THE ANTIINFLAMMATORY EFFECT OF SOME PROTEINIC HYDROLISATES.

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Summary. The anti-inflammatory effect of some proteinic hydrolisates (PL) was tested on the experimental models of the inflammation with carrageen on rats and Freud Adjuvant Arthritis on rats and on guineea pigs. The PL-s are: PL1 (horse serum hydrolysate), PL2.1 and PL2.2 (caseine hydrolisate). The hydrolisate has as active agent a certain quantity and quality of polypeptides with little molecular weight avoided of antigenicity, avoided of pyrogenic substance and with a very little toxicity. All three PL-s tested had anti-inflammatory effects. PL1 and PL2.1had an extremely favorable therapeutically value.
These results can open a new therapeutically way in inflammatory syndromes, especially in rheumatical diseases.

Key words: Polypeptides, Anti-inflammatory effect.

Introduction.

The purpose of this paper is to check the anti-inflammatory effect of some proteinic hydrolisates (PL) for which we had preliminary experimental and clinical studies (2,3,4,5,6,7,8,11,13,15). The hydrolisates we tested were obtained from the horse serum the PL1 (Institutul Cantacuzino - Bucuresti), from pure Fluka caseine PL2.1 and from the industrial caseine PL2.2 (ICCF-Bucuresti). We used the test of the inflamation with Carrageen and the Freund Adjuvant Arthritis on the Wistar rats and guineea pigs.

Material and Methods.

The PL-s were obtained by acidic hydrolysis. Here are invariable chemical constants for each PL. They are avoided ot antigenicity and pyrogenic substances too. They have no proteins (tested with three chlor acetic acid, sulphosalicilic acid, perchloric acid reactives) but they have polypepeptides with little molecular weight (phosphotungstic reactive in acidic medium). Aminic nitrogen content (Moore and Stein methods), nitrogen content (Lowry and Biuret methods), molecular weight in Sefadex G75 filtration compared with pure peptides curves) were established (1,10).

For inflammation with Carrageen they were taken three groups of ten Wistar male rats, 200-+10g weight, to which was administrated 0.1 ml. Carrageen 1% in the paw. PL-s were administrated peritoneal 5-ml/Kg-weight animal thirty minutes before the phlogistic agent was administrated. For double comparison we use saline. They were made plethysmometrical determination of the volume of the posterior paw (injected) at the initial moment, the start of the experiment, at 2,4, and 24 hours from the carrageen inoculation. The doses of PL-s, showed intables, were expressed in milligrams mdrying residium without NaCl. This is the active substance. The results were expressed by Newbould formule (9), which consider the reducing of the inflammation in regard to the initial value obtained at the witness animals.
For the Complete Freund Adjuvant inflammation it was taken a number of six Wistar mare rats for each group. They were injected at the basis of the tale with the Complete- Bacto Adjuvant Difco (inactivated Mycobacterium butiricum in Freund Adjuvant) 0.1 ml in the first and the 28-th days, to the first and second group of animals. PL2.1 was administrated 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 565 days. It was followed the weight curve, the pletismometrical measurement of the right posterior paw and at the 54-th days the anatomico - pathological damages.

For the experiment with the guineea pigs we take five animals, 350-+50g.weight, for each group. The first and the second group were injected with 0.5 ml. Freund Adjuvant in the posterior paw, the first and the seventh days (14). The third group was with normal witness animals. The experiment lasted 14 days. At the end of the trial were made anatomico - pathological observations.

Results.
The anti-inflammatory effects of PL-s on the Carrageen experiment.

Anti-inflammatory action of PL-s on the Carrageen trial.
Percentage effects on reducing inflammation.

Table 1

	Mg/Kg	*2H*	*4H*	*24H*
PL1	**5**	**8**	**12**	**43**
PL2.1	**1**	**36**	**50**	**34**
PL2.2	**1**	**19**	**24**	**4**
Saline	**5 ml.**	**0**	**0**	**0**

Table 2
Anti-inflammatory action of PL2.1 on the Carrageen trial.
Percentage effects of the reducing the inflammation.

Doses	*mg/Kg*	*2H*	*24H*
	0.1	**27**	**22**
	1.0	**36**	**34**
	3.5	**71**	**55**
	35.5	**92**	**13**

From the Table 1 we may observe that three PL have anti-inflammatory effect in different degrees. The efficient doses in active substances are very low and the effects are still good 24 hours from the Carrageean administration. The results presented in the Table 2 show the dependence of the anti-inflammatory action of PL2.1 on the doses and allow to compute the efficient doses 50% (ED50) by the graphic method of the probit. The ED50 at two hours is 1.8 mg/Kg. at 24 hours. From the first table we can see also that PL2.2 which is a proteinic hydrolisate, produced from the industrial caseine, has an anti-inflammatory effect, but a low one (compared with PL1 and with PL2.1), as well as we may see even in Table 1 that the anti-inflammatory effect of little doses of polypeptidic solution (all the three PL-s) towards the null effect of saline solution. For PL2.1 it has been determined the acute toxicity at the intraperitoneum administration on mice. It showed a lethal dose for 50 per cent (LD50) of 576 mg/Kg. body. In such a case the therapeutical value (LD50/ED50) is extremely favorable.

From the Table 3 we can follow some experimental results of the trial with the Freund Adjuvant inflammation on rats.

Table 3

The value (mean on 10 animals) of the of the wright posterior paw, in cubic millimeters.

Groups	*Day of experiment*							
	initial	*8*	*10*	*15*	*20*	*30*	*56*	*increasing %*
PL2.1 +								
Fr.Adj.	**39**	**38**	**38**	**38**	**39**	**42**	**42**	**+7**
Fr.Adj.	**35**	**40**	**40**	**40**	**40**	**41**	**43**	**+23**
Witness	**38**	**38**	**38**	**38**	**39**	**39**	**40**	**+5**

The evolution of the weight curve (means on 10 animals).

Day of experiment							
Groups	*initial*	*8*	*15*	*20*	*27*	*56*	*increase %*
PL2.1+							
Fr.Adj.	**150**	**142**	**145**	**160**	**156**	**185**	**+23**
Fr.Adj.	**159**	**148**	**155**	**156**	**159**	**177**	**+11**
Witness	**152**	**160**	**165**	**168**	**170**	**180**	**+18**

We may know that the anatomical- pathological damages with the experimental arthritis with Freund Adjuvant on rats as the experimental miositis on guineea pigs with Freund Adjuvant showed dystrophic alteration, necrosis, limphocitis infiltration in: liver, spleen, kidney, lungs, the damage of the cartilage and the capsula of the joint. All the damages were less important at the animals protected by the PL-s

Conclusions.

The experiment that has been the object of this paper shows that a certain quality of polypepotides with a little molecular weight, realized by the proteic hydrolisis of the horse serum or the caseine, has anti-inflammatory effects. This property is present at a very little doses. It is possible to have in view a cellular intervention by improving the enzymatic damage directly, by chemical mediators or indirectly by the thermodinamic level.

The little molecular weight of that polypeptides and their thermical stability (at 120 Celsius degree) make sure the parenteral administration (in injection). The absence of antigenicity and no pyrogenic substances is another advantage.

We are thinking that this result may open an interest in that new therapeutic way of the rheumatical diseases and other inflammatory complains.

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