REDVET Rev. electrón. vet. http://www.veterinaria.org/revistas/redvet
2013 Volumen 14 Nº 2 - http://www.veterinaria.org/revistas/redvet/n020213.html

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504

Comparison of the protective effects of Omega 3 fatty acids and D-002 (beeswax alcohols) on the ethanol-induced gastric ulcer in the rat - Comparación del efecto protector del Omega-3 y el D-002 (alcoholes de la cera de abejas) sobre la úlcera gástrica inducida por etanol en ratas

CARBAJAL QUINTANA DAISY, MOLINA CUEVAS VIVIAN, RAVELO CALZADO YAZMIN AND MAS FERREIRO ROSA

Pharmacology Department, Centre of Natural Products, National Centre for Scientific Research (Havana City, Cuba)

*Author For Correspondence: Dr. Daisy Carbajal - E-mail: cpn.sup@cnic.edu.cu

Resumen

D-002, es una mezcla de de 6 alcoholes alifáticos purificados de la cera de abejas y ha sido demostrado en estudios experimentales que posee un efecto gastrotprotector y antiinflamatorio. El aceite de pescado Omega-3 posee efectos antiinflamatorios demostrados y mas recientemente se describe un efecto antiulceroso. Tanto el D-002 como el Omega-3 previenen las úlceras inducidas por etanol, aspirina, estrés y ligadura de píloro. Sin embargo no existen estudios previos que comparen el efecto de ambos. El objetivo de este trabajo consiste en comparar el efecto preventivo del D-002 y el omega-3 en la úlcera inducida por etanol. Ratas Sprague Dawley fueron distribuídas en 7 grupos (10 ratas por grupo); un control negativo, 3 grupos tratados con D-002 y 3 con omega-3 (25, 50 y 250 mg/kg) respectivamente durante 15 días. Las úlceras fueron inducidas por la administración oral de etanol al 60 %. A la hora fueron sacrificadas y las úlceras cuantificadas. Los resultados demuestran que: el pretratamiento con omega-3 (50 y 250 mg/kg) reduce significativamente el tamaño de las úlceras en un 43 y 45%, respectivamente comparado con el grupo control, mientras la dosis menor (25 mg/kg) fue inefectiva. (2) Todas las dosis de D-002 (25 – 250 mg/kg) redujeron las úlceras significativamente ($p < 0.05$ for 25 mg/kg, $p < 0.01$ for 25 and 250 mg/kg) en un 51, 56 and 58% respectivamente. En conclusión, los hallazgos de este estudio demuestran que tanto el D-002 como el omega-3 inhiben significativamente y comparativamente similar las lesiones gástricas inducidas por etanol en ratas.

Palabras claves: D-002 | alcoholes de la cera de abejas | Omega-3 | gastroprotección | úlcera por etanol .

Abstract

D-002, a mixture of six higher aliphatic alcohols purified from beeswax, has been shown to produce both gastroprotective and anti-inflammatory effects in experimental studies. Antiinflammatory effects of Omega-3 fatty acids (ω-3 FA)

derived from fish oil have been documented. Also, recent experimental studies have found that ω-3-FA has produced antiulcer effects. Both D-002 and ω-3 FA have been able to prevent aspirin-, cold-restraint stress-, ethanol- and pylorus ligation-induced ulcers. No previous study, however, has compared the gastroprotective effects of these substances. The objective of this work was to compare the preventive effects of D-002 and Ω-3 FA on ethanol-induced gastric mucosal injuries in rats. Male Sprague Dawley rats were randomly into seven groups of 10 rats: a negative vehicle control, three D-002-treated groups (25, 50 and 250 mg/kg, respectively) and three groups treated with the same doses of ω-3-FA during 15 days. The gastric ulcers were induced by instilling 1 ml 60% ethanol into the stomach. One hour later, the stomachs were removed and the gastric lesions were quantified. The findings of our study were as follows: (1) the ω-3-FA (50 and 250 mg/kg) pretreatment was found to provide significant ($p < 0.01$) reductions of ulcer sizes (43 and 45%, respectively) as compared to the control group, while the lowest dose (25 mg/kg) was ineffective; (2) all doses of D-002 (25 – 250 mg/kg) produced significant ($p < 0.05$ for 25 mg/kg, $p < 0.01$ for 25 and 250 mg/kg) reductions (51, 56 and 58%, respectively) of ethanol-induced ulcer sizes. In conclusions, the findings of this study showed that both ω-3-FA and D-002 significantly and comparably inhibited the ethanol-induced gastric lesions in rats.

