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EFFECT OF DIFFERENT VEHICLES IN CARRAGEENAN SUSPENSION ON THE RATE OF THE INFLAMMATORY RESPONSE OF CHICKS

EFEITO DE DIFERENTES VEÍCULOS NA SUSPENSÃO DE CARRAGENINA SOBRE A RESPOSTA INFLAMATÓRIA EM PINTOS

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SUMMARY

This paper describes the pattern of edema, increased vascular permeability and cellular exudation elicited by the injection of different carrageenan suspensions into the foot pad of 80 male chicks, three to four-week old. Carrageenan suspensions at 0.5% were prepared in: Ringer Locke solution (RL), glucose aqueous solution 0.1% (G), demineralized water (W) or phosphate buffered saline (PBS). The foot pad volume and vascular permeability were evaluated by pletismography and by Evans blue extravasation, respectively, before and at 0:15, 0:30, 1:00, 1:30, 2:00, 2:30, 3:00, 3:30 and 4:00 hours after injury. Cellular exudation was observed in thin sections of stained tissue 0:30, 1:30, 2:30 and 4:00 hours after injection of the carrageenan or vehicle only. The inflammatory response varied according to the carrageenan suspension used. Suspension C/PBS induced a less intense inflammatory response in foot pads of chicks than C/W, C/G and C/RL suspensions.

UNITERMS: Carrageenan; Hens; Inflammation.

INTRODUCTION

Carrageenan is a wellknown sulfated polyssacharide largely used for experimental studies of the inflammatory response in mammals. Few studies done in chickens (TALIAFERRO; BLOOM[15], 1945; CARLSON[4], 1982) have suggested that the inflammatory response in birds is both less intense and lasting than that observed in rats. The chemical modulation seems to be different in both species, too. In rats, bradykinin (DI ROSA; SORRENTINO[7], 1970; BHALLA; TANGRI[2], 1970), prostaglandins (FERREIRA et al.[9], 1974; BONTA et al.[3], 1978) and complement (DI ROSA et al.[6], 1971; MC CALL; YOULTEN[13], 1974) play an important role in the carrageenan-induced inflammatory response, while in chickens, histamine and serotonin seem to be the main mediators (ITO et al.[11], 1989). This fact could explain why the inflammatory process elicited by carrageenan injection in chickens lasts less in rats. In addition, low rate intensity of carrageenan-induced response in birds could be attributed to other factors, as well. It is known that the rate of the inflammatory process in rats is influenced by the structural presentation (DI ROSA[5], 1972; ANDERSON et al.[1], 1984) and the concentration (ZANIN; FERREIRA[17],1978) of carrageenan used. In chickens the concentration of carrageenan does not modify the intensity of the process (ITO et al.[11], 1989). The purpose of this paper is to study the influence of the vehicle used to prepare carrageenan suspension on the pattern of edema, increased vascular permeability responses and cellular exudation in chicks.

MATERIALS AND METHODS

Chicks

Three to four-week old, Hyssex Brown, male chicks were used. The birds were fed with commercial food and water "ad libitum" throughout the experiment.

Edema production

Potasium carrageenan (C) (Sigma, Batch C 1013) 0.5% suspension in phosphate buffered saline (PBS), Ringer Locke solution (RL), sterile demineralized water (W), or glucose aqueous solution 0.1% (G), were prepared at room temperature. Newly prepared carrageenan suspensions, and their respective vehicles (control) were injected into the subcutaneous tissue of the left and right foot pads of the chicks (0.1 ml/foot), respectively.

Edema evaluations

Eight groups of nine randomly taken chicks were used to evaluate the edema response. The foot pad volume was evaluated by pletismography, as described by WINDER et al[16] (1957) and modified by LEME at al.[12] (1973). The measurements were made before and 0:15, 0:30, 1:00, 1:30, 2:00, 2:30, 3:00, 3:30, and 4:00 hours after injury. The foot, up to the vestige of the fifth digit, was immersed in the cuvette of

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the apparatus. The differences in the volumes of the foot pads observed before anf after carrageenan-injection were considered as the values of edema.

Vascular permeability evaluation

Eight groups of six randomly taken chicks were used to evaluate the changes in vascular permeability. Evans blue (Merck) at 2,5% was prepared in phosphate buffered saline 0.01M and used as a vascular permeability tracer. The dye was injected by intravenous route at 0:15, 0:30, 1:00, 1:30, 2:00, 2:30, 3:00, 3:30, and 4:00 hours after injury. Thirty minutes after tracer injection chicks were killed, feet were cut off at the vestige of the fifth digite, immersed in a tube containing 4 ml of formamide (Merck), and incubated at 37ºC during 24 hours. Evans blue extracted by formamide from the site of injury was measured by spectrophotometry at 618 nm. The concentrations of extracted Evans blue were estimated in relation to a curve obtained with the Evans blue serial concentrations diluted in formamide. The vascular permeability was expressed in g of Evans blue/ ml formamide.

