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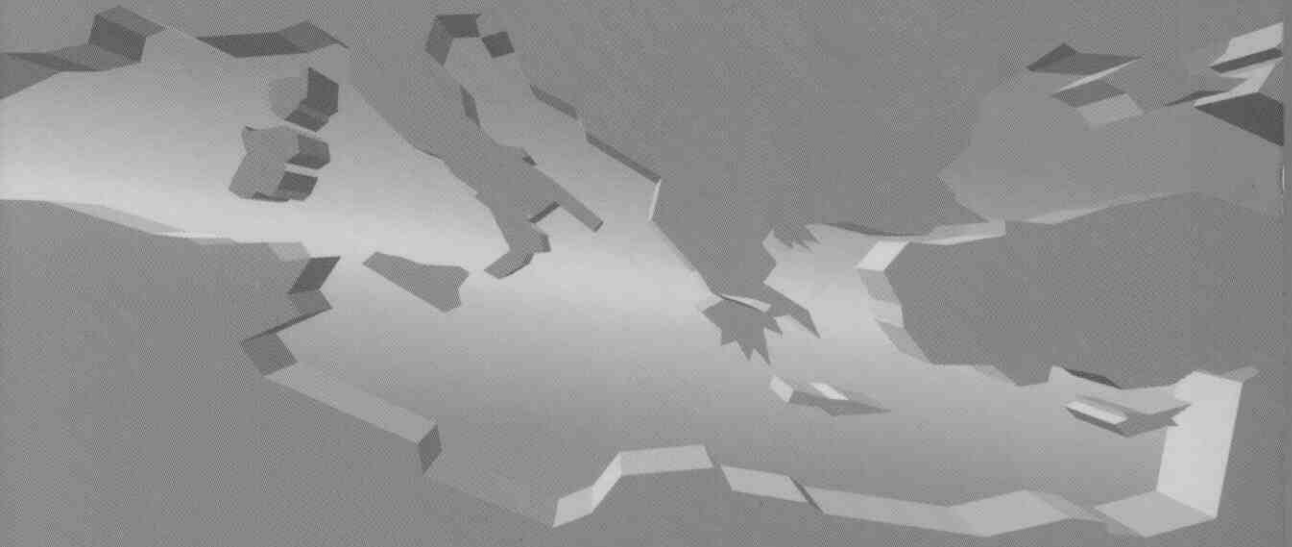

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Effects of high dilutions of histamin and other natural compounds on acute inflammation in rats

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INTRODUCTION

The aim of this work was to evaluate the possible pharmacological activity of various dilutions of homoeopathic compounds in the rat during an acute inflammatory process.

Apis-Homaccord, Arnica Compositum, Histamin are often used in homoeopathy for treating inflammatory conditions as edema, trauma, contusion. These compounds have been also studied in experimental and clinical research and we report below some results on a rat model.

Several pharmacological studies demonstrated the protective action of Apis-Mellifica on the development of erythema induced by U.V. radiation on guinea pigs, either when given intraperitoneally[1] and orally[2]. Intra-articular injections of Arnica compositum were shown to significantly reduce the recovery time in patients with haemarthro[3].

In vitro experiments have shown that the anti IgE-induced degranulation of basophil is significantly inhibited by high dilutions of Lung Histamine[4]. In experiments with isolated guinea pig heart it has been reported that high dilution of histamine are able to mantain vasodilating activity increasing the coronary flow[5].

We choosed as first model the carrageenan foot oedema, which is the classical test utilized to screen non-steroidal antiinflammatory drugs[6]. The oedema which develops in rat paw after carrageenan injection is a biphasic event involving several mediators of inflammation as histamine, serotonine, kinins and prostaglandins.

Thereafter, the research was focused to find a more simple model of inflammation and we used histamine as oedema inducing agent. The injection of 0.1 mg of histamine in the rat paw induces a local, quick reaction with a swelling of paw reaching the maximum after 15-30 minutes from injection. The study of the activity of Histamin in different dilutions in rats with histamine oedema may represent a good model also to verify the principle of similarity in this experimental conditions.