Keywords: D-002 | beeswax alcohol | Omega-3 | gastroprotection | ethanol ulcers.

INTRODUCTION

Anti-inflammatory effects and cardiovascular benefits of omega-3 fatty acids (ω-3 FA) derived from fish oil have been documented, [1-3] which competitively inhibit the biosynthesis of eicosanoids via the arachidonic acid cascade, linked to inflammation and cell proliferation. [4 – 6] Also, experimental studies have found gastrointestinal benefits of ω-3 FA that has exhibit promising effects to treat non-alcoholic fatty liver disease, [7] and has displayed protective effects against gastric ulcers induced by several noxious stimuli. [8] Administration of ω-3 FA reduced the severity of aspirin-, cold-restraint stress (CRS)-, alcohol-, and pylorus ligation-gastric ulcers by acting on both aggressive and defensive gastric mucosal factors. Therapy with ω-3 FA has been shown to decrease the offensive acid-pepsin secretion, and to increase mucin secretion and gastric mucus following rat pylorus ligation, significantly increasing the activity of anti-oxidant enzymes and lowering lipid peroxidation in the rat gastric mucosa. [8]

D-002, a mixture of six high molecular weight aliphatic primary alcohols (C_{26}, C_{26}, C_{28}, C_{30}, C_{32} and C_{34}) purified from the beeswax, wherein triacontanol (C_{30}) is the most abundant component, [9] has been shown to protect against gastric ulcers induced by non-steroidal antiinflammatory drugs (NSAIDs), ethanol, CRS and pylorus ligation in the rat. [10 – 17] The gastroprotective effects of D-002 are associated to a multifactorial mechanism that involves the increase of defensive factors, like gastric mucus secretion, increase of the protein content of such mucus, [11, 12] and antioxidant effects exerted on the gastric mucosa, [14] as well.

No previous study, however, has compared the gastroprotective effects of these

substances.

This study, therefore, was undertaken to compare the effects of ω-3 FA and D-002 on ethanol-induced gastric ulcers in rats.

MATERIALS AND METHODS

The batch of D-002 used in the experiment was supplied by the Plants of Natural Products of the National Centre for Scientific Research (Havana City, Cuba). The batch composition, assessed with a validated gas chromatographic method, [18] was as follows: tetracosanol (7.1%), hexacosanol (11.2%), octacosanol (13.8%), triacontanol (31.4%), dotriacontanol (22.1%) and tetratriacontanol (2.5%). Purity (total content of these 6 alcohols) was 88%.

The batch of ω-3-FA, supplied by Rainbow & Nature, LTD (Sydney, Australia) had a complex fatty acid composition, in which eicosapentanoic acid (EPA) and docohexapentanoico (DHEA) are in 44 and 37%, respectively, while others ω-3 FA were present in lower concentrations.

Animals Adult male Sprague Dawley rats (250-300g) were acquired in the National Centre for Laboratory Animal Production (CENPALAB, Havana) and housed in temperature-controlled rooms (22-23 ^{o} C, humidity 55-60%, 12 hours dark/light cycles) for 7 days. Free access to water and standard chow (rodent pellets from CENPALAB) was allowed. The study was conducted in accordance with current Cuban Guidelines for Good Laboratory Practices and for the care of laboratory animals. An independent ethic board for animal use approved the protocol for the study.

Treatments (D-002, ω-3 FA) were suspended in Tween 20/water (2%) vehicle, and sucralfate (Merck) was dissolved in distilled water (20 mg/ml) and administered orally via gastric gavage (1 ml/200 g) for 15 days.

Rats were randomized into eight groups (10 rats per group): a negative vehicle control (treated only with the vehicle), three D-002-treated groups (25, 50 and 250 mg/kg, respectively), three groups treated with the same doses of ω-3-FA and one group positive control with sucralfate (100 mg/kg).

