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Dual effects of a homeopathic mineral complex on carrageenan-induced oedema in rats

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Carrageenan oedema, a classical experimental model commonly used to test activity of anti-inflammatory drugs, was used to evaluate the therapeutic activity of a low-potency mineral complex (MC). The MC was administered in the right plantar surface of albino rats 60 min before, simultaneously and 30 min after injection of carrageenan, an irritant which causes a local, transitory increase of fluid volume. The administration of the MC 60 min before the injection of carrageenan primed the animal to enhanced inflammatory response to the irritant. The administration of MC contemporarily to carrageenan did not modify the kinetic and the extent of the oedema, while the administration of the MC 30 min after the induction of the oedema significantly reduced the early phase of the inflammatory reaction. This indicated that the therapeutic action of this MC is not due to conventional anti-inflammatory effect but to activation of endogenous regulatory mechanisms, a phenomenon which may be regarded as a simple application of the 'similia rule'.

Keywords: carrageenan oedema; inflammation; paw volume; mineral complex; animal model; reactivity

Introduction

Inflammation is one of the most important problems of pathology and various therapeutic procedures aimed at its treatment have been proposed. Whereas thinking according to 'contraria contrariis' principle has always referred to antiphlogistic treatment using high doses of inhibitors, thinking according to 'similia similibus' principle has always considered inflammation as a therapeutic reaction which should be imitated, regulated by biological means but not blocked.

Oedema is one of the main effects of inflammation: although it is a defensive reaction of biologic organisms, sometimes it represents the main pathology. Carrageenan oedema is a laboratory model commonly used to test activity of anti-inflammatory drugs.[1,2] It is induced in rats by injecting in the plantar of hind paw a 1% solution of carrageenan (a mucopolysaccharide from an Irish sea moss) in sterile saline. This compound causes a mild, self-limiting, increase of paw volume which peaks three hours after injection of the irritant.

This experimental model was used to test the activity of a low potency homeopathic complex, named 'Ultima Ratio'. This is a complex solution containing a number of mineral salts, prepared according to the Homeopathic Pharmacopoeia, which was developed empirically in clinical studies[3–6] and showed no toxicity in cellular models,[7] but which so far has never been tested in animals. This mineral complex (MC) is used as a local infiltration to regulate inflammatory processes and reduce pain, with the rationale of activating the lymphatic system and to promote the reabsorption of the oedematous liquid, but its precise mechanism of action is unknown; it is conceivable that it has a non-specific action on the modulation of inflammation, allowing the restoration of the physiologic homeostasis.

In order to find the best treatment schedule, the MC was injected intra-paw either 60 min before, or at the same time, or 30 min after carrageenan oedema induction and its effect compared with a non-homeopathic saline solution. In this report we show that the results of the three treatment schedules not only differed quantitatively, but showed opposite effects.

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Materials and methods

Animals

A total of 307 male Sprague-Dawley rats (Harlan, Italy) weighing 150–175 g were used. Animals were kept under standardised conditions (12 h dark/light cycle) on a standard diet and water *ad libitum*. Authorisation for animal experiments was obtained by Italian Ministry of Health.

Carrageenan-induced paw oedema

Carrageenan oedema was induced by injecting in the plantar surface of the right hind paw 0.1 ml of a suspension of carrageenan 1% in sterile saline. The time of carrageenan injection was designated as time zero.

The paw volume was measured using a water plethysmometer (Ugo Basile, MI, Italy), and oedema was assessed by the difference of paw volume at various times after carrageenan injection minus the paw volume of the same animal, measured before the beginning of the experiment.[8] The mean paw swelling in every treatment group was compared to its relative control group and the percentage of inhibition was calculated.

$$\%\ \text{inhibition} = \frac{\text{Saline treated} - \text{MC treated}}{\text{Saline treated}} \times 100$$

A technician who was unaware of the treatment schedule of the animals did all plethysmometric measurements.

Statistical significance was evaluated using Student's *t*-test.