Histologic sections

Microscopical analysis of the injured tissue was at 0:30, 1:30, 2:30 and 4:00 hours after carrageenan or vehicle injection. Five randomly taken chicks were used for each period of time. The injected feet were cut off at the vestige of the fifth digit and fixed in formaline 10%. Slices of the fixed tissue were parafin-embedded and thin sections (5-6 um) of the included tissue were stained by haematoxilin-eosin method.

Statistical Analysis

Edema and vascular permeability results were submitted to analysis of variance (ANOVA) using Dunan's test (DUCAN8,1955) ($p<0.05$).

RESULTS

Edema formation

Comparing the results obtained from the subcutaneous injection of different carrageenan suspensions and their respective controls (Fig. 1), it was possible to observe that the beginning of the responses, as well as the time they lasted, were different one from another. C/RL, C/W and C/G responses began around 15 and 30 minutes after injury, while C/PBS reaction started only 1:00 hour after injury. The C/PBS and C/W - induced responses lasted 2:00 and 2:15 hours, respectively, while C/G and C/RL responses lasted 2:45 hours and 2:30 hours respectively. The ending-time of oedema induced by C/PBS, C/RL and C/G was after 3:00 hours, while the one induced by C/W was after 2:30 hours.

Also, comparing mean values for the increased volumes of the foot pads minus the edema values after dluent injection (Fig.2), it was possible to observe significant differences ($p < 0.05$) in the responses induced by the various carrageenan suspensions; C/RL results were higher than C/PBS ones at 1:00, 2:00, and 2:30 hours, as well as they were higher than C/W and C/G ones at 2:30 and 3:00 hours, respectively. It was observed also that response to C/ PBS was more discrete and had its peak 2:00 hours after injury. The peaks after C/W and C/G injections occurred at 1:00 and 1:30 hours respectively, while peaks after C/RL injection took place at 1:30 and 2:30 hours later.

In addition, comparing the rsults obtained from the subcutaneous injections of the vehicles only (Fig. 3), it was possible ro observe that in the beginning of the respones, between 15 minutes and 1 hour after injury, RL response was larger than PBS response ($p < 0.05$), and at 30 minutes RL response was significantly bigger than those induced by the other vehicles.

Vascular permeability

All carrageenan suspensions were able to induce dye extravasation. The C/PBS - induced increased vascular permeability was observed 30 minutes after injury, while the C/W and C/G - induced permeability were observed 15 minutes after injury. The ending-time of vascular responses induced by C/G was at 2:30 hours, and the ones induced by C/RL and C/W were at 3:30 hours, and that induced by C/PBS was at 4:00 hours after injection. Therefore, the lasting-time for the increased vascular permeability was 2:15 hours for C/G, 3:00 and 3:15 hours for C/RL and C/W, respectively, and 3:30 hours for C/PBS (Fig. 4).

Comparing duration and intensity of responses induced by C/RL and C/PBS (Fig.4), C/PBS induced a longer vascular reaction, though less intense than that induced by C/RL. It was significantly smaller at 1:30, 2:00 and 3:00 hours after injury. Going further on with comparative studies of the vascular response curves, induced by different carrageenan suspensions after discounting the effect of diluent (Fig. 5), it was possible to see that C/RL determined a response higher than C/W and C/G,respectively, at 2,2:30, 3 and 3:30 hours after injury ($p < 0.05$). On the other hand, C/PBS suspension presented a response peak at 2:30 hours, C/W had its peak at 1:00 hour, and both C/G and C/RL showed their response peaks 2 hours after injury.

Comparing the effect of the vehicles in the vascular permeability, it was possible to observe that permeability responses were different, and that PBS induced a less intense vascular reaction (Fig.6).

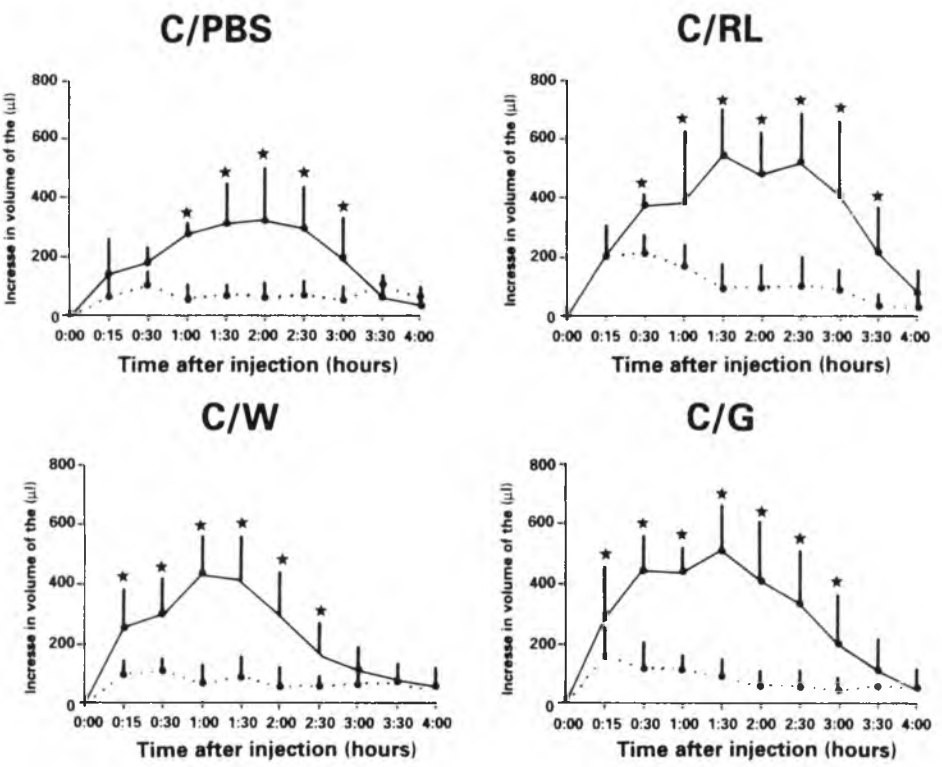

FIGURE 1

Edema induced by s.c. injection of C/PBS,C/RL, C/W, and C/G in the foot pad of chicks. The results are expressed as mean standard deviation and represent the difference in the values obtained before and after the injection of irritant and/or vehicle. ( ) irritant, ( ) diluent, (*) difference statistically significant in relation to the control ($p < 0.05$) (n = 36).

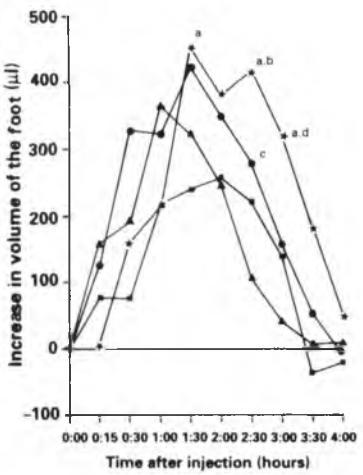

FIGURE 2

Edema induced by s.c. injection of C/PBS,C/RL, C/W, and C/G in the foot pad of chicks. The results are expressed as mean and represent the differences in the values, obtained before and after the injection of irritant, subtracted from the values obtained in the respective diluents. (■) C/PBS, (✱) C/RL, (▲) C/W, (●) C/G. (a) difference statistically significant between C/RL and C/PBS; (b) between © C/RL and C/W; C/RL and C/G; (d) C/G and C/AD. ($p < 0.05$) (n =36).

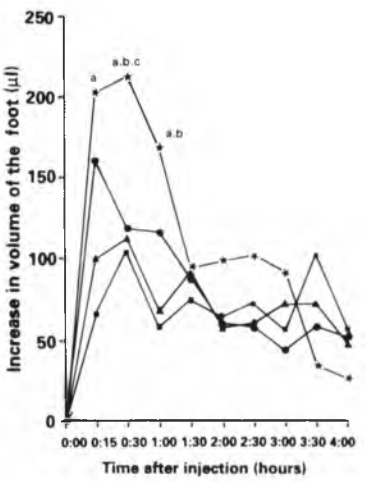

FIGURE 3

Edema induced by s.c. injection of PBS, RL, W and G in the foot pad of chicks. The results are expressed as mean and represent the differences in the values, obtained before and after the injection of diluents. (■) PBS, (✱) RL, (▲) W, (●) G. (a) difference statistically significant between RL and PBS; (b) between RL and W; © RL and G ($p < 0.05$) (n =36).

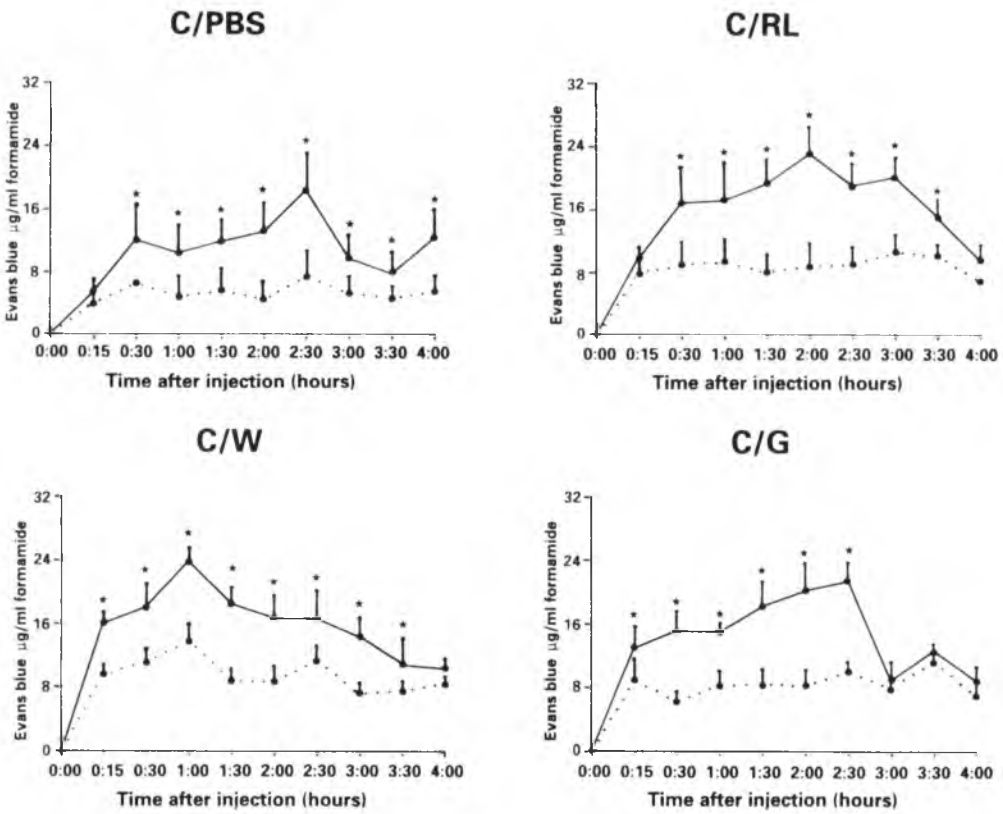

FIGURE 4

Vascular permeability induced by s.c. injection of C/PBS,C/RL, C/W, and C/G in the foot pad of chicks. The results are expressed as mean standard deviation and represent the amount of Evans blue extracted per ml of formamide ( ) irritant, ( ) diluent, (*) difference statistically significant difference in relation to the control ($p < 0.05$) ($n = 24$).

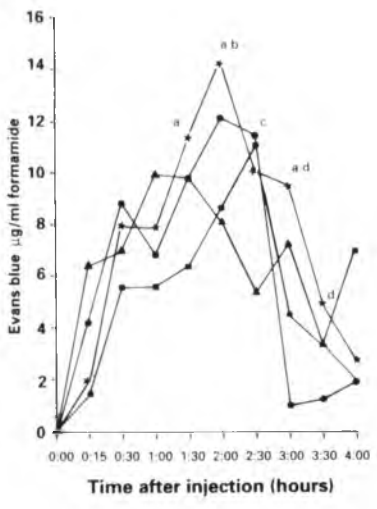

FIGURE 5

Vascular permeability induced by s.c. injection of C/PBS,C/RL, C/W, and C/G in the foot pad of chicks. The results are expressed as mean and represent the amount of Evans blue extracted per ml of formamide after the injection of irritant, subtracted from the values obtained in the respective diluents. (■) C/PBS, (✱) C/RL, (▲) C/W, (●) C/G. (a) difference statistically significant between C/RL and C/PBS; (b) between C/RL and C/W; © C/RL and C/G; (d) C/G and C/W. ($p < 0.05$) ($n = 24$).