The gastric ulcers were induced by instilling 1 ml of 60% ethanol by orogastric gavage to the stomach of fasted rats. We sacrificed animals under ether anesthesia 45 min after treatment with ethanol, and removed their stomachs, which were opened along the greater curvature and examined for lesions developed in the glandular portion under dissecting microscope (10) with a square grid. The numbers of ulcer lesions (U. No.) in the glandular portion of the stomach were noted. The ulcer area (mm^2) were measured and expressed as the ulcer size.

Statistical analysis

Results were expressed as mean ± SEM (standard error of mean).Comparisons among groups were conducted with the nonparametric Kruskal Wallis test, while paired comparisons between treated and control groups were performed with the

Mann-Whitney U test. Statistical significance was chosen for α= 0.05. Data were processed with the Statistics Software for Windows (Release 4.2 Stat Soft Inc, Tulsa OK, US).

RESULTS

Table 1 shows the results of the experiment. Pretreatment with ω-3-FA (50 and 250 mg/kg) significantly ($p < 0.01$) reduced the ulcer sizes (43 and 45%, respectively) as compared to the control group. The lowest dose (25 mg/kg), however, was ineffective. All the doses of D-002 (25 – 250 mg/kg) produced significant ($p < 0.05$ for 25 mg/kg, $p < 0.01$ for 25 and 250 mg/kg) reductions (51, 56 and 58%, respectively) of ethanol-induced ulcer sizes. The effects of both ω-3-FA and D-002 were statistically similar. Sucralfate a positive control (100m/kg) produce reductions (55.8%), in ulcer lengths when were compared with controls (vehicle).

Table 1.
Effects of ω-3-FA and D-002 on the ethanol-induced gastric ulcer in the rat

Treatment and doses	Mucosal lesions Ulcer sizes (mm^2) mean ± SEM	Reduction (%)
Vehicle control	78.75 ± 15.24	-
ω-3-FA 25 mg/kg	46.24 ± 11.71 n.s	41..2
ω-3-FA 50 mg/kg	44.35 ± 10.59*	43.6
ω-3-FA 250 mg/kg	43.05 ± 10.90*	45.3
D-002 25 mg/kg	38.28 ± 10.45 *	51.3
D-002 50 mg/kg	34.44 ± 11.51**	56.2
D-002 250 mg/kg	33.24 ± 10.59*	57.8
Sucralfate 100 mg/kg	34.8 ± 6.50 *	55.8

The values represent the means ± SEM. * $p < 0.05$, ** $p < 0.01$.
Comparisons among groups were conducted with the nonparametric Kruskal Wallis test, while paired comparisons between treated and control groups were performed with the Mann-Whitney U test.

According to its objectives, this study did not add new pieces on the mechanisms whereby ω-3-FA and D-002 exert their protective effects on ethanol-induced gastric ulcers, but our findings are compatible with the mode of action proposed for them.

The gastric mucosa maintains structural integrity and function despite continuous exposure to factors capable of digesting this tissue, like acid and pepsin. Under normal conditions, mucosal integrity is maintained by defensive mechanisms: a mucus-bicarbonate-phospholipid *"barrier"*, an epithelial *"barrier"* of linked surface epithelial cells that generates bicarbonate, mucus, phospholipids, peptides, prostaglandins (PGs) and heat shock proteins, continuous proliferation of progenitor cells, continuous blood flow, sensory innervation and generation of PGs and nitric oxide. [19] Mucus, which continuously coats over the gastric mucosa, protects the mucosa against the injury of noxious agents and is implicated in scavenging oxygen-derived free radicals. [20, 21] Mucus glycoproteins and lipids, which account for antiradical activity, [22, 23] and endogenous antioxidants are relevant as protective factors of gastric mucosa. The pool of endogenous non-protein sulfhydryl groups, which contains high concentrations of reduced glutathione, displays a protective role due to their oxygen-derived free radicals scavenging [24, 25] and their influence on the production and characteristics of the mucus. [26, 27] Unbalance between mucosal defensive mechanisms and offensive factors may lead to mucosal injury.

We tested the effects of ω-3-FA and D-002 in the model of ethanol-induced gastric mucosal lesions in the rat, in which the mechanisms involved in the pathogenesis of the gastric damage include increased oxidative stress, [28-30] decreased concentration of NP-SH content in gastric mucosa, [31] direct damage to the mucin layer, [32] and increased gastric cell's apoptosis. [33] Since the effect of ω-3-FA on this model has been associated to inhibition of offensive mucosal factors (oxidative stress acid-pepsin secretion) as well as to the increase of defensive (mucin secretion, gastric mucus secretion, anti-oxidant enzymes -catalase and glutathione peroxidase- activities) in the rat gastric mucosa, [8] the present results are logical. In turn, oral administration of D-002 decreased some offensive factors like lipid peroxidation and concentrations of thromboxane B_2 in the gastric mucosa, unchanging acid secretion, and markedly increased defensive factors such as the gastric mucus secretion and glycoprotein concentration in the gastric mucus. [11,12] Also, increase of antioxidant enzymes and total plasma antioxidant status has been induced by D-002. [34 – 39]

The comparable efficacy of ω-3-FA and D-002 here found is consistent with the similarity of the mechanisms whereby that support their efficacy on this model. The fact that ω-3-FA, not D-002, has been shown to reduce luminal acid secretion, [8] meanwhile the opposite occurs with the reduction of stomach thromboxane B_2 concentrations, supports the interest of study the effects of the combined therapy with both substances on this model, specifically the therapeutic effect.

In conclusion, the findings of this study have confirmed that both ω-3-FA (50 and 250 mg/kg) and D-002 (25-250 mg/kg) significantly inhibited the ethanol-induced gastric lesions in rats. Further studies are warranted to evaluate if combination therapy with both substances can confer some advantage over each monotherapy regarding to gastric protection for noxious stimuli, which should include the assessment of different dosage schemes and the use of different ulcer models.

REFERENCES

1. Bays, H.E., Tighe, A.P., Sadovsky, R., Davidson, M.H. Prescription omega-3 fatty acids and their lipid effects: physiologic mechanisms of action and clinical implications. *Expert Review of Cardiovascular Therapy*, 2008, nº 6, p. 391-409.
2. Harris, W.S., Miller, M., Tighe, A.P., Davidson, M.H., Schaefer, E.J. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. *Atherosclerosis,* 2008, vol.197, p.12-24.
3. Marik, P.E., Varon, J,. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. *Clinical Cardiol*ogy, 2009, vol. 32, .365-72.
4. Larsson, S.C., Kumlin, M., Ingelman-Sundberg, M., Wolk, A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *American Journal of Clinical Nutrition*, 2004, vol.79, p. 935–45.
5. Cheng, J.L., Ogawa, K., Kuriki, K., Yokoyama, Y., Kamiya, T., Seno, K., Okuyama, H., Wang, J., Luo, C., Fujii, T., Ichikawa, H., Shirai, T. Increased intake of n-3 polyunsaturated fatty acids elevates the level of apoptosis in the normal sigmoid colon of patients polypectomized for adenomas/tumors. *Cancer Letters*, 2003, vol. 193, p. 17-24.
6. James, M.J., Gibson, R.A., Cleland, L.G. Dietary polyunsaturated fatty acids and inflammatory mediator production. *American Journal of Clinical Nutrition,* 2000, Vol. 71, p. 343–8S.
7. Masterton, G.S., Plevris, J.N., Hayes, P.C. Review article: omega-3 fatty acids - a promising novel therapy for non-alcoholic fatty liver disease. *Alimentary Pharmacology and Therapeutics.* 2010, vol.31, nº 7, p. 679-92
8. Bhattacharya, A., Ghosal, S., Bhattacharya, S.K. Effect of fish oil on offensive and defensive factors in gastric ulceration in rats. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 2006, vol. 74, p.109-116.
9. Más, R. D-002: A product obtained from beeswax. *Drugs of the Future*, 2001, vol. 26, p. 731-44.
10. Carbajal, D., Molina, V., Valdés, S., Arruzazabala, M.L., Más, R. Anti-ulcer activity of higher primary alcohols of beeswax. *Journal of. Pharmacy and Pharmacology,* 1995, vol. 47, p.731-33.
11. Carbajal, D., Molina, V., Valdés, S., Arruzazabala, M.L., Rodeiro, I., Más, R., Magraner, J. Possible cytoprotective mechanism in rats of D-002 an anti-ulcerogenic product isolated from beeswax. *Journal of Pharmacy and Pharmacology, 1996*, vol.48 , p. 858-60.
12. Carbajal, D., Molina, V., Noa, M., Valdes, S., Arruzazabala, M.L., Aguiar, A., Más, R. Effects of D-002 on gastric mucus composition in ethanol-induced ulcer. *Pharmacological Research*, 2000, vol.42, p. 329-32.
13. Molina, V., Valdés, S., Carbajal, D., Arruzazabala, M.L., Menéndez, R., Más, R. Antioxidant effects of D-002 on gastric mucosa of rats with experimentally-induced injury. *Journal of Medicinal Food,* 2001, vol. 4, p. 79-83.
14. Molina, V., Carbajal, D., Arruzazabala, M.L., Más, R. Therapeutic effect of D-002 (Abexol) on gastric ulcer induced experimentally in rats. *Journal of Medicinal Food* , 2005, vol. 18, p. 59-62.
15. Hano, O., Illnait, J., Más, R., Fernández, L., Piñol, F., Fernández, J. Effects of D-002, a product isolated from beeswax, on duodenal ulcer: a double-blind, placebo-controlled study. *Current Therapeutic Research, Clinical and Experimental, 2001*, vol. 62, p. 394-07.