Drugs

Ultima Ratio (Alexander Arzneimittel, Hamburg) is a mineral complex constituted of different salts, at various potencies, prepared according to the German Pharmacopoeia.[9] 1000 ml of the MC contain: 3.7 ml Potassium bromide D3, 7.0 ml Potassium sulphate D3, 12.7 ml Calcium chloride D2, 5.0 ml Strontium chloride D5, 5.0 ml Molybdic acid D5, 6.0 ml Barium chloride D5, 6.0 ml Manganese sulphate D5, 3.5 ml Iron sulphate D4, 1.0 ml Cobalt sulphate D6, 5.0 ml Copper sulphate D6, 12.0 ml Magnesium sulphate D2, 5.0 ml Chromium chloride D6, 3.0 ml Ytterbium chloride D6, 3.0 ml Lanthanum chloride D7, 2.0 ml Cerium chloride D6, 3.4 ml Zirconium nitrate D4, 1.4 ml Tungsten chloride D4, 1.0 ml Nickel chloride D6, 2.0 ml Ruthenium chloride D6, 6.0 ml Iridium chloride D7, 6.0 ml Tin chloride D6, 5.0 ml Rubidium chloride D6, 2.0 ml Cesium chloride D7, 7.0 ml Selenic acid D7, 7.0 ml Antimony chloride D7, 2.0 ml Silver chloride D7, 2.0 ml Gold chloride D7, 1.0 ml Zinc chloride D6, 3.0 ml Europium perchlorate D7, 4.0 ml Lead chloride D6, 3.0 ml Cadmium chloride D7, 2.0 ml Mercury chloride D7, 4.0 ml Arsenic chloride D7, 3.1 ml Aluminium chloride D5, 7.8 ml Silicic acid D3.

In this series of experiments we used the stock no. 9516254, exp. Date 31.12.2004. In order to rule out a possible effect by the injection procedure itself, (that is independent of composition of the drug), rats of control group were treated by an identical volume of isotonic, sterile NaCl solution (control saline). The osmotic pressure of the MC solution and of the control saline solutions were almost identical (290 mOsm and 285 mOsm respectively), indicating that the different effects could not be due to osmotic effects.

To test the effectiveness of a classical anti-inflammatory drug on our model, 0.1 ml of 3 μM indomethacin[10] were injected subcutaneously 1 h before, at the same time and half an hour after carrageenan oedema.

Treatments

All the treatment schedules were repeated in seven different experiments.

The mineral complex was injected in the plantar surface of the right hind paw 1 h before inducing oedema, at the same time and 0.5 h after oedema induction.

The results in each treatment group were compared with a group of animals receiving intra-paw saline at the same time (every treatment schedule was so compared with its control).

- Group 1 received 0.1 ml of control saline 60 min before carrageenan.
- Group 2 received 0.1 ml of MC 60 min before carrageenan.
- Group 3 received 0.1 ml of control saline simultaneously with carrageenan.
- Group 4 received 0.1 ml of MC simultaneously with carrageenan.
- Group 5 received 0.1 ml of control saline 30 min after carrageenan.
- Group 6 received 0.1 ml of MC 30 min after carrageenan.

The paw volumes were measured before any injection and 1, 3 and 5 h after carrageenan injection.

Results

First of all the direct effect of the tested drugs on healthy animals (in the absence of carrageenan treatment) was assessed, by measuring hind paw volume of rats 60 min after injection of either MC or control saline. The volume increase was 0.136 ± 0.07 ml for saline receiving rats, 0.105 ± 0.014 ml for MC receiving rats ($P < 0.05$, Student's *t*-test). Since the injected volume was 0.1 ml, this result indicates that saline caused a very small extra-increase of volume ($0.136 - 0.1 = 0.036$ ml), which was presumably due to trauma of injection. On the other hand, the increase of volume due to MC was negligible ($0.105 - 0.1 = 0.005$ ml),

Table 1 Oedema volume and percentage of inhibition of MC *vs* saline during the seven separate experiments and the pooled data

