THE ANTIINFLAMMATORY EFFECT OF SOME PROTEIC HYDROLISATES

(Published in "VIATA *MEDICALA", Nr.* 6, *1989, p.277-281)*
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SUMMAliy.The antiinflammatory effect of some proteic hydrolisates (PL) were tested on the experimental models of the inflammation with carrageen on rate and the Freund adjuvant arth ritis on rats and on guineea pigs. The PL-s are; *PL* —horse serum hydrolisate (Cantacuzino Institute Bucharest), *PLa* and *PLc* — caseine hydrolisatcs (Chemical-Pharmaceutical Reserch Institute Bucharest). The hydrolysates have as active agent a certain quality of polypeptides with little molecular weight avoided of antigenicity, avoided of pyrogenic substances and with a very little toxicity. All three PL-s tested had antiinflanunatory effect. *PL* and *PLa* had an extremely favorable therapeutical value.

These results can open a new therapeutical way in inflammatory syndroms, especialy in rheumatical disease*.

Key *words:* polypeptides, antiinflanunatory effect.

Introduction

The purpose of this paper is to the check the antiinflamatory activity of some proteic hydrolysates (PL) for which we had preliminary experimental and clinical studies [2-8,11,13,15]. The hydrolisates we tested were obtained from the horse serum —*PL* (Cantacuzino Institute Bucuresti), from pure Fluka caseine Hammerstein .without vitamines —*PLa* and from industrial caseine —*PLc* (Institutui de cercetari chimico-farmaceutice Bucuresti). We used the test of the inflamation with carrageen and the Freund Adjuvant arthritis **on** the Wistar rats and guinee pigs.

Material and methodes

The PL-s were obtained by acid hydrolysis. There are invariable chemical constants for each PL. They are avoided of antigenicity and of pyrogenic substances too. They have not proteins (threechlor acetic acid, sulphosalicilic acid, perchloric acid re-actives) but they have polypeptides with little molecular weight (phosphotungstic reactive in acid medium). Aminic nitrogen content (Moore and Stein methodes), nitrogen content (Lowry and Biuret methodes) molecular weight (Stephadex G75 nitration compared with pure peptides curves) were established [1,10].

For the inflammation with carrageen they were taken three groups of ten Wistar male rats, 200 ± \0*g* weight, to which was administrated O.l ml carrageen 1% in the paw. PL-s were administrated intraperitoneum, 5 ml/kg animal, thirty minutes before the phlogistic agent was administrated. For comparison we used saline solution. They were made plethysmometrical determination of the volume of the posterior injected paw at the initial moment, at 2, 4, 24 hours from in-oculation with carrageen. The dosis of PL-s showed in tables, was expressed in mg. drying residium without NaCl. This residium is active substance. The results were expressed by Newbould formule [9] which consider the inflammation reducing in regard to initial value obtained at the witness animals.

For the complet Adjuvant Freund inflammation it was taken a number of six Wistar male rats for each group. They were injected at the basis of the tale with complet —Bacto adjuvant Difco (inactivated Mycobacterium butiricum in Freund adjuvant) O.lm/ at the first and at the 28-th days, to the first and second group of animals. *PLi* was adminis trated intraperitoneum, 1 mg/kg animal (active substance), only at the first group, each day from the first day, 43 days long. The third group was of witness, normal animals.

The experiment lasted 56 days. It was followed the weight curve, the pletysmometrical mesurment of the right posterior paw, and on the 56-th day the anatomico-pathological damages.

For the experiment with guinee pigs it was taken a number of five animals, 350g ± 5O*g* weight, for each group. The first and the second group were injected with 0.5ml Adjuvant Freund in the posterior paw, the first and the seventh days [14]. The third group was of normal witness ani mals. The experiment lasted 14 days. At the end of the trial were made anatomico pathological observations. "

Results

The antiinflammatory effects of PL-s on the carrageen experiment were noted in the Tab.l and 2.