16. Illnait,J., Terry, H., Más, R., Fernandez, L., Carbajal, D. Effects of D-002, a product isolated from beeswax, on gastric symptoms of patients with osteoarthritis treated with piroxicam: a pilot study. *Journal of Medicinal Food*, 2005, vol. 8, p. 63-8.
17. Rodríguez, I., Illnait, J., Terry, H., Más, R., Fernández, L., Fernández, J.C., Gámez, R., Mesa, M., Mendoza, S., Ruiz, D., Cruz, Y. Effects of Abexol® (beeswax alcohols) on gastrointestinal symptoms of middle-aged and older subjects. *Revista CENIC Ciencias Biológica*s, 2009, vol. 40 p.147-54.
18. González, V., Marrero, D., Sierra, R., Velázquez, C., Vicente, R. Nuevo método por cromatografía gaseosa capilar para el análisis del ingrediente activo D-002. *Revista CENIC Ciencias Químicas*, 2008, vol. 39, p.123-25.
19. Laine, L., Takeuchi, K., Tarnawski, A. Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology*, 2008, vol.135, p. 41-60.
20. Garner, A., Flemstrom, G., Allen, A., Heylings, J.R., McQueen, S. Gastric mucosal protective mechanisms: role of epithelial bicarbonate and mucus secretions. *Scandinavian Journal of Gastroenterology,* 1984, vol. 101, p. 79-86.
21. Grisham, M.B.**,** Von Ritter, C., Smith, B.F., Lamont, J.T., Granger, D.N. Interaction between oxygen radicals and gastric mucin. *American Journal of Physiology,* 1987, vol. 253, p. G93-6.
22. Cross, C.E.**,** Halliwell, B., Allen, A. Antioxidant protection: a function of tracheobronchial and gastrointestinal mucus*. Lancet,* 1984, vol. 1, p. 328-30.
23. Gong, D.H.**,** Turner, B., Bhaskar, K.R., Lamont, J.T. Lipid binding to gastric mucin: protective effect against oxygen radicals. *American Journal of Physiology,* 1990, vol. 259, p. G681-86.
24. Hiraishi, H., Terano, A., Ota, S., Mutoh, S., Sugimoto, T., Hirada, T. Role for mucus glycoprotein in protecting cultured gastric mucosal cells against toxic oxygen metabolites. *Journal of Laboratory and Clinical Medicine,* 1993, vol. 121, p.570-78.
25. Allen, A., Cunliffe, W.J., Pearson, J.P., Sellers, L.A., Ward, R. Studies on gastrointestinal mucus. *Scandinavian Journal of Gastroenterology,* 1984, vol. 93, p. 101-13.
26. Salim, A.S. Sulfhydryl-containing agents: new approach to the problem of refractory peptic úlceration. *Pharmacology,* 1993, vol. 46, p. 281-88.
27. Szabo, S., Goldberg, I. Experimental pathogenesis: drugs and chemical lesions in the gastric mucosa. *Scandinavian Journal of Gastroenterology,* 1990, vol. 174, p.1-8.
28. Li, T., Zhang, X.J. Involvement of sulfhydryls in the protective mechanism of gastric mucosa. *Acta Pharmacologica Sinica*, 1990, vol. 42, p.571-77.
29. Pihan, G., Regillo, C., Szabo, S. Free radicals and lipid peroxidation in ethanol- and aspirin-induced gastric mucosal. injury. *Digestive Diseases and Sciences,* 1987, vol. 32, p. 1395-01.
30. Szelenyi, I., Brune, K. Possible role of oxygen free radicals in ethanol-induced gastric mucosal damage in rats. *Digestive Diseases and Sciences,* 1988, vol. 33, p. 865-71.
31. Mutoh, H., Hiraishi, H., Ota, S., Ivey, K.J., Terano, A., Sugimoto, T. Role of oxygen radicals in ethanol-induced damage to cultured gastric mucosal cells. *American Journal of Physiology,* 1990, vol. 258, p. G603-09.
32. Slomiany, A., Morita, M., Sano, S., Piotrowski, J., Skrodzka, D., Slomiany, B.L. Effect of ethanol on gastric mucus glycoprotein synthesis, translocation,