	− 60 min treatment			*0 min treatment*			*+ 30 min treatment*		
	1st group	*2nd group*	*%*	*3rd group*	*4th group*	*%*	*5th group*	*6th group*	*%*
First experiment ($n=42$ rats)									
1st hour	0.38 ± 0.12	0.54 ± 0.14	+ 42.1*	0.49 ± 0.11	0.57 ± 0.14	+ 16.3	0.50 ± 0.07	0.55 ± 0.10	+ 10.0
3rd hour	0.76 ± 0.24	0.96 ± 0.25	+ 26.3	1.02 ± 0.20	0.97 ± 0.22	− 5.0	0.83 ± 0.20	0.86 ± 0.17	+ 3.6
5th hour	0.69 ± 0.24	0.96 ± 0.20	+ 37.7	0.93 ± 0.24	0.87 ± 0.13	− 6.5	0.92 ± 0.25	0.77 ± 0.20	− 16.0
Second experiment ($n=48$ rats)									
1st hour	0.33 ± 0.10	0.51 ± 0.10	+ 54.5**	0.61 ± 0.14	0.52 ± 0.13	− 14.7	0.76 ± 0.17	0.58 ± 0.10	− 24.2*
3rd hour	0.87 ± 0.20	0.97 ± 0.14	+ 11.5	1.01 ± 0.14	0.95 ± 0.17	− 6.0	0.96 ± 0.13	0.88 ± 0.12	− 8.3
5th hour	0.80 ± 0.13	0.93 ± 0.15	+ 16.2	0.94 ± 0.12	0.88 ± 0.18	− 6.0	0.91 ± 0.15	0.80 ± 0.09	− 12
Third experiment ($n=45$ rats)									
1st hour	0.56 ± 0.08	0.53 ± 0.18	− 5.3	0.57 ± 0.10	0.61 ± 0.10	+ 7.0	0.68 ± 0.12	0.64 ± 0.11	− 5.6
3rd hour	1.15 ± 0.14	1.05 ± 0.08	− 8.7	1.11 ± 0.14	1.08 ± 0.12	− 2.7	1.14 ± 0.10	0.98 ± 0.16	− 14.5*
5th hour	1.07 ± 0.12	1.03 ± 0.13	− 3.7	1.03 ± 0.15	0.99 ± 0.13	− 3.8	0.99 ± 0.16	0.82 ± 0.20	− 17.0
Fourth experiment ($n=42$ rats)									
1st hour	0.42 ± 0.08	0.54 ± 0.10	+ 21.4*	0.65 ± 0.06	0.66 ± 0.08	+ 2.3	0.62 ± 0.08	0.66 ± 0.05	+ 5.7
3rd hour	0.79 ± 0.09	0.80 ± 0.17	+ 1.2	0.86 ± 0.12	0.85 ± 0.16	− 1.2	0.68 ± 0.1	0.87 ± 0.18	+ 27.9*
5th hour	0.73 ± 0.13	0.78 ± 0.21	+ 6.8	0.82 ± 0.13	0.86 ± 0.14	+ 4.8	0.77 ± 0.08	0.87 ± 0.13	+ 12.9
Fifth experiment ($n=42$ rats)									
1st hour	0.29 ± 0.2	0.25 ± 0.09	− 13.8	0.36 ± 0.13	0.50 ± 0.14	+ 40.4	0.62 ± 0.13	0.56 ± 0.08	− 9.6
3rd hour	0.74 ± 0.24	0.60 ± 0.19	− 18.9	0.84 ± 0.18	0.87 ± 0.12	+ 3.6	1.20 ± 0.1	0.97 ± 0.14	− 4.9
5th hour	0.88 ± 0.19	0.65 ± 0.23	− 26.1	0.67 ± 0.14	0.73 ± 0.1	+ 9.0	0.83 ± 0.16	0.96 ± 0.13	− 13.5
Sixth experiment ($n=44$ rats)									
1st hour	0.65 ± 0.21	0.80 ± 0.14	+ 23.1	0.62 ± 0.14	0.63 ± 0.13	+ 2.1	0.70 ± 0.11	0.49 ± 0.17	− 30.6*
3rd hour	1.21 ± 0.14	1.14 ± 0.09	− 5.7	1.14 ± 0.14	1.16 ± 0.14	+ 2.0	1.10 ± 0.05	0.98 ± 0.12	− 10.5*
5th hour	1.20 ± 0.09	1.21 ± 0.13	+ 0.8	1.03 ± 0.18	1.04 ± 0.12	+ 0.7	0.90 ± 0.14	0.94 ± 0.17	+ 4.8
Seventh experiment ($n=44$ rats)									
1st hour	0.71 ± 0.17	0.64 ± 0.11	− 9.8	0.65 ± 0.20	0.58 ± 0.07	− 11.5	0.69 ± 0.15	0.53 ± 0.15	− 23.5
3rd hour	1.37 ± 0.12	1.31 ± 0.13	− 4.3	1.15 ± 0.10	1.16 ± 0.14	+ 0.5	1.01 ± 0.12	0.92 ± 0.15	− 8.4
5th hour	1.26 ± 0.16	1.11 ± 0.14	− 11.9	1.06 ± 0.14	1.14 ± 0.12	+ 7.3	1.05 ± 0.08	1.04 ± 0.11	− 1.0
Pooled data ($n=307$ rats)									
1st hour	0.48 ± 0.21	0.55 ± 0.19	+ 14.6*	0.56 ± 0.16	0.58 ± 0.12	+ 3.57	0.66 ± 0.14	0.57 ± 0.13	− 13.63**
3rd hour	0.99 ± 0.29	0.99 ± 0.26	+ 0.0	1.02 ± 0.18	1.01 ± 0.19	− 0.98	0.99 ± 0.22	0.91 ± 0.16	− 8.1*
5th hour	0.95 ± 0.26	0.96 ± 0.24	+ 1.1	0.93 ± 0.20	0.93 ± 0.18	+ 0.0	0.91 ± 0.16	0.90 ± 0.21	− 1.1

*$P < 0.05$; **$P < 0.01$ student's *t*-test.

indicating that this drug does not cause any inflammatory reaction but, instead, reduces the trauma-induced volume increase.