MATERIALS AND METHODS

Materials

The homoeopathic drugs used in this series of experiments were from Heel Gmbh (Baden-Baden, Germany) and were made in 0.9% NaCl according to the german pharmacopoeia[7]. Indomethacin was from Chiesi (Liometacen); promethazin was from Farmitalia (Farganesse); carrageenan and histamine were from Sigma, St. Louis, Mo, Usa. The composition of homoeopathic compounds was as follows: Histamin Injeel forte: Histamin D6, D12, D30, D200; Histamin Injeel: Histamin D12, D30, D200; Histamin D30: only 30th decimal dilution of histamine; Apis Homaccord: Apis mellifica D4, D10, D30, D200, D1000, Apisinum D8, D30, Scilla D4, D10, D30, Tartarus stibiatus D4, D10, D30, D200; Arnica compositium: Arnica D2, Calendula D2, Chamomilla D3, Symphytum D6, Millefolium D3, Belladonna D2, Aconitum D2, Bellis prennis D2, Hypericum D2, Echinacea angustifolia D2, Echinacea Purpurica D2, Hamamelis D1, Mercurius solubilis D6, Hepar sulfuris D6.

Oedema measurement

For each animal, oedema was assayed as foot volume increase with respect to the foot volume measured before irritant. Measurements were done with a plethysmometer (mod. 7150- Ugo Basile, Milano, Italy), by a technician who was unaware of the experimental protocol. The average foot swelling in groups of treated animals is compared with that of a group of saline treated animals and the percentage inhibition of oedema is determined. Statistical analysis was done with Student's t test.

Carrageenan oedema

Female Sprague-Dawley rats from Nossan s.r.l., Correzzana, Milano, weighting 150-180 g, were used. The oedema was induced by injecting 0.1 ml of 1% carrageenan suspended in sterile 0.9% NaCl (saline), into the plantar surface of the right hind-foot of each rat. Foot volume (ml) was measured at 0, 1, 3, 5, 7, and 24 hours after the carrageenan injection, and oedema volume was calculated as described above. The rats (50 each experiment) were divided in five groups of 10 rats and received the following substances:

— sterile saline;
— indomethacin 3 mg /Kg;
— Histamin Injeel forte;
— Apis Homaccord;
— Arnica Compositum.

Indometacin was administered once orally, 1 hour before carrageenan; saline, Histamin, Apis and Arnica were injected in two times intraperitoneally: 0.25 ml 30 minutes before and 0.25 ml 30 minutes after carrageenan.

Histamine oedema

Female Sprague-Dawley rats from Nossan s.r.l., Correzzana, Milano, weighting 210-225 g, were used. The oedema was induced by injecting 0.1 mg of histamine suspended in 0.1 ml of sterile saline into the plantar surface of the right hind-foot of each rat. Foot volume (ml) was measured at 0, 0.5, 1, 2, 3, hours after the histamine injection. Oedema volume was calculated as described above. The rats (50 each experiment) were divided in five groups of 10 rats and received the following substances:

— sterile saline;
— promethazine 10 mg/kg;
— Histamin Injeel forte;
— Histamin Injeel;
— Histamin D30.

All compounds were administered intraperitoneally in two times: 0.25 ml :30 minutes before and at the same time that histamine injection in the paw. In each experiment one group of animal treated with saline and with promethazine was used as control.

RESULTS

Table 1 reports the percentage of modification of carrageenan oedema in animals treated with Histamin Injeel forte, Apis Homaccord and Arnica Compositum, with respect to control animals, in three independent experiments.

Histamin slightly inhibited the development of oedema in two experiments; Apis showed a repetitive inhibition in the first phase of reaction and after 5 hours, while in the other time intervals both stimulatory and inhibitory responses

TABLE 1 - *Effects of Histamin Injeel forte, Apis Homaccord and Arnica Compositum on carrageenan oedema*

		1h	3h	5h	7h	24h
Histamin Injeel forte	Exp. 1	-15.4	-1.6	-10.8	-12.3	-13.6
	Exp. 2	-4.6	+13.2	-18.4	-9.5	-12.5
	Exp. 3	n.m.	+4	n.m.	n.m.	-3.2
Apis Homaccord	Exp. 1	-12.8	-4.9	-13.8	-29	-40.9
	Exp. 2	-18.6	+22	-7.9	+5	+8
	Exp. 3	-31.2	n.m.	-9.8	-15.9	-16.6
Arnica compositum	Exp. 1	-5.1	+14	+8	-14.5	-22.7
	Exp. 2	n.m.	+14	-13.1	n.m.	-29
	Exp. 3	+29	n.m.	-8.4	+11	-13

-=% inhibition; + = % increase; n.m=no modifications of oedema volume versus control; in each experiment 10 animals in treated and control groups were used.

were noted. Arnica inhibited the oedema after 24 hours, while in the other phases contrasting effects were observed. The reference drug indomethacin caused the expected inhibition of oedema in all experiments (data not shown).