transport, glycosylation and secretion. *Alcoholism, Clinical and Experimental Research,* 1997, vol.21, p. 417-23.

33. Hoshino, T., Takano, T., Tsutsumi, S., Tomisato, W., Tsuchiya, T.I, Mizushima, T. Effects of prostaglandin E2 on gastric irritant-induced apoptosis. *Digestive Diseases and Sciences,* 2002, vol. 47, p. 2370-79.
34. Menéndez, R., Amor, A.M., González, R.M., Jiménez, S. ,Más .R. Inhibition of rat microsomal lipid peroxidation by the oral administration of D-002. *Brazilian Journal of Medical and Biological Research,* 2000, vol. 33, p. 85-90.
35. Menéndez, R., Más, R., Amor, A.M., Pérez, Y., González, R.M., Fernández, J.C., Jiménez, S. Antioxidant effects of D-002 on the *in vitro* susceptibility in healthy volunteers. *Archives of Medical R*esearch, 2001, vol. 32, p.436-41.
36. Menéndez, R., Más, R., Illnait, J. Pérez, J., Amor, A.M., Fernández, J.C., González, R.M. Effects of D-002 on lipid peroxidation in older subjects. *Journal of Medicinal Food,* 2001, vol.4, p. 71-7.
37. López, E., Illnait, J., Molina,,V., Oyarzábal, A., Fernández, L., Pérez, Y., Más, R., Mesa, M., Fernández, J.C., Mendoza, S., Gámez, R., Jiménez, S., Ruiz, D., Jardines, Y. Effects of D-002 (beeswax alcohols) on lipid peroxidation in middle-aged and older subjects. *Latin American Journal of Pharmacy,* 2008, vol. 27, p. 695-03.
38. Rodríguez I., Illnait J., Molina V., Oyarzábal A., Fernández L., Fernández JC., Mesa M., Más R., Mendoza S., Gámez R., Jiménez S., Ruiz D. Comparison of the antioxidant effects of D-002 (Beeswax Alcohols) and Grape Seed Extract (GSE) on plasma oxidative variables in healthy subjects. *Latin American Journal of Pharmacy*. 2010, vol. 29, n°. 2, p. 255-262.
39. Pérez, Y., Gonzalez, R.M., Amor, A.M., Jiménez, S., Menéndez, R., D-002 on antioxidant enzymes in liver and brain of rats. *Revista CENIC Ciencias Biologicas,* 2002, vol. 33 ,p. 3-5.

REDVET: 2013, Vol. 14 Nº 2

Recibido 07.06.2012 / Ref. prov. NOV1103C_REDVET / Revisado 22.12.2012/ Aceptado 08.01.2013 / Ref. def. 021303_REDVET / Publicado: 02.02.2013

Este artículo está disponible en http://www.veterinaria.org/revistas/redvet/n020213.html concretamente en http://www.veterinaria.org/revistas/redvet/n020213/021303.pdf

REDVET® Revista Electrónica de Veterinaria está editada por Veterinaria Organización®.