Table 1 reports the volume of oedema after carrageenan injection in the six different groups of rats during each of the seven experiments. The administration of the MC 60 min before the induction of the oedema (groups 1 and 2) did not have any anti-inflammatory effect but, on the contrary, it seemed to act by priming the tissue to an increased oedema induced by the carageenan. In fact, the increase of volume of the paw in the group of animals treated with MC was higher in comparison to the control group in four of seven experiments. The relative increase was 14% at the first hour ($P < 0.05$).

The administration of MC contemporaneously with the induction of oedema (groups 3 and 4) did not have any notable effect on the inflammation.

The scheme of treatment which showed the best therapeutic effects was the administration of MC 30 min after induction of oedema (groups 5 and 6). The drug reduced oedema in five experiments out of seven, with varying effectiveness. The pooled data show a modest but significant decrease of carrageenan-induced oedema ($P < 0.01$ after 1 h, $P < 0.05$ after 3 h) in rats treated with MC compared to animals treated with control saline (Figure 1).

Discussion

Inflammation is the response of living tissues to damage, which may be of a physical, chemical or biological nature. It is a complex response, consisting of a number of biological events, which is at the same time physiological and pathological and is in some way implicated in all diseases. The tissue where the most of the inflammatory process takes place is the connective tissue. When a noxious event occurs many biological phenomena come into operation, the most important of which are: the relaxation, after initial contraction, of the smooth muscle cells of arteries, causing hyperaemia ('calor' and 'rubor'). Then the mast cells present in the tissue release inflammatory mediators (histamine, serotonin etc.) causing formation of exudate (oedema); then there is the action of the white blood cells, primarily granulocytes. In the last phase we also find lymphocytes, monocytes, and macrophages (chronic inflammation).

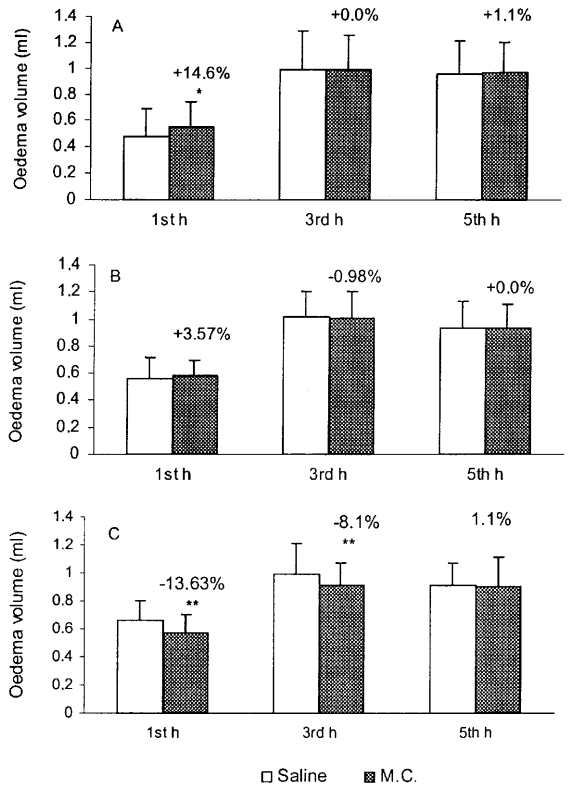

Figure 1 Graphic representation of pooled data oedema volume ($n = 307$ rats) in animals treated with MC or saline as follows: A. 60 min before carrageenan, B. simultaneously with carrageenan, C. 30 min after carrageenan. $*P < 0.05$; $**P < 0.01$ Student's *t*-test.

The inflammation induced by carrageenan simulates the events of a typical acute inflammatory response and recognized biochemical mechanisms including increase of serotonin (1st phase) and prostaglandins (2nd phase).