We then turned to a model where the first phase of oedema (presumably the histamine phase) is investigated. The percentage of inhibition of histamine oedema in animals treated with three preparations containing different dilutions of histamine versus animals treated with saline, is shown in Fig. 1. Each experiment was analyzed separately and the figure shows the results of all experiments performed (9 with Histamin Injeel forte, 5 with Histamin Injeel and Histamin D30).

The solutions seemed to have pharmacological effects, (inhibition of oedema), but the results showed marked variations in the extent of the observed effects between the experiments. Moreover, in two of these experiments (marked with * in the figure) some differences in the experimental setting occured. In particular, the drugs were not injected into animals directly from the ampoules, but after pouring them into tubes. In order to deal with data more standardized as possible, these two experiments have not been considered in the statistical analysis, reported in Fig. 2.

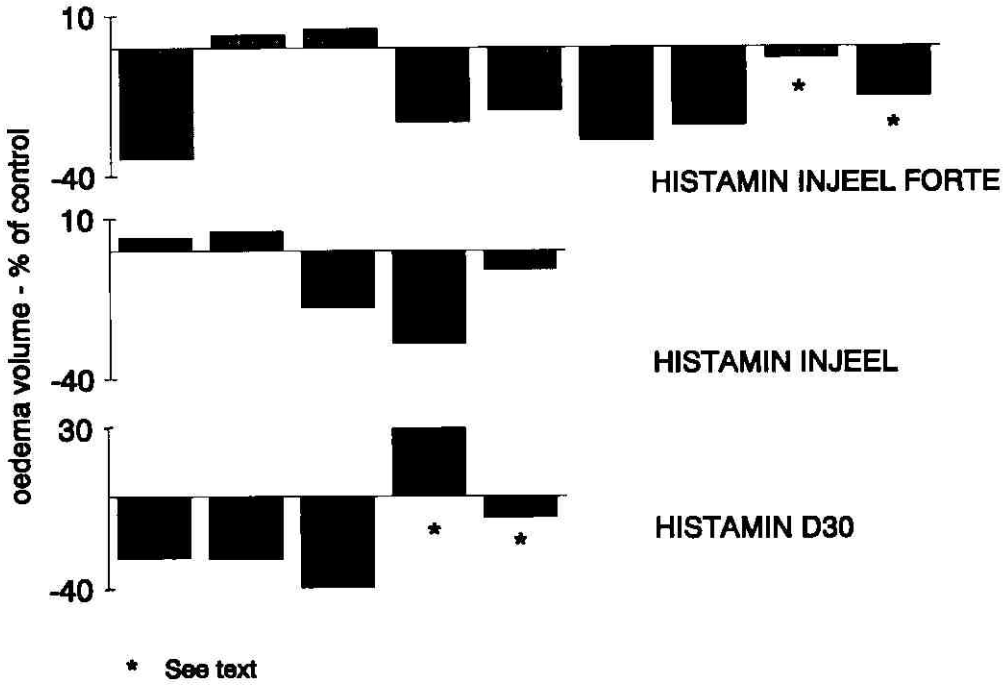

Figure 1 - Histamine oedema: inhibition pattern of each experiment 0.5 hours after irritant, in animals treated with Histamin Injeel forte, Histamin Injeel and Histamin D30.

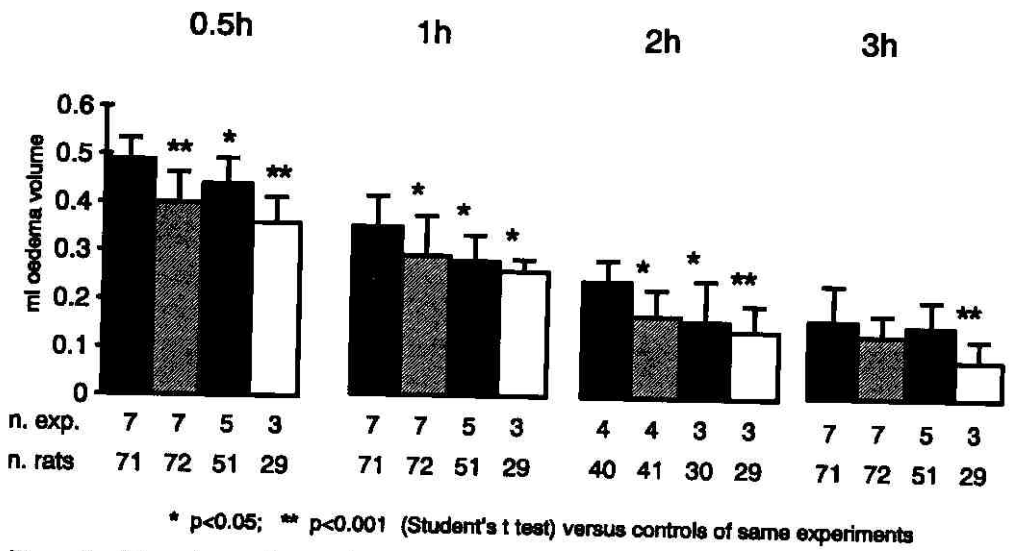

Figure 2 - Histamine oedema volume: mean of experiments and statistical analysis.