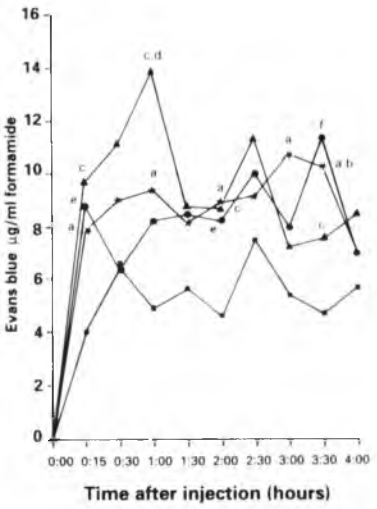

FIGURE 6

Vascular permeability induced by s.c. injection of PBS,RL, W, and G in the foot pad of chicks. The results are expressed as mean and represent the amount of Evans blue extracted per ml of formamide after the injection of diluents. (■) PBS, (✱) RL, (▲) W, (●) G. (a) difference statistically significant between RL and PBS; (b) between RL and W; ©W and PBS, (d) W and G, (e) G and PBS, (f) G and W ($p < 0.05$) ($n = 24$).

Histologic features

C/PBS produced a less intense cellular exudation in the injured tissue than those elicited by C/W, C/G and C/RL. The cellular response foundat 30 minutes post injury with C/PBS was characterized by vascular margination of heterophils and mononuclear cells infiltration in the connective tissue. At this time, large number of heterophils and mononuclear cells were found in the connective tissue injured by C/W, C/G and C/ RL.

Heterophils were the predominant cells, and edema was a common finding during the period of 1:30 to 4:00 hours after carrageenan injection, irrespective of the type of vehicle used. All vehicles used, except water, produced a discrete migration of mononuclear cells and heterophils, and absence of edema in the connective tissue. Demineralized water induced predominant infiltration of heterophils and edema 0:15 to 4:00 hours after injection.

DISCUSSION

Despite the fact that all carrageenan suspensions induced edema, increased vascular permeability, and infiltration of heterophil and mononuclear cells in injured tissue, the present data show that the intensity and duration of the inflammatory response in the foot pads of chicks varied according to the solution used. Carrageenan prepared in RL induced an inflammatory response higher than those elicited by C/G, C/W and C/PBS. Among the studied vehicles, only the demineralized water induced inflammatory cellular exudation similar to the carrageenan. This could be related to an injuring effect caused by watr hipotonicity,that induces larger migration of mononuclear cells and heterophils to the site of injury. The fact that PBS determined a mild response, and that Rlinduced a higher response suggest that potassium and calcium salts present in RL solution might increase the pattern of the inflammatory response. According to HERSH; BODEY [10] (1970), bivalent anions and cations produce a more ntense inflammatory response than monovalent ions.

Differences in the rate of inflammatory responses induced by carrageenan prepared in different suspensions could be related to an alteration of the biological activity of this substance. According to MORRIS et al.[13] (1980), the viscosity and the elastic properties of carrageenan are altered in the presence of a cation. These authors found that the helicoidal configuration of carrageenan does not occur in the presence of sodium, but in the presence of potassium in which there is aggregation, with subsequent increase of molecular weight. As the biological activity of carrageenan depends on the molecular weight, size, charge, molecular configuration and hidration ability of the 6- sulphated or 2,6- sulphated galactose residues (ANDERSON et al. [1], 1984), the predominance of the determined ions in the different vehicles could have an important role in the carrageenan phlogogenicity.

It is known that many factors can change drug effects; the present paper is pointing to one of these.

Therefore, before starting a study on inflammatory process induced by carrageenan in chicks, it is important not only to choose carrageenan vehicle, but also to know the effect of the vehicle in the phlogogenicity these irritants.

RESUMO

Este trabalho descreve o padrão de resposta de edema, aumento de permeabilidade vascular e exsudação celular induzidos pela injeção de diferentes suspensões de carragenina nos coxins plantares de 80 pintos machos, de três a quatro semanas de idade. As suspensões de carragenina 0,5% foram preparadas em: solução de Ringer-Locke (RL), solução aquosa de Glicose (G), água desmineralizada (W) ou tampão fosfato salino (PBS). Antes, e às 0:15, 0:30, 1:00, 1:30, 2:00, 2:30, 3:00, 3:30, e 4:00 horas após a injúria, o volume da pata e a permeabilidade vascular foram avaliados através de pletismografia e extravasamento de Azul de Evans respectivamente. A exsudação celular foi observada em cortes finos de tecido corado, 0:30, 1:30, 2:30 e 4:00 horas após a injeção de carragenina ou somente do veículo. A resposta inflamatória variou de acordo com a suspensão de carragenina utilizada. A suspensão C/PBS induziu uma resposta inflamatória menos intensa nos coxins plantares do que as suspensões de C/W, C/G e C/RL.

UNITERMOS: Carragenina; Galinhas; Inflamação.

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