With the aim of evaluating the therapeutic activity of a low-potency homeopathic mineral complex under standard conditions, we used this experimental system, which was already in use in our laboratory. Our hypothesis was that MC caused a decrease of carrageenan-dependent oedema development. We experimented with three different treatment schedules. We were surprised by the results which often showed an increase of oedema, instead of a decrease, when the drug was administered before carrageenan. Classical non-steroidal anti-inflammatory drugs, administered intra-paw either before or after the injection of carrageenan, are known to exert a profound inhibition in this model.[1,2,11,12]

With MC, a slight, but significant, therapeutic effect confirming the starting hypothesis was obtained only when the drug was administered after carrageenan injection. These results clearly indicate that the effect of MC tested in this experiment is quite different from the inhibitory effect of a typical anti-inflammatory agent. This series of experiments showed a marked variability of the measured parameter. This variability may be due to a series of chronobiological factors that influence a dynamic process such as inflammation and which may particularly affect the results when very low doses of test compounds are used. Taking into account these limitations, the overall results show that the effect of the tested compound is qualitatively different according to the state of the animal: the inflammatory reaction is worsened by pretreatment with MC, i.e. when MC is given to healthy animals which are subsequently treated with carrageenan, while animals already affected by carrageenan-induced inflammation are improved.

These results are in keeping with the homeopathic theory and with the possible ways by which the 'simile principle' can be seen to operate in biological systems. The occurrence of dual or inverse effects of agonists, antagonists or toxins have been described in various experimental systems and may have a number of explanations at the level of receptors, signal transduction mechanisms, enzyme regulation, and gene expression, according to the test compound and system involved.[13–15]

The mineral complex injected alone did not cause any inflammation. This fact may appear in contrast with the similia law, where the drug cures in the diseased person the same symptoms that it causes in the healthy person. However, this apparent contradiction disappears when the homeopathic effect is regarded as a 'change of sensitivity'. The very low doses of minerals contained in the preparation used in this study do not have a *direct pathological effect*, rather they 'prime' the healthy animal to an increased pathological response to a subsequent challenge. This may be in keeping with the effect of homeopathic drugs, whose action on a healthy living system is not to cause direct harm, but a change of sensitivity (for example, to light, to cold, to specific foods). In fact, any 'disease' (or, in homeopathic terms, any pattern of symptoms) is the resultant of both internal factors (such as genetic predisposition or complex changes of sensitivity due to neuro-endocrine regulation) and external, or triggering factors (for example, diet, trauma, microbial agents, heat). According to this view, it is quite conceivable that the level of action of the drugs working according to the similia principle, either in healthy or in diseased living system, is represented by subtle changes in the sensitivity of regulatory systems, not by direct pathological modifications in cells or tissues.

Our data do not provide definite proof regarding the mechanism(s) of the dual action of MC. A possible hypothesis could be that MC increases the diffusion of liquids through connective tissue and lymphatic microcirculation; if this is the case, one could speculate that when the drug is present before the injection of carrageenan, it facilitates the diffusion of this irritating agent, thus accelerating the development of oedema. On the other hand, when the drug is injected after the beginning of the reaction to carrageenan, it could facilitate the scavenging of acute-phase inflammatory mediators, thus reducing the oedema

development. Since this is a low-potency mixture of several minerals, it is possible that some of these components have a direct effect on the mechanisms of inflammation. For example, it cannot be excluded that magnesium salts may act through a catalytic effect on (in healthy animals) or by interference with (during inflammation) the complement cascade; on the other hand, it is also possible that manganese may change the sensitivity of calcium channels, which play a key role in the cell excitation. Copper or zinc may regulate free radical reactions, which undoubtedly play a major role in inflammatory processes. Whatever the mechanism may be, we suggest that the similia principle 'in action', as shown by the phenomenon described in this work, is a manifestation of cybernetical regulation of living systems. This regulation may occur either at a biochemical-physiological level, at a neuro-immuno-endocrine level and at a biophysical-bioelectromagnetic level. Obviously, our model brings into focus only the most elementary, biochemical and local, level of regulation.

Another problem of the interpretation of these results is represented by the complex mixture of several minerals, utilised at different potencies, which composes the drug we have utilised in this study. Clearly this is a major obstacle for the identification of the active chemical principle(s), because it is practically impossible to carry on studies of individual components and using a sufficient number of animals. On the other hand, this is not a problem restricted to homeopathic complexes, involving also plants, animal extracts and venoms, which are composed by a number of active principles, often, but not always acting in a synergistic way. Therefore, our data should be regarded neither as a proof of the clinical efficacy of this MC, nor as a clarification of the action mechanism of homeopathic drugs. The present study showed an important feature of a classical inflammatory reaction, which can be modulated in opposite way by a low-potency homeopathic mineral solution, according to the timing of the administration. The change of the 'reactivity' of the host to the same drug in healthy or diseased state is one of the cornerstones of the homeopathic theory and here we have provided an experimental example.

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