returning in 20 minutes. Giving the patient 1 or 2 glasses of water. Observing him / her all the time. On that day, no laboratories are taken. Then, auxiliary 2 hands him / her over the bottle with 15 capsules of Vitamin C, which contains 0gr of Vitamin C per capsule. He / she has to take 1 a day, during 15 days. The patient is not informed on the amount of grams he / she is taking.

**DAY 3 (auxiliary 2)**

- **Group A (experimental), or MEGADOSE:** In 15 days from the second dose, the patient has to come back, and urine and blood sample will be taken. The questionnaire 1 has to be repeated. The physician will evaluate the patient anew. The intervention is ended.
- **Group B (control), or Low Dose:** In 15 days from the second dose, the patient has to come back, and urine and blood sample will be taken. The questionnaire 1 has to be repeated. The physician will evaluate the patient anew. The intervention is ended.

**3.8.7 Follow up**

- Evolution of the patient was carried out, by way of a format especially designed for this purpose.
- One of the researching physicians called the patient and inquired about new things related to the application - within the 24 hours from the dose being applied.
- The auxiliary 1, who put away the blind, was the one in charge of administering the patient oral doses.
- The blind of the research was revealed when the size of the sample was achieved.
- Gathering of data and consolidation
• Analysis.
• Results and Conclusions.

5.7 RANDOMIZED AND MASKING

60 bottles of 100 ml with 30 grams of liquid Sodium Ascorbate each one, were used, and 60 -
5 ml ampoules of 500 mg of liquid Sodium Ascorbate, which were donated and sent from
Australia, at a temperature between 2 and 8 degrees centigrade to preserve the chain of cold,-
by the laboratory: Biological Therapies of Australia. 10 bottles and 10 ampoules were
destined as reserve for covering the possible losses. Biological Therapies has all the licenses
and certificates of Australia for the manufacture, distribution and sale - for human intake - of
Vitamin C, including the GMP certificate (Good Manufacture Practice).

Once received, the 60 bottles of liquid Sodium Ascorbate, and the 60 ampoules of liquid Sodium
Ascorbate, they were handed over to the research medical auditor, Dr. Guillermo Pérez,
gastroenterologist, from the Samaritana Hospital, University Professor, who was in charge of
separating them into 2 groups, and he was not part of the research.

The random assignment to the two groups was carried out by way of the use of the EPITABLE,
Simple / Random List Program, for the generating of the random - number table of 40 units.
The custody of the register of the random, as well ads the putting into sealed and opaque
envelopes marked with sequential numbers from 1 to 40 according to the distribution into the
groups carried out by the program, was by the auditor of the research.

With the purpose of guaranteeing the keeping of the blind, the auditor of the research handed
over to the Nurse Auxiliary #1 the 40 bottles / ampoules along with the 40 marked envelopes.
Once received the nurse auxiliary 1 was the one in charge of keeping the blind.

5.7.1 Procedure:
The patient was admitted the out-patient consult service, at the reception of Grupo Gales
Medicina Preventiva © with format: numbering and vignette Preventive Medicine.©, which was the
only authorized place of the study.
The auxiliary # 1 received each patient who reviewed whether he / she fulfilled criteria for
inclusion. In order of arrival, she assigned them a number (starting from 1 and ending with 40).
She opened clinical record for them, with data on contact, and she wrote the number assigned for the study, in the Clinical Record.

Then, the auxiliary # 1 accompanied the patient to the medical office assigned for the research (number 2), where the auxiliary # 2 received him/her, takes signs, and introduces him/her to the physician in charge for evaluation, - who will be one of the three physicians of the research. The physician in charge corroborated that each one of patients fulfill the inclusion criteria, and did not have any of exclusion, for including into the study. At this stage, it is only excluded the criteria of C hypovitaminosis, which is evaluated latter on.

Each patient read, understood, and signed the informed consent to be part of the study.

Aside from the mandatory clinical record, a special format was filled out (questionnaire 1) which evaluated the condition of the individual who was admitted to the study.

Before the intervention, the auxiliary # 2 took from each patient a urine sample. The physician evaluated it by way of reactive strips (Strips for evaluation of urinary Vitamin C) the presence of Vitamin C. If the response be positive, (presence of Vitamin C), it meant that the individual did not suffer from C hypovitaminosis; and therefore, he was ruled out. Additionally, she took a blood sample from all the participants, for the measuring (of) Blood Test and Lipidic Profile.

The blood and urine samples were carried out in the amount of two: one, before the application of the first Vitamin C dose, and the second one, 15 days after the second dose of Vitamin C. Likewise, the entrance questionnaire (1) was repeated. The results were registered.

The person responsible for the preparing and distribution of the dose (auxiliary # 1) will relate the numbers of lot of each bottle according to distribution and random, and will hand them over to the auxiliary # 2.

The auxiliary # 1, then, will hand over the participant, to auxiliary # 2; she opened the sealed envelope equivalent to the number assigned (For example: patient 1 with envelope 1; patient 2 with envelope 2, etc.), after knowing the dose that corresponded (A= High or megadose; B= Low or standard dose). The assigned dose, be it high or low, will be the same for each patient during the two days. She kept under key the envelope with the data from the patient, and carried out - behind doors - the mixtures, as follows: For Megadose, the first day 15 gr. of the sodium ascorbate solution (50ml) were dissolved in 250 cc of SSN 0.9%. The remaining is put away in
the refrigerator, marked with the number of the patient; next day, the application will be repeated in an identical manner - to the patient - from the same bottle; for the low dose, 100 mg of sodium ascorbate (1 ml) in 250 cc of SSN 0.9%. The remaining will be put away in the refrigerator, marked with the number of the patient; next day, the application will be repeated in an identical way to the patient - from the same bottle.

Afterwards, the nurse auxiliary # 1 handed over to the nurse auxiliary # 2, the mixture of Vitamin C (liquid Sodium Ascorbate) with SSN 0.9% for its application to each one of the patients in office number 2, which was assigned for the carrying out of the study. According to asepsis and antisepsis 35 protocol - annexed, the auxiliary # 2 will canalize vein (wearing gloves), and will apply the solution according to what the clinical record indicates. 36

In the refrigerator, at a temperature of from 2 to 4 degrees Centigrade, and under key, the auxiliary of nurse # 1 kept each bottle of 100 ml of liquid Sodium Ascorbate, marking it with the number of the patient, with the purpose of using it with the same patient, the next day. Likewise, each opened ampoule was thrown away, and was not used again.

5.7.2 Initial considerations for the intravenous managing of Vitamin C Megadose

Hyperosmolar solution administered very quickly in a small vein causes pain. It ought to be administered slowly.

When dissolving 30 gr. of sodium ascorbate in 1 liter of distilled water, isotonic solution is obtained.

The sodium content in sodium ascorbate does not increase the levels of plasmatic sodium, neither does it alter arterial pressure, nor the blood pH.

The period of administration for intravenous treatment of 15 gr. is 30 minutes.

The bottle of 30 gr. of sodium ascorbate comes from the manufacturing laboratory dissolved into 100 ml of saline solution, or distilled water. the 500 mg ampoule of sodium ascorbate is dissolved into 5 ml of saline solution or distilled water. Both presentations are ready for being directly used into the vein, if required.

1 glass of water should be given to the patient during the passing of the solution.

After removing the needle, pressure should be applied at the point of puncture during 3 minutes, except if the patient is canalized.
The 30 gr. sodium ascorbate products have a reserve life of 12 months from the manufacturing date.

The solution should be slightly yellow.
The solutions have to be applied at room temperature, or slightly warm.
A solution dissolved in a plastic bag of liquids (LR, SSN) can be kept 6 weeks if it is preserved by refrigeration of from 2 to 8 degrees centigrade.
The solutions in the 100 ml - bottles and the 5 ml-ampoules should be kept refrigerated, odorless and keep a transparent color or slightly yellow. Not frozen.
A very concentrated yellow color is an indication of expiration of the solution - independently of the time passed since its manufacture.
Occasionally, some symptoms appear during treatment, such as pain in the vein, tingling sensation, coldness in the arm, headache or thirst.
The size of the veins, the concentration of the solution, the speed of the injection and the individual sensibility of the patient are important factors that can cause these symptoms.
Pain can disappear by diminishing the speed of the administration of the solution and / or diluting the solution more.
The objectives in the application of the venous catheter are: to place a peripheral catheter in one vein, keep a permeable venous access, obtain venous blood samples, avoid complications, and give comfort and safeness to the patient and his family.

5.8 VITAMIN C PRESENTATION
5.8.1 Intravenous Presentation

- 100 ml bottle, with 30 gr. of Sodium Ascorbate
- 500 mg ampoule of Sodium Ascorbate.
Laboratory: Biological Therapies, Victoria, Australia

5.8.2 Handling of the product
Preservation by refrigeration of from 2 to 8 degrees centigrade.
5.8.3 GATHERING OF INFORMATION

- Questionnaire of numerical assignation
- Clinical Record
- Laboratory data
- Format of 24-hour evolution.

5.9 VARIABLES

- For the purpose of this study, it has been defined as the only independent, qualitative (categorical) dichotomous, Vitamin C dose.
- The presence of ascorbate in urine is the variable of main conclusion. It is an independent, qualitative (categorical) and dichotomous variable.
- The side effects of smoking (dislipidemia and changes in the concentration of hemoglobin) as well as the adverse effects of Vitamin C, are the side conclusions. They are independent, qualitative (categorical and dichotomous variables.
- Diet will be a confusion or control variable, because diets rich in Vitamin C can alter the results. In order to control this variable of confusion, we carried out some dietary recommendations for the experimental group, as well as for the control group.

Table 1 Classification of Variables

<table>
<thead>
<tr>
<th>INDEPENDENT VARIABLE</th>
<th>DEPENDENT VARIABLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of Vitamin C</td>
<td>1. Presence of ascorbate in urine (Main Conclusion)</td>
</tr>
<tr>
<td></td>
<td>2. Side Effects of Smoking</td>
</tr>
<tr>
<td></td>
<td>3. Adverse Effects of Vitamin C</td>
</tr>
<tr>
<td></td>
<td>DIET (confusion)</td>
</tr>
</tbody>
</table>
### Manual of Variables

<table>
<thead>
<tr>
<th>TYPE OF VARIABLE</th>
<th>NAME</th>
<th>INDICATOR</th>
<th>CODE</th>
<th>CODE OF EXCEPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>INDEPENDENT</td>
<td>Vitamin C Megadose</td>
<td>340 mg / kg d</td>
<td>1 = Yes</td>
<td>9 = SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 = No</td>
<td>0 = NA</td>
</tr>
<tr>
<td>DEPENDENT</td>
<td>Levels of Ascorbic Acid in urine</td>
<td>According to Criteria</td>
<td>1 = More than</td>
<td>9 = SD</td>
</tr>
<tr>
<td></td>
<td>(Main conclusion)</td>
<td></td>
<td>2 = Less than</td>
<td>0 = NA</td>
</tr>
<tr>
<td>DEPENDENT</td>
<td>Side Effects of Smoking</td>
<td>According to Criteria</td>
<td>1 = Yes</td>
<td>9 = SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 = No</td>
<td>0 = NA</td>
</tr>
<tr>
<td>DEPENDENT</td>
<td>Adverse Effects of Vitamin C</td>
<td>Diarrhea</td>
<td>1 = Yes</td>
<td>9 = SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 = No</td>
<td>0 = NA</td>
</tr>
<tr>
<td>DEPENDENT</td>
<td>Smoking Habit</td>
<td>Diminishing of 10 cigarettes smoked a day</td>
<td>1 = Yes</td>
<td>9 = SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 = No</td>
<td>0 = NA</td>
</tr>
<tr>
<td>CONTROL OR CONFUSION</td>
<td>Weight</td>
<td>Kilos</td>
<td>1 = Yes</td>
<td>9 = SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 = No</td>
<td>0 = NA</td>
</tr>
</tbody>
</table>

* 9 = SD (Without datum. When there is no information); 0 = NA (Not Applicable)

### 5.10 BIAS CONTROL

#### 5.10.1 Bias of Selection

In order to avoid risk in the selection, care has been taken so that the chosen individuals be representatives of the objective population, from which the conclusions will be drawn. A questionnaire, for evaluating the smoking habit and its associated effects, will be used with the purpose of confirming that the group of selected participants be representatives and fulfill the
criteria of inclusion and of exclusion.
In addition, an open convoking will be carried out, in order to draw voluntary participants in universities and schools, as well as centers that manage programs of smoking and the general public, with the purpose that the sample would not come from an only group of individuals.

5.10.2 Bias of Information

We have noticed in the pilot test, that some participants exaggerate the information with respect to the amount of cigarettes they smoke, in the hope of receiving the Vitamin C megadose free. In order to avoid this bias, patients will be random, and it will be handled as a double-blind essay. On the other hand, it will be included in the informed consent, the commitment to administer the Vitamin C megadose, once revealed the blind, and - if the study is positive -, megadose will be administered to the control group, if it requires it.
The **diet** will be a variable of confusion or control, because diets rich in Vitamin C can alter the results. In order to control this variable of confusion, we have made some recommendation for the diet in the experimental group as well as the control group.

5.11 STATISTICAL ANALYSIS

The statistical analysis was carried out with the Epi-Info Vr. 6.04 d software for the capture of data with load parameterization in order to minimize fingering (typing) errors. The information was analyzed with SPSS Vr. 15, corporate license from the Universidad del Rosario

A descriptive uni-varied and di-varied analysis was carried out in order to compare the homogeneity of the two groups with respect to the variables of: age, systolic and diastolic arterial tension, cardiac frequency, weight Body Mass Index (IMC), Low density Cholesterol (C-LDL) and of high density (C-HDL), *hematocrito* - hematocrit - and hemoglobin. It included measures of central tendency, dispersion and the distribution of the sample.

The homogeneity of the sample was analyzed by way of T test or Mann Whitney or of homogeneity of chi square, according to the case.

In order to compare the changes in the Vitamin C levels in urine, hemoglobin, hematocrit, C-LDL, H-HDL nonparametric tests of Wilcoxon or T, according to the case before and after Mann’s test to establish if - after the intervention - there were differences.
in the medians of the two groups for the Vitamin C levels in urine, hematocrit and hemoglobin.

5.12 ETHICAL CONSIDERATIONS

5.12.1 SPECIAL CONSIDERATIONS:

The research: “effectiveness of Vitamin C megadose, over the standard dose, as treatment of clinical hypovitaminosis in smokers”

- **Previous Studies**: It is going to be performed in Colombian patients, supported by previous studies in human beings and animals, developed in other countries, which fulfill all the scientific methodology, and that are found in medical publications.

- **Ethical Committee Evaluation**: This design is formulated in a protocol with the adequate methodology, which will be submitted for consideration to the ethical committee of the Universidad del Rosario, who will give the pertinent observations; and, it is worth noting, that this committee has the scientific and legal powers to undertake this work.

- **Researchers**: This research is carried out by personnel who belong to the health area, trained in various fields which are guided by Researchers who are medical doctors trained in the subject of megadose of Vitamin C, who are responsible for the results of the study.

- **Objective**: The objective of this research is the one of making known to the Colombian scientific community the benefits of the Vitamin C megadose, in patients with heavy smoking, and its impact on antioxidant levels in their body reserve.

- **Side Effects**: All the contraindications, and all the side effects that Vitamin C in its megadose way could produce, which were given by the world-wide literature, and by the experience of the researchers in the subject; as well as the benefits which this way of administration of Vitamin C, can bring about to the burned patient. According to research made in world - with human beings -, the side effects and
contraindications that the patients - to whom it is applied - may undergo, are described; and, at no time an uncontrollable complication that might put at risk the life or integrity of the patient - is found; on the contrary, the benefits are quite superior to the negative effects of the megadose.

- **Type of Risk:** This present one, is a clinical essay, controlled, double-blind. According to resolution 8430 of 1993: “Technical and administrative scientific norms for research in health”, the type of risk of this study is a: **research with risk over the minimum one** 37.

- **Criteria of Selection:** A selecting will be carried out, which is described in the project as: criteria for inclusion and criteria for exclusion, in order to determine - according to the contraindications and the side effects - which patients are the most adequate ones for entering into the study; as long as, they be totally convinced about the possible risks and benefits - through an informed consent that they will understand, and will sign without any kind of pressure -, taking care also of the confidentiality and identity of each one of the participants in the study.

- **Publication:** When the study be ended, the results will be published without any type of modification, and with the exactness which this type of inform study requires, even, if the result of the study would accept the null hypothesis.

- **Informed consent:** An informed consent will be made, the patient will be informed on the methodology of the study, the risks, the benefits and on the possible result that would be carried out, and they will be given free option to decide under all their reasoning power, whether to participate or not. The informed consent will be taken by a physician, who will have the adequate training for being able to explain to the patient everything concerning the study, and who will be in charge of responding any question that might be originated; and he - besides - will be detached from the study, in order not to intervene in the decision of the patient. When a patient will not be able to give his consent (whether he be incapacitated or that he may not have the mental faculties for being able to give his consent) for the participation in the study, it will be requested
that the person responsible, sign the consent - with the previous pertinent explanations; and, if a responsible person were not to be found, it will be proceeded to excluding the patient from the study.

- **Well-being**: During the research process, there will be total freedom - on the part of the medical doctor in charge to use every type of therapeutic method - that be necessary - to keep the patient’s well-being.

- **Protocol**: In the research study, there will be two groups of patients who are being treated with the same managing protocol for this pathology; but, it will be differentiated because one group will have a Vitamin C megadose, and another group will only have the dose which is standardized in the protocol of official managing.

- **Negative**: Denial - on the part of the patient - to participate in the study, will not interfere in the physician-patient relation, and the research will be interrupted if - in the opinion of the researchers - harmful results for the patient be evidenced, even if the study has not been concluded.

- **Priority for the well-being**: Well-being of the person participating in the study, will be above the scientific and medical results.

- **Exclusion criteria**: The studio will not be made in pregnant women, minors, mentally diseased, or nursing mothers.

- **External Auditing**: A delegate(s) will be appointed, from the Universidad del Rosario, as an auditor(s) of the research.

- **Legal Protection**: The availability for acquiring a protection policy, for the researcher's action during the study, as well as for indemnification for the possible risks that the patients might have - will be searched for.

6 **RESULTS**

From 54 patients who fulfilled the criteria for inclusion, 4 were excluded for not fulfilling the inclusion criteria; being random assigned 30 patients of GMD and 20 to GDE. At the end of the follow up on day 17, 4 patients did not show up for taking of laboratory (samples) (8%), 3 (10%) of GMD and 1 (5%) of GDE. Figure 1.
### Figure 1 Recruiting and Following up of Patients

Clinical Essay, controlled, random, double-blind.

<table>
<thead>
<tr>
<th>Confirmation Inclusion / Exclusion Criteria</th>
<th>54 patients Excluded: 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C Test in Urine.</td>
<td>Randomized</td>
</tr>
<tr>
<td></td>
<td>GMD  Vit C: 30</td>
</tr>
<tr>
<td></td>
<td>GDE  Vit C: 20</td>
</tr>
<tr>
<td></td>
<td>Data Gathering, Physical Examination and Laboratories</td>
</tr>
<tr>
<td></td>
<td>Double-blind</td>
</tr>
<tr>
<td>17 days (15 days VO)</td>
<td>Beginning of Vitamin C IV Intervention and Orally</td>
</tr>
<tr>
<td></td>
<td>Losses: 4</td>
</tr>
<tr>
<td></td>
<td>GMD  Vit C: 27</td>
</tr>
<tr>
<td></td>
<td>GDE  Vit C: 19</td>
</tr>
</tbody>
</table>

**Losses:** 4 patients who did not attend for stage 2 after treatment on day 15.

With the purpose of guaranteeing compatibility of the two groups, statistical analysis of homogeneity was carried out, with parametric and nonparametric tests, according to distribution of each variable.
Table 3 Characteristics of the patients at the beginning of the study for the two groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GDE N = 19</th>
<th>GMD N = 27</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masculine Sex (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic ** arterial pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic * arterial pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-LDL**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-HDL**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin **</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* GDE Group Standard Dose and GMD: Megadose Group.
** Characteristics not normally distributed. Homogeneity was analyzed with nonparametric statistics - Man Whitney’s test.

With the purpose of establishing differences before and after the intervention, an analysis with Wilcoxon’s test was carried out for level of Vitamin C, hemoglobin and C-LDL in urine, and test T for related samples for hematocrit. Table 2.

Table 4 Comparison before and after the GDE and GMD intervention

<table>
<thead>
<tr>
<th>Variable / Group</th>
<th>GDE n = 19</th>
<th>GMD n = 27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C in Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-LDL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
From the result, we highlight that in the GMD of Vitamin C, there are statistically significant differences between the measures before and after the intervention, and in the measure on day 17 in Vitamin C in urine, hemoglobin and the hematocrit; while in group GDE, significant differences are observed only in Vitamin C in urine.

Even though there are significant changes in levels of Vitamin C in urine in the 2 groups shown by Wilcoxon’ test, Mann Whitney’s test, it shows that there are statistically significant differences between the GMD and the GED, on day 17, p=0.000. This difference can be appreciated in graphic 2.

**Graphic 1** Comparison of Levels of Vitamin C in Urine in the two groups at the end of the follow up, day 17.

<table>
<thead>
<tr>
<th>VC DOSE</th>
<th>GDE</th>
<th>GDE</th>
</tr>
</thead>
</table>
* N2VITCORIN: Levels of Vitamin C in urine mg / ml on day 17
** VC DOSE: Groups GDE and GMD.**
7 DISCUSSION

The role of antioxidants and specifically the one of Vitamin C, has been controverter in the treatment of several diseases such as: cancer, the handling of burned patients, of smoking, amongst others 29. When reviewing the database of Pub Med, Cochrane, Proquest, Ovid, Ebsco and Hinary, we did not find specific studies on the effect of megadose of Vitamin C in the treatment of hypovitaminosis in smokers, its action on hematocrit and hemoglobin, which are increased in smokers.

This present study demonstrated statistically significant changes (p<0.05) in concentrations of Vitamin C in urine (indirect measure of the plasmatic concentrations (ref 37)) in groups GDE and GMD, before the intervention, and on day 17 of the first IV dose, being superior the concentrations of Vitamin C in the second measure. This change was significantly superior (p>0.05) in the group of the megadose (GMD), than the ones that were administered 30 g IV oral by day for a total of 45 g, which demonstrates the effectiveness of the administration of megadose. In the GDE, the measure changed from 26.79 ± 13.76 before the intervention to 39 ± 19.74 mg / 10 ml on day 17 vs. in the GMD, which shows a change of 21.85 ± 11.9 to 71 ± 20.6 mg / 10 ml after the intervention. This type of results, are not informed in the literature for smokers, which makes it being a first approximation to this problem.

The change in urinary concentrations of Vitamin C in the GDE before and after the intervention on day 17 of the study (group that received the standard dose and oral placebo) is statistically significant, which makes us think that there can exist a lasting effect, with time, of Vitamin C - even with low dose IV, which allows to keep for more than two weeks a rising of the levels of ascorbic acid in plasma, which has been documented 38. Nevertheless, this has to be confirmed with additional studies. The above finding is not confirmed for the GMD, by reason of this group not receiving oral placebo, but a dose of 1 g daily up to the last day, which allows measuring the effect sustained in the time of the IV dose.

The concentrations of hemoglobin and hematocrit found increased above normal levels - at the start, were corrected in the GMD by use of 30 gr. of endovenous Vitamin C and 1 gr. of Vitamin C orally (1gr by day) during a period of 2 weeks. Likewise, when comparing data obtained from hemoglobin and hematocrit in the laboratory, differences statistically significant were found.
(p=0.002 and p= 0.016 respectively) only in the GMD.

Although it is true that we did not find studies specifically of Vitamin C correlated with changes in the hematocrit and the hemoglobin in smokers, the multiple antioxidant mechanism of ascorbate through the intracellular sweeping of free radicals, could be related with our findings, which has to be broadened by future studies 13-30.

In one study 39, there were not found significant changes in the levels of serum Lipids, specifically of the LDL, which correlates itself with our findings, in which we observed that there are no significant differences in the levels of C-LDL between the 2 groups before and after our intervention.

The strength of this study include: the size of the sample n=46, superior to the studies applied on effectiveness of Vitamin C and smoking of 27 individuals, in total 13. Likewise, it is about an random clinical double-blind essay in patients who are smokers, homogeneous in variables such as: age, sex, weight, and the IMC, with an additional control of intake of food rich in Vitamin C by standardized dietary recommendations to patients included in it.

Within the weaknesses of the study, it ought to be considered: the determining of the levels of Vitamin C by way of reactive strips of ascorbate in urine, as an indirect measure of the plasmatic levels. The ideal method, is the measuring of leukocytes, (Jacob et al., 1987) 28, which is not feasible in Colombia.

In order to give continuity to this first approximation in the determining of the effectiveness of Vitamin C megadose, complementary studies should be carried out - with a greater size of samples, longer periods of follow up, with the use of other methods for determining the levels of Vitamin C, such as the one of leukocytes.
8 ACKNOWLEDGMENTS

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