In this figure the oedema volume of the control and the treated groups is shown, at different times, during the development of inflammatory reaction. It can be seen that the oedema was maximal at 0.5 hour after injection of histamine and then rapidly decreased.

All treatments caused a decrease in oedema volume with respect to saline, in particular Histamin D30 caused a reduction of oedema statistically significant at all times considered; Histamine Injeel forte and Histamin Injeel caused a reduction statistically signiflcant 0.5, 1 and 2 hours after paw irritation. In all experiments the reference drug promethazine (a histamine antagonist) caused a statistically significant inhibition of oedema at all time considered (data not shown).

CONCLUSIONS AND DISCUSSION

In this work the effects of some homoeopathic preparations, commonly used in men on inflammatory conditions, have been tested on two models of acute inflammation in rats, carrageenan and histamine foot oedema.

Carrageenan is a mucopolysaccaride from Irish sea moss, wich causes an oedema when injected in subplantar zone of rat paw. The local reaction is the result of increased vascular permeability of blood vessel and of the migration of leucocytes in the site of irritation. The time course of the development of oedema can be divided in two phases, on the basis of drug sensitivities. The early swelling could have a serotonin and histamine component, even if histamine an-

tagonist appear inactive in this phase. Histamine has been reported to be present in the inflammatory exudate in the foot induced by carrageenan. Others possible mediators of this phase are kinins, as shown by inhibition with antikinin substances as aprotinin, cellulosa sulfate and saliva. An important role in the development of carrageenan foot edema in the second phase, is played by prostaglandins; high concentrations of PGE_2 were found in oedema fluid squeezed out from rat foot between 18 and 24 hours. A major role of prostaglandins in oedema formation is confirmed by the ability of prostaglandin production inhibitors, to inhibit the carrageenan-induced oedema. This oedema can be inhibited by at least two mechanisms: inhibition of the primary mediators involved in the swelling or inhibition of the prostaglandin amplification mechanism[8].

Despite their large clinical use, the mode of action of homoeopathic substances is completely unknown; as preliminary approach we tested some commercially available solutions with possible antiinflammatory properties in this well established model. In this model, only Apis showed small and transient inhibitory effects. However, taken together, the results of Histamin Injeel forte, Apis Homaccord and Arnica Compositum, tested on carrageenan oedema seem not to be very helpful in clarification of their mechanism of action. Probably other biological tests are to be looked for.

More significant results have been obtained with Histamin in various dilutions and combinations on histamine induced oedema test. In this model, even high dilutions of histamine (Histamin D30) showed consistent regulatory properties, but marked variations of the effects, between experiments, were noted.

The experimental approach that we have reported raises some general questions that may be summarized as follows:

a) the administration of small doses or even high dilutions of histamine, an agent known as inflammatory mediator, is able to modulate a histamine-induced reaction *in vivo*. This conclusion is in agreement with other experimental evidence in different models[1, 2];

b) the observed effects of homoeopathic preparations have been quite low, if the percentage of inhibition is considered. In conventional pharmacology, an antiinflammatory compound is expected to show a minimum of 30% inhibition in order to be considered active. However, the action mechanism of highly diluted natural compounds is supposed to act by a fine regulation of natural reactions instead to have a strong inhibitory activity over specific enzimatic systems;

c) we have demonstrated that small but significant inhibitions of oedema may be obtained both in the first phase of carrageenan oedema and in the development of histamine oedema. This fact suggests that in order to study effects of highly diluted compounds (whose action mechanism is probably regula-

tory and not inhibitory), models of inflammation simple and relatively weak should be used. However, in our experience, histamine model has proven to be quite difficult because the reaction is rapid and short-lasting. As a consequence, the intra-experiment and between experiments variations are remarkable.

For these reasons we cosidered this work as a pilot study in which the basis of the principle of similitude in homoeopathy have been observed in an acute model of inflammation in rats.

The inhibitory effect of highly diluted histamine, in particular Histamin D30 in histamine oedema, is an *in vivo* example of this principle.

ACKNOWLEDGEMENTS

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