

Experimental Studies on the Anti-Inflammatory Activity of a Homeopathic Preparation

A. Conforti, S. Bertani, H. Metelmann¹, S. Chirumbolo², S. Lussignoli³, and P. Bellavite²

Institute of Pharmacology, University of Verona, Italy

¹Biologische Heilmittel Heel GmbH, Baden-Baden, Germany

²Institute of Clinical Chemistry and Microscopy, University of Verona, Italy

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Summary

The anti-inflammatory effects of *Arnica montana*, either alone or in combination with other homeopathic substances, have been observed in experimental and clinical studies. However, its mechanism of action has not been elucidated. This paper presents the findings of a study of the anti-inflammatory effects of *Traumeel*®S (a preparation containing *Arnica montana* and other plant extracts as well as minerals that could potentially work synergistically) using various *in vitro* and *in vivo* models.

The effect of *Traumeel*®S on two important cellular functions, namely superoxide anion production and human platelet adhesion, was tested. *Traumeel*®S did not affect either of these cellular functions, suggesting that its anti-inflammatory effects are not due to granulocyte and platelet inhibition. These findings also suggest that the antimicrobial functions of immune cells such as granulocytes are not disrupted by *Traumeel*®S.

In vivo experiments using models of chronic and acute inflammation show that in the case of submaximal activation of the inflammatory response, *Traumeel*®S significantly reduces the development of local edema (such as that seen in the first phase of adjuvant arthritis) and causes a 15% reduction of the carrageenan-induced edema when administered locally. These results suggest that the anti-inflammatory effects of *Traumeel*®S are not due

to its action on a specific type of immunomodulating cell or due to a biochemical mechanism associated with conventional anti-inflammatory drugs. Instead, *Traumeel*®S appears to inhibit the acute inflammatory process at the local level.

1. Introduction

The blossoms of *Arnica montana* are used therapeutically primarily for their anti-inflammatory and analgesic properties. There are more than 150 chemical substances in *Arnica* blossoms and, of these, helenalin and its derivatives and flavonoids are the most important. The anti-inflammatory and antimicrobial activities of these substances have been demonstrated both *in vitro* and *in vivo* [1,2]. A medicinal preparation containing active substances from *Arnica montana* together with other plant extracts and minerals that could potentially act synergistically has been developed by Heel and is called *Traumeel*®S. The primary indications of *Traumeel*®S include various types of lesions and inflammatory processes of the muscles and joints such as sprains and bruises. Its efficacy has been demonstrated in a number of experimental and clinical studies [3,4], including randomized, double-blind, placebo-controlled trials. However, the mechanism(s) by which *Traumeel*®S exerts its anti-inflammatory effects have heretofore not been clarified.

In this paper we report a series of studies on animal models to improve the understanding of *Traumeel*®S and its mechanism of anti-inflammatory action in comparison with classical anti-inflammatory drugs.

Considering the different uses of *Traumeel*®S in humans, two animal

models of inflammation have been employed. For assessing its effect on chronic inflammation *Traumeel*®S was administered to rats with adjuvant arthritis (AA), which shares with human rheumatoid arthritis many immunological, biochemical, and clinical characteristics. Adjuvant arthritis may be induced in rats by injecting into the hind paw or tail a variety of killed mycobacteria suspended in mineral oil. The sequence of physiological effects is characterized by early local inflammation and, after two weeks, disseminated arthritic lesions. Because of its reproducibility, AA is widely used for studying anti-inflammatory and immunomodulatory agents.

For assessing its effect on acute inflammation, *Traumeel*®S was tested in carrageenan-induced edema in rats; a test that is currently employed for studies of conventional anti-inflammatory drugs. In the carrageenan-induced edema test, the early swelling may have a serotonin component since it can be inhibited using serotonin antagonists. Histamine does not appear to play a significant role in this type of acute inflammation, while prostaglandins (PGs) play an important role in the development of the second phase of carrageenan-induced foot edema.

A series of additional experiments was performed on the basic functions of neutrophils and platelets to investigate possible effects of *Traumeel*®S on cellular functions and possible cytotoxicity.

2. Materials and methods

2.1. *In vivo* experiments

Adjuvant arthritis was induced in Lewis rats weighing 270-290 g by inject-

ing into the plantar surface of the hind paw 0.1 ml of a suspension containing 0.3 mg of heat-killed *Mycobacterium butyricum* (Mb) in mineral oil. Previous experiments using a suspension of 0.6 mg of Mb showed slight inhibitory effect by *Traumeel*®S, so we decided to use lower arthritogenic concentration of Mb in order to find the experimental conditions to better emphasize this modulatory effect.

Traumeel®S was given i.m. (0.15 ml/rat) every two days from day 2 to day 28 after arthritis induction according to therapeutic protocol. Control rats received an equal volume of 0.9% NaCl.

Every two days the animals were weighed and their contralateral paw swelling was measured by a water plethysmometer (mod. 7150, Ugo Basile, Milan, Italy). The paw edema was assessed by the increase in paw volume compared to paw volume measured before arthritis induction.

Fourteen, 21, 28, and 35 days after the arthritogenic injection, arthritis development was evaluated by the same observer using an arbitrary scale as follows: left and right hind paws, each 0-7; left and right forepaws, each 0-4.5; tail 0-5; ears 0-2; nose and eyes, each 0-1. The mean paw swelling and arthritis index in the groups of treated animals were compared with that obtained in the control group and the percentage of inhibition was then calculated [5].

Carrageenan-induced edema was induced by injecting 0.1 ml of 1% carrageenan (suspended in sterile saline) into the plantar surface of the hind paw of 40 Sprague-Dawley rats weighing 210-230 g. The first group (n=24 rats) received 0.2 ml of *Traumeel*®S in their hind paw one hour before the carrageenan injection and the second group (n=8 rats) was treated i.m. with 2 injections of 0.2 ml of *Traumeel*®S half an hour before and after edema induction. Each of the control rats (n=21) received an identical volume of 0.9% NaCl in their hind paw.

The paw volumes were measured with a water plethysmometer before and 1, 3, and 5 hours after the carrageenan injection,

and edema was assessed by measuring the increase in paw volume compared with the paw volume before the injection of carrageenan [5]. The mean paw swelling in the treated group was compared with that of the control group. The response was expressed as percentage inhibition (reduction) of inflammation.

2.2. *In vitro* experiments

The neutrophil is one of the most important cells involved in the inflammatory response, particularly when tissue damage or bacterial products are present. Therefore, it was of interest to test the possible effects of *Traumeel*®S on the functions of the neutrophil cells *in vitro*.

Two important functions of granulocytes were measured in the presence and in the absence of *Traumeel*®S, namely superoxide anion production (which reflects the ability of the cells to mount an exudative metabolic response necessary to kill microorganisms) and adhesion to serum-coated plastic surfaces (which reflects the ability of cells to express and activate membrane adhesion molecules, a prerequisite for chemotaxis and phagocytosis). It should be pointed out that many conventional anti-inflammatory and analgesic compounds are known to inhibit one or both of these functions.

Human blood neutrophils from healthy volunteers were isolated as described previously [6]; superoxide anion was measured by the SOD-inhibitable reduction of ferricytochrome c, and adherent cells were quantified by measuring the membrane enzyme acid phosphatase [6]. The percentage of adherent cells was calculated by means of a standard curve obtained with known numbers of neutrophils.

Inflammatory and homeostatic events are strictly interlinked and it is well known that platelets are involved in inflammatory reactions. One important mechanism by which blood platