Vardenafil: a Phosphodiesterase-5 Inhibitor with protective action against the development of Multiple Organ Dysfunction Syndrome (MODS)

Vardenafil: um inibidor da Fosfodiesterase-5 com ação protetora contra o desenvolvimento da Síndrome Da Disfunção de Múltiplos Órgãos (SDMO)

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Abstract

Introduction: MODS is a pathology associated to nonspecific and severe trauma, characterized by elevated morbidity and mortality. The intense inflammatory MODS-related reactions generates disseminated micro-thrombi, the most relevant pathophysiologic component in the induction of this syndrome. Nitric oxide concentration elevation has the potential of counteracting the typical systemic vasoconstriction, as well as the platelet-induced hyper-coagulation. A phosphodiesterase 5 inhibitor-vardenafil, would, possibly, act protectively by inducing a NO system elevation resulting in diffuse vasodilatation, besides reduction of endothelial lesions and platelet-induced hyper-coagulation, thus, preventing MODS development. **Methods**: MODS was induced through the micro-neurosurgical bilateral anterior hypothalamic lesions model. Experimental groups of 10 Wistar rats were divided into: a) Non-operated control; b) Operated control group; c) Pre-treated with vardenafil operated group; d) Post-treated with vardenafil operated group. The animals were sacrificed after 24 hours and submitted to histopathologic examination of five organs: brain, lungs, stomach, kidneys and, liver. **Results**: The anterior hypothalamus stereotaxic electrolytic lesions resulted in a full picture of MODS with disseminated multiple-organs lesions, provoked, primarily, by diffusely spread micro-thrombi. The pre-treatment with vardenafil 2 hours before the micro-neurosurgical lesions reduced significantly ($p>0.01$) the lesions development in 66.37%, as compared to the operated-control. The post-treatment with vardenafil, 2 hours after the micro-neurosurgical lesions, also reduced significantly ($p>0.01$) the lesions

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development in 59.64% as compared to the operated control. **Conclusion**: Vardenafil, in the doses and timing utilized, showed to act as a protective agent against the experimentally-induced MODS.
Key words: Multiple Organ Dysfunction Syndrome, Nitric oxide, vardenafil, Systemic Inflammatory Reaction Syndrome, phosphodiesterase-5 inhibitors.

Resumo

Objetivo: SDMO é uma patologia associada ao trauma inespecífico e grave, caracterizada por elevada morbidade e mortalidade. As intensas reações inflamatórias relacionadas geram micro-tromboses disseminadas, o componente fisiopatológico mais relevante na indução desta síndrome. A elevação do óxido nítrico tem o potencial de reverter a vasoconstrição sistémica típica, bem como a hiper-coagulação induzida por plaquetas. O inhibitor da fosfodiesterase-5, vardenafil, poderia agir de forma protetora induzindo uma elevação sistêmica das concentrações de NO, resultando em vasodilatação difusa, além de redução de lesão endotelial e hiper-coagulação induzida por plaquetas ,evitando, assim, o desenvolvimento da SDMO. **Métodos:** A SDMO experimental foi induzida através do modelo de lesões micro-neurocirúrgicas bilaterais do hipotálamo anterior. Os grupos experimentais de 10 ratos Wistar foram divididos em: a) Grupo Controle não-operado; b) Grupo Operado; c) Grupo Operado Pré-tratado com vardenafil; d) Grupo Operado Pós-tratado com vardenafil. Os animais foram sacrificados após 24 horas e submetidos ao exame histopatológico de cinco órgãos: cérebro, pulmões, estômago, rins e, hepáticas. **Resultados**: lesões eletrolíticas estereotáxicas do hipotálamo anterior resultaram em um quadro patológico típico da SDMO, com lesões disseminadas em múltiplos órgãos, provocadas, principalmente, por micro-trombos difusos. O pré-tratamento com vardenafil 2 horas antes das lesões micro-neurocirúrgica reduziu significativamente (p> 0, 01) o desenvolvimento de lesões multiorgânicas em 66,37%, em comparação com o controle operado. O pós-tratamento com vardenafil, 2 horas após as lesões micro-neurocirúrgica, também reduziu, significativamente, (p> 0,01) o desenvolvimento de lesões multiorgânicas, de 59,64% em comparação com o controle operado. **Conclusão**: O vardenafil, nas condições experimentais utilizadas,, mostrou funcionar como um agente de protecção contra a indução de lesões multiorgânicas da SDMO experimental..
Palavras Chaves: Síndrome da Disfunção de Múltiplos Órgãos, Óxido nítrico, Vardenafil, Síndrome da Resposta Inflamatória Sistêmica, Inibidores da Fosfodiesterase.

Introduction

Modern industrial societies have trauma as a relevant epidemiologic factor of morbidity and mortality.[1] In the United States, for example, trauma is the leading cause of morbidity and mortality for persons from birth to 44 years of age. Every year, more than 13 million adults, in the range of 20-49 years of age were injured, and nearly 80,000 were killed, with a loss of US$406 billion in lost productivity and medical bills in the last decade.[2,3,4] Parallel to this scenario, the prevalence of Multiple Organ Dysfunction Syndrome (MODS) has been very high, affecting one-third of all in-hospital patients and more than 50% of all ICU patients.[5,6]

Moon[7], in 1948 and Mallory et al.[8], in 1950, had described pathological findings secondarily associated to trauma in soldiers that were wounded in military actions during the World War II. These papers described the occurrence of a picture of universal, diffuse organic deterioration, typical of MODS. They reported the presence of diffuse endothelial lesions in several organs, besides capillary and venules engorgement. The development of disseminated micro-thrombosis with scattered tissue hemorrhages were the most frequent secondary post-traumatic findings. De Oliveira et al.,[9] in 1972, working on their hypothesis of gastric mucosal ischemia as the main cause of stress ulcers, infused a solution of norepinephrine intraperitoneally in restrained guinea pigs. This procedure induced, not only, the development of stress ulcers, but also scattered histopathologic organ lesions, similar to those found in patients with the, yet unknown, MODS, suggesting the occurrence of a ischemia-reperfusion process in the genesis of disseminated micro-infarcts.

Indeed, the classic Virchow's [10] work, published in 1853, proposed the mechanisms for the induction of tissue micro-infarcts, the basis of MODS pathophysiology: a) Low blood flow; b) Endothelial lesion; c) Hypercoagulability. This classical study gave De Oliveira [11] the support for the proposal of the peripheral pathophysiologic core of MODS. Therefore, the following Selye's [1] Alarm Reaction (AR) associated peripheral factors would be the responsible for the MODS induction process. Then, the sequence of peripheral factors participating in the MODS ischemia-reperfusion pathophysiology, as proposed by De Oliveira [2,9,10,11], would be:

1. Phase of Vasospasm, Thrombosis and Ischemia:

1.1. Factor of vasoconstriction and low flow.

1.2. Factor of endothelial lesion.

1.3. Factor of hypercoagulability.

2. Phase of Reperfusion, Failure and Vascular Necrosis:

2.1. Factor of vascular metabolic deterioration.
2.2. Factor of vascular hypo-response, dilatation, reperfusion and hyper-permeability.
2.3. Factor of vascular necrosis and micro-hemorrhagic.

Maire & Patton, in 1956 [12], reported the development of multiple organic lesions in rats submitted to hypothalamic preoptic nuclei stereotaxic lesions. These lesions were histopathologically identical to those found in MODS, an unknown syndrome at that time. Afterwards, in 1975, 1979 and 1981, De Oliveira et al.[11,13] through the utilization of Maire & Patton's experimental micro-neurosurgical techniques, reached to the hypothesis that occurrence of a trauma-induced multiple-system derangement pathophysiologic process, that they called Secondary Post-Traumatic Syndrome, would be, possibly, based on a Central Nervous System(CNS) imbalance. [1,12] These authors proposed, based on the Wiener's Theory of Systems [14], that unspecified trauma would result in a complex adaptation Alarm Reaction(AR) process. This process could, secondarily damage the CNS itself resulting in uncontrollable system-deterioration process: the basis of MODS.

In 1987, Ignarro [15] and Moncada [7] detected that the Endothelium-Derived Relaxing Factor (EDRF) was, indeed, a simple molecule: nitric oxide. It proved to have several physiologic actions. Actions such as: a) regulation of vascular tone, influencing tissue blood flow and arterial pressure; b) platelet function, by controlling its adhesion and aggregation; c) neurotransmission at the level of some motor neurons of the parasympathetic branch of the autonomic nervous system that modulates intestinal peristalsis and penile erection; d) Acts, also, at NO-sensitive neurons of the respiratory centers of medulla oblongata; e) NO stimulates NMDA receptors at hippocampal area, improving mechanisms of long term memory; f) Activation of immunity/inflammatory systems, aiding to kill various pathogens, such as virus and bacteria; g) Inhibits inflammation and exocytosis of various mediators from endothelial cells, macrophages and cytotoxic T lymphocytes; h) Increase of renal blood flow, with improvement of the filtration rate and urine formation; i) Stimulating endocrine secretions, such as gonadotropin-releasing hormone (GnRH) from hypothalamus, adrenaline from adrenals or exocrine secretions such as amylase from the pancreas. [15-20]

Considering this pathophysiologic context, it was hypothesized that nitric oxide (NO) would present several beneficial actions, especially on cGMP-induced systemic vasodilatation. Other important actions would be the reduction of platelet aggregation and

platelet disaggregation, through the same cGMP-induced mechanism. In addition, the inhibition of platelet adhesion to collagen fibers to endothelial cell matrix and to endothelial cell monolayers, as well, would reduce the development of micro-thrombosis: the MODS basic inducing-factor. [15-19,21]

NO is a labile lipid soluble gas synthesized from the amino-acid l-arginine by NO synthase (NOS) in most areas of the organic system. During this synthesis, NO is transformed into a monomer, that in the presence of tetrahydrobiopterin, it turns into a dimer. Therefore, in the presence of calmodulin and molecular oxygen, it converts l-arginine to NO and citrulline as a by-product. [15-17,19,21]

In addition, three isoforms of the synthesis enzymes (NOS) were described. a) Neuronal n-NOS(or NOS-1); b) Endothelial NOS(E-NOS or NOS1); c) Inducible NOS(i-NOS or NOS2). Several co-factors are involved in the NOS actions, such as calmodulin, FAD, FMN, NADPH and tetrahydrobiopterin. Heme structure is, indeed, part of the NOS molecule.[22,23]

Ischiropoulos et al.,in 1994 [24]; Preiser et al., in 1995 [25] and Wong et al., 1995 [26] were the first to suggest the therapeutic utilization of inhaled NO in the prophylaxis and treatment of Adult Respiratory Distress Syndrome. Offner at al. [27] reported that inhaled NO-induced improvement in the pulmonary hypertension associated to experimental septic shock in pigs, with concomitant elevation in the cardiac ejection fraction.

It is important to emphasize that inhaled NO has very short half-life, varying between 6 and 40 seconds. However, when released into the intrapulmonary spaces and pulmonary vessels, NO is inactivated very quickly by combination with hemoglobin, yielding methemoglobin as end-product. In addition, there are 11 phosphodiesterases, that are also NO important metabolizing systems [22,28]. The phosphodiesterase 5 has the most desirable characteristics to be inhibited. Therefore, the important vasodilator effect of inhaled NO is rapidly neutralized, remaining restricted to the lung vascular and airways systems, with minimal, or, no systemic effects. Therefore, if systemic actions are intended, as the needed in MODS, it must be used a phosphodiesterase 5 inhibitor(PDE5), that will maintain NO actions for a long period, through its half-life extension. [23,29]

Therefore, it becomes obvious that, instead of utilizing inhaled NO, a short half-life gas, the most effective way to elevate tissues levels of NO would be through the utilization of phosphodiesterase 5 inhibitors such as vardenafil, sildenafil, tadalafil, udenafil or avanafil [28-30]. Vardenafil would be one of

the best PDE5-I candidate to inhibit NO degradation and thence, enhancing the system NO circulating levels, for a prolonged period. Therefore, it was decided to ascertain the potential protective effects of vardenafil in the MODS development utilizing an experimental neurosurgical model in this syndrome induction. [22,28-31]

The final choice of vardenafil was pharmacologically-based on its easy and fast absorption, favorable pharmacokinetic profile, such as low first-pass degradation, large volume of distribution, and relatively slow metabolism and excretion. In addition, and especially, for its long half-life and benign therapeutic profile, it was finally chosen as the drug that, for its characteristics of inducing long-term NO tissue concentrations, would result in of vasodilation and reduction of the coagulation profile, [28,29,30,31] that would act reducing the potential of development of micro-thrombosis, the pathologic basis for the MODS development.

Methods

Animals, Environment and General Procedures

The *in-vivo* experiments protocol was approved by the specific institutional review committee and was performed on Wistar male rats weighing from 180 to 220g that were kept separate cages placed in a thermally stable room (21^0 degree Celsius) with free access to water and balanced food. Each group was composed by 10 animals. These animals were submitted to the following procedures.

Micro-neurosurgical Procedures

The animals were anesthetized with ethyl ether (anesthesia wakening time of 9 minutes$\pm$ 3 minutes) and placed in a stereotaxic neurosurgical frame (David Kopf 1404, S. Paulo, SP). After appropriate antisepsis, parietal bones were exposed and holes (0.5mm of diameter) were drilled bilaterally in specific places. The Surgical Group followed specifically these procedures:

Stereotaxic electrolytic lesions (EL) were placed in the anterior (AH) hypothalamus nuclei; by using an anodal constant current of 5mA for 20 seconds through a monopolar stainless-steel insulated electrode (0.3 mm diameter and 30u of tip diameter) previously calibrated in an optical microscope. The stereotaxic parameters were extracted from the Konig and Klippel Atlas [1,10,12,32]. Sham-operated(S) animals were submitted to the same procedures, except for the placement of hypothalamic lesions. The electrode was stained with methylene blue for further histological lesion precision placement evaluation.

Pharmacological Procedures

The animals were divided into four groups:

Basic Control Group: This group was composed by 10 animals, that were injected intraperitoneally, with a 2mL of NaCl 0,9%. 24 hours after, these animals were sacrificed through by abdominal vessels section, under ethyl ether anesthesia.

Surgical Control Group: composed by 10 animals submitted to the above mentioned protocol of stereotaxic electrolytic lesions [2] under ethyl ether anesthesia. These animals received 2 mL of NaCl 0.9% intraperitoneally. These animals were sacrificed 24 hours after the surgical procedure.

Vardenafil 2 hours Before Group: composed by 10 animals submitted to the same above mentioned stereotaxic electrolytic procedure under ethyl ether anesthesia. A solution of vardenafill (Ely Lilly Co. Indianapolis, IN) 0,3mg/Kg dissolved in 2mL of a solution of NaCl 0,9% was injected intraperitoneally 2 hours before the hypothalamic lesions placement. These animals were sacrificed 24 hours after the surgical procedure.

Vardenafil 2 hours After Group: composed by 10 animals submitted to the same above mentioned stereotaxic electrolytic procedure, under ethyl ether anesthesia. A solution of vardenafil (Ely Lilly Co. Indianapolis. IN) 0,3mg/Kg dissolved in 2 mL of a solution of NaCl 0,9% was injected intraperitoneally 2 hours after the placement of the hypothalamic lesions. These animals were sacrificed 24 hours after the surgical procedure.

Histopathology Procedures

The animals were sacrificed 24 hours after the neurologic stereotaxic electrolytic procedure, under ethyl ether anesthesia, by sectioning the abdominal vessels. In our laboratory experience, this period was enough to allow the development of intermediate-to-severe gravity MODS pattern. In addition, intermediate-to-severe levels of multi-organ involvement would make possible the evaluation of, either attenuation or potentiation of MODS evolution. Aorta washing was not performed in order to preserve the already-formed micro-thrombosis in several organs. The following organs were removed. and fixed in neutral 10% formaldehyde solution and after embedded in paraffin and stained with hematoxylin-eosin for further histological examination: brain, lungs, heart, spleen, liver, kidneys(and adrenals), and stomach-duodenum. Spleen, duodenum, heart and adrenals histology were not studied in this work due to its minimal MODS-related deterioration. Animals with inappropriately placed cerebral lesions were discarded and replaced in the experimental

series. MODS evolution will be presented as follows,

The histopathological assessment was quantitated by reviewing ten microscopic fields, the following an arbitrary grading scale, basing on literature and to our previous experience:

Lungs Scores (LS):

(microscopic examination:64 X and 250X augmentations)

Grade O - No alteration.

Grade I - Minimal interstitial edema.

Grade II - Interstitial edema, scattered fibrin microthrombi .

Grade III - Interstitial edema, fibrin microthrombi, interstitial microhemorrhages.

Grade IV - Major edema. interstitial and intra-alveolar hemorrhages. fibrin and leukocyte microthrombi, hyaline membranes.

Stomach Scores (SS):

(microscopic examination:64 X augmentation)

Grade O - No alteration.

Grade I - Mucosal petechiae.

Grade II - Petechiae, less than two stress ulcers.

Grade III - Petechiae, between 2 and 5 stress ulcers.

Grade IV - Petechiae, more than 5 stress ulcers, major hemorrhage.

Kidney Scores (KS):

(microscopic examination:64 and 250 X augmentations)

Grade O - No alteration.

Grade I - Minimal parenchymal edema.

Grade II - Edema, scattered fibrin microthrombi.

Grade III - Edema, microthrombi, interstitial microhemorrhage.

Grade IV - Edema, microthrombi, interstitial extensive hemorrhage, hemoglobinuria, incipient

tubular necrosis.

Brain Scores (BS):

(microscopic examination:64 and 250X augmentation).

Grade O - No alteration.

Grade I - 1-5 microthrombi.

Grade II - Neuron and glial edema, 6-10 microthrombi.

Grade III - Neuron and glial edema, 11-25 microthrombi.

Grade IV - Neuron and glial edema and cytoplasmic vacuoli, more than 26 microthrombi. interstitial hemorrhages.

Liver Scores (LS):

(microscopic examination: 64 and 250X augmentation).

Grade O - No alteration.

Grade I - Centrilobular turgence.

Grade II - Centrilobular turgence, microthrombi.

Grade III - Centrilobular turgence, microthrombi, rare tissue hemorrhage.

Grade IV - Centrilobular turgence, microthrombi, tissue extensive hemorrhage, leukocyte infiltration, hepatocyte edema and degeneration.

The histopathologic examination was performed by a "blind" physician (pathologist). The microscopic slides were identified only by a computer-generated randomized numbers, kept under a code broken after finished slide examination. The scores were attributed to every slide, basing on the sum of each grade of ten 64X microscopic neighbor fields.

Therefore, the minimum field damage score (absence of lesion) would be 0 (zero) and the maximum field damage score would be 4 (four). Since were examined 10 fields per slide, that multiplied by each field damage scores, would reach 40 per each organ, reaching 00 or 40. This value multiplied by 5 organs, would reach a maximal value of 200. And, then, multiplied by 10 slides per group, we would have a final minimal score of 0000, and a maximum score of 2000. The 250X magnification was utilized, in cases of doubt, only in order to detect specific qualitative tissue damage for grading classification.

Statistical Analysis

The results (mean$\pm$SD) were evaluated through the gravity consolidated organ damage scores (CGS) of all the organs scores for each experimental setting of n=10 animals, being 00 the baseline and 40 the maximal gravity values. We utilized the non-parametric method of Kruskall-Wallis. For the comparisons between two different groups were utilized X^2 test. It were considered significant values of $p<0.05$ or, highly significant of $p<0.01$.

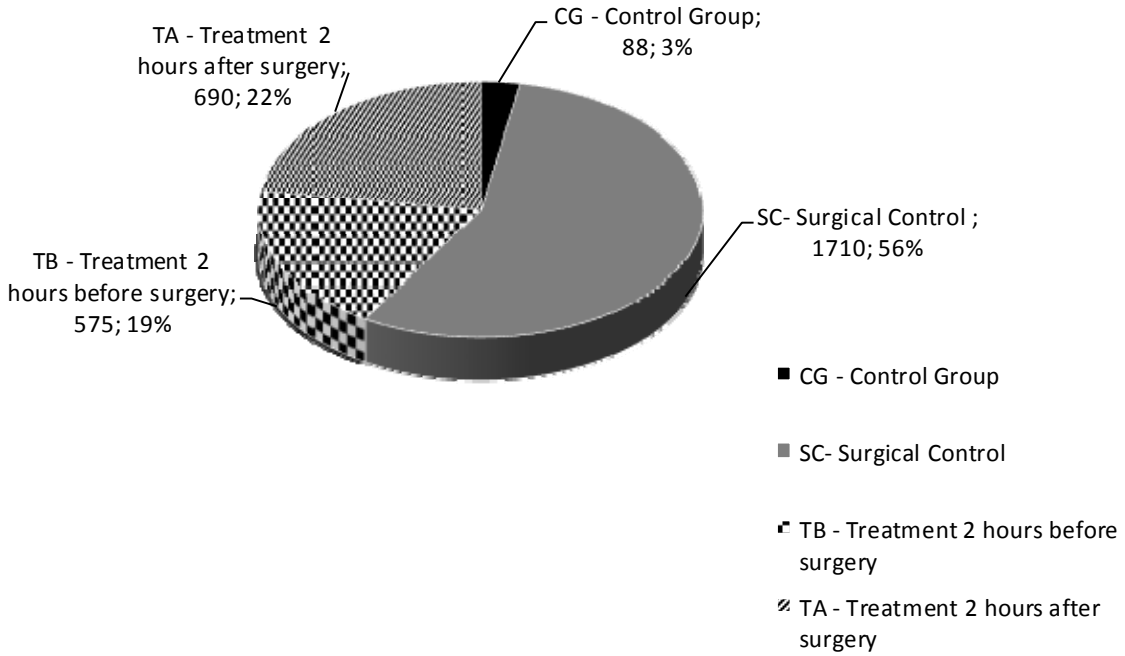
Results

A comparison between the groups demonstrated the following results (graphic):

a) Control Group ($X\pm sd= 88\pm 37$) compared to Surgical Control Group ($X\pm sd= 1710\pm 105$): $p<0.001$;

b) Surgical Control Group compared to 2 Hours Before Group ($X\pm sd= 575\pm 65$): $p<0.01$;

c) Surgical Control Group compared to 2 hours After Group($X\pm sd= 690\pm 55$): $p<0.01$.



Graphic – Number and consolidated gravity scores (cgs) comparison of organ damage in different groups

Discussion

Despite being detected by medical teams, shortly after ICU concept emergence, in 1958, the secondary post-traumatic organ failure was only officially recognized in 1983, when the global term "Multiple Organ Failure" (MOF) first appeared in Index Medicus [2]. However, its existence could be found in very remote descriptions, such as that done in 1823 by William Cumin [33], Surgeon of the Royal Infirmary and the Asylum of Lunatics of Glasgow and by Joseph Swan, Physician of the Lincoln County Hospital in the same number of the Edinburgh Medical and Surgical Journal. They reported the pathological lesions in several organs not primarily damaged of six patients of burned patients that died days after the trauma. Cumin observed, with high accuracy: ***"...distant internal parts sympathized with the burned surface and suffer inflammation…".*** Other XIX Century authors, such as Cooper [34], in 1839; Long [35], in 1840; or

Curling, [36] in 1842, among others, had described similar findings, with no repercussion in the scientific community.

MODS development, as above mentioned, is associated to multiple systems activation that could be a post-trauma CNS-induction [2], or by peripheral exogenous substances, such as endotoxin or drugs. Some of the systemic alterations are:

1) **Increase of vascular permeability**: induced by histamine, bradykinin, C3a, C5a, LTD4, LTC4, PGE2, PGI; activated Hageman Factor; high molecular weight kininogen fragments; etc.

2) Vasoconstriction: epinephrine; norepinephrine; dopamine; histamine(lungs); serotonin; endothelins I, II and III; Clowes Factor; thromboxane A2; leukotrienes: LTC, LTB4, LTD; formil peptides; vasopressin; angiotensin II; endothelium-derived contracting-factors (EDCF); cyclo-oxygenase-dependent factor (EDCF1); hypoxia-dependent factors (EDCF3); cyclo-oxygenase-independent free oxygen radicals (EDCF4); endothelium-derived hyperpolarizing factor (EDHF); activated complement C5a; endotoxin, among others.

3) **Smooth muscle contraction**: activated complement: C3a; C5a; histamine; leukotrienes: LTB4, LTC, LTD; thromboxane A2; acetylcholine,;bradykinin, etc.

4) **Leukocyte proliferation**: IL-1; C3a, CSFs, endotoxin, catecholamines, TNF-alpha; etc.

5) **Endothelial adhesiveness augmentation**: Il-1; LTB4; TNF- . ;etc

6) **Bone marrow leukocyte recruiting**: C3a; IgG; fibronectin; epinephrine, etc.

7) **Leukocyte adhesiveness and aggregation augmentation**: C5a; LTB4; Il-8; PAF (platelet activating factor); histamine; laminin; formyl-peptides; collagen fragments; lymphocyte-derived chemotactic factor, etc.

8) **Release of lysosomal enzymes**: C5a; PAF; formyl-peptides; phagocytosis, etc.

9) **Oxygen toxic radicals formation**: C5a; PAF; Il-8; Inf-gama ; TNF-alpha.

10) **Phagocytosis:** C3a; Ig G (Fc fraction); fibronectin; Inf-gama, etc.

11) **Fever**: Il-1; PGE2; Inf-gama; Inf-alpha; TNF-alpha, etc.

12) **Pain:** bradykinin; P substance; PGE2, etc.

13) **NO formation:** catecholamines; endotoxin, hypoxia, drugs, etc.

14) **Hypercoagulation**: catecholamines (elevation of >50% circulating platelets; ADP-induced platelet aggregation; long-chain free fat acids; Factor V; Hageman Factor(XII); PAF; thromboxane B4; LTB4, serotonin [2,11,37-40]

As considered above, vardenafil showed a favorable profile in the MODS protection, because its pharmacologic and pharmacokinetic characteristics, such as an 36% absorption of

oral dose through the gastrointestinal tract. [28,22] In addition, CYP3A proved to act as the major hepatic metabolizing enzyme for the phosphodiesterase inhibitors, having a lower effect on the agent tadalafil, which is responsible for its longer half-life.[18,20,23,29] Vardenafil is lipophilic molecule responsible for its elevated membrane absorption, which is translated into a large volume of distribution with high tissue uptake and binding.[29] Moreover, vardenafil is highly protein-bound, being transported to the whole system with a free concentrations of only 4 to 6% of the plasma fractions. [18,20,23,29] Vardenafil, indeed, has been detected in plasma even 5 days after oral administration due to its long half-life. This suggests the possibility of accumulation if taken regularly and at short intervals. [28,29] All these aspects are responsible for efficacy profile of vardenafil.

The most common MODS pathologic findings in the rats submitted to CNS electrolytic lesions, were: multiple micro-thrombi, tissue edema and disseminated hemorrhages. [31,37-40]

The results presented in this work shows definitely that vardenafil acts protectively against the experimentally-induced MODS development, especially when the drug is administered before the neurosurgical MODS-induction procedure. In the 2 hours vardenafil pre-treatment group, it was possible to detect a highly significant CGS ($p>0.001$) reduction of 66.37% in the gravity in the MODS typical multiple organs damage. In the 2 hours vardenafil post-treatment group, it was also detected a less intense, but equally highly significant CGS ($p>0.01$) reduction of 59.64% of MODS organ damage gravity. The difference between the two groups, probably, is due to the spontaneously developing MODS reactions that occurs in the 2 hours after brain lesions.

Considering specifically the five organs examined in this work, the highest grades of histopathological damage (CGS) detected in the vardenafil 2 hours-Before Group showed the following low percentages:

1) Liver- Grade I- centrilobular turgence (8,23%); 2) Kidney- Grade I- minimal parenchymal edema (11,10%); 3) Lung- Grade I-minimal interstitial edema (19,78%) ; 4) Brain- Grade I-5.45 %; 5) Stomach- Grade I-mucosal petechiae (14.56%); Grade II:mucosal petechiae, 1 stress ulcer (7.4%). These percentages show that the pre-treatment has induced a protective effect.

Conclusion

Indeed, the drug continues to show its protective characteristics even starting to act two hours after the neurosurgical induction.

Therefore, it suggests that the protective vardenafil effect continues to occur even after a period of time after trauma occurred, a situation that usually happens in clinical conditions.

Therefore, vardenafil, as above considered, might have protective actions against experimentally-induced MODS that are based, possibly, on its vasodilation actions, platelet aggregation inhibition and inflammatory modulation effects.[30,31] This work creates, possibly, the scientific basis for utilizing vardenafil in clinical trials in post-traumatic conditions,

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