Tab. l

Antiinflammatory action of PL-s on the carrageen experiment. Percentage effects of the reducing of inflammation

	Dosis mg/kg	2	*4h*	*24h*
PL	*5*	*8*	12	*43*
PLa	1	36	50	*34*
PLc	1	19	24	*4*
Sal.Sol.	5ml	0	0	*0*

Tab. 2

Antiinflammatory action of *PL* on the carrageen experiment. Percentage effects of the reducing of inflammation

Dosis mg/kg	2h	***24h***
0.1	**27**	**22**
1.0	**36**	**34**
3.5	**71**	**55**
35.5	**92**	**13**

From the Tab.l we can observe that all the three PL have antiinflammatory effect in different degrees. The efficient dosis in active substances are very low and the effects are still good 24 hours from the carrageen administration. The results presented in Tab.2 show the dependance of the antiinflammatory action of *PL* of the dosis and allow to compute the efficient dosis 50% *{ED5o)* by the graphic methode of the probit. The *ED50* (computed) at two hours is *1.8mg/kg* and *2.e)mg/kg* at 24 hours. From the first table we can also see that *PLc* which is a proteic hydrolisate, produced from an industrial caseine, has an antiinflammatory effect, but a low one, as well from the Tab.l we can see the antiinflammatory effect of little dosis of polypeptides in solution towards the nule effect of the saline solution. For *PL* it has been determined the acute toxicity, at the intraperitoneum administration on mice, which showed a letal dosis for 50 per cent *{LD5o)* of 250*mg/kg* animal. The therapeutic value *(LD5o/ED5o)* is extremely favorable.

From the Tb.3 and 4 we can watch some results of the complete Adjuvant Freund experiment on rats.

Tab3

The value of posterior right paw (on mm) mean on 10 animals

Groups	*Days of experiment*							
	Ini tial	5	10	15	25	40	56	inc. %
PL+Adj.Fre	39	38	38	38	39	*42*	42	+7
Adj. Freund	35	40	40	40	40	*41*	43	+23
Witness	38	38	38	38	39	39	40	+5

Tab. 4

The evolution of the weight curve (mean on tlie 10 animals)

Groups	*Days of experiment*						
	Ini-tial	*5*	*15*	*2O*	*27*	*56*	inc. %
PL+Adj.Fre	150	142	146	160	156	185	+23
Adj.Freund	159	148	155	156	159	177	+11
Witness	152	160	165	168	170	180	+18

From the evolution of the weight curve we can see a breeds of 23% of the animals protected with *Pi* instead of 11% of the animals unprotected. The same protection of *PL* takes place with the posterior right paw (Tab.3).

We can follow the anatomico-pathological damages on Tab.5 (experimental arthritis with Freund Adj. on rats) and Tab.6 (experimental miositis with Freund Adj. on guinees pigs). The dystrophic alterations, the necrosis, the limphocitis infiltratibn of the liver, of the spleen, of the kidney, of the lung, of the musculi, the damages of cartilage of the capsula articularis, a.s.c. were less important at the animals protected by PL-s (conventional notation).

Tab. 5

Anatomico-pathological damages at 56 days from the first inoculation of Freund Adjuvant and PL treatment (mean on 6 rats)

Wit-ness	*Fre. Adj.*	*Freu. Adj. + PL*		Group*
0.0	1.0	*1.0*	Hyperemia, edema	
0.5	2.0	*0.0*	Lymphomonocit infiltrat	
0.7	3.0	*1.0*	Lymphoreticular hyperplasia	Liver
0.3	1.0	*0.0*	Granular dystrophia	
0.0	1.0	*o.2*	Fibrinoid necrosis	
0.0	2.0	*1.0*	Megakariocitis	
0.0	1.5	*1.0*	Ly mphoret icular hyperplasia	Spleen
0.0	2.0	*1.0*	Hyperemia edema	
0.0	0.7	*0.3*	Glomerulitis edema	Kidney
0.0	1.0	*0.5*	Glomerular dystrohia	
0.0	1.0	*0.0*	Glomerular thrombosis	
0.5	2.0	*0.?*	Hematic infiltrat	
0.3	1.3	*0.6*	Lymphomonocit infiltrat	Lung
0.3	1.0	*0.5*	Hyperemia, medular edema	Adrenal
0,0	1.5	*O.S*	Hyperemia, cortical edema	gland
0.0	1.0	*1.0*	Chronic inflammation	Tale
0.0	1.0	*O.3*	Edema, hematic infiltrat	
0.0	1.0	*1.0*	Lymphomonocit infiltrat	Anterior paw
0.0	1.0	*O.3*	Fibrinoid necrosis striated fibrae	
0.0	1.5	*O.3*	Edema, hematic infiltrat	•
0.0	2.0	*1.0*	Lymphomonocit infiltrat	Posterior paw
0.0	2.0	*O.3*	Fibrinoid necrosis striated fibrae	
0.0	1.0	*0.2*	Arthodial edema and capsula articularis	

Tab. 6

Anatomico-pathological damages at 14 days from the first administration of Freund Adjuvant and PL treatment (first and second groups). The third • group was administrated only PL dayly (mean on 5 guineea pigs)

Wit ness	Fre. *Adi*	Fre. *Adj. + PL*		Grony*
0.0	1.0	*O.3*	Hyperemia, edema	
- 0.0	1.5	*0.7*	Hep&tocytis edema	Liver
0.0	1.0	*O.3*	Clear intumescence centrolobular zone	
0.5	2.0	*O.3*	Glomerular	
0.0	2.0	*O.3*	Turbid intumescence epitelium of renal tubuli	Kidney
0.0	2.0	*O.3*	Hyperemia	
0.0	2.0	*O.3*	Submucosa infiltrat	Spleen
0.0	2.0	*0.7*	Hemorragic bronchitis	Lung
0.0	1.0	*O.3*	Medullar hyperemia	Adrenal
0.0	1.0	*0.5*	Cortical edema	gland
0.0	2.0	*0.5*	Akantosis	
0.0	2.0	*1.0*	Dermal and hypodermal edema	
0.0	2.0	*0.5*	Lymphomonocytes infiltrat	Anterior paw
0.0.	1.0	*0.0*	Fibrinoid necrosis striated fibrae	
0.0	2.0	*O.3*	Interfascicular edema striated fibrae	
0.0	2.0	*1.0*	Interfascicular edema	(1)
0.0	2.0	*1.0*	Interfascicular edema	(2)
0.0	2.0	*O.3*	Akantosis	
0.0	1.5	*O.3*	Dermal and hypodermal edema	
0.0	2.0	*0.7*	Lymphotrombocy te infiltrat	
0.0	2.0	*1.0*	Fibrinoid necrosis striated fibrae	Posterior paw
0.0	2.0	*1.0*	Mucofibrinoid dystrophia striated fibrae	
0.0	2.0	*O.3*	Interfascicular edema	
0.0	2.0	*O.3*	Articular cartilage edema	
0.0	2.0	0.2	Capsula articularis edema	•
0.0	2.0	*O.3*	Sinusal lymphadenitis	(3)
0.0	2.0	*1.O*	Simusal lymphadenitis	(4)

Conclusions

The experiment that has been the object of the present paper shows that a certain **quality of polipeptides with a little molecular weight, realised by the proteic hydrolisis of the horse serum or the caseine, has antiinflammatory effects. This property is present at a very little dosis. It is possible to have in view a cellular intervention by improving** th« **enzymatic damage directly, by chemical mediators, or indirectly by the thermodynamic level.**

The little molecular weight of that polypeptides and their thermical stability (at the sterilisation) make sure the parenteral administration of the therapeutical medium. We add at these advantages the absence of antigenicity and of pyrogenic substances.

We think that this results are sufficient to open an interest in this new therapeutic way **in inflammation, especialy in rhumatismatical diseasis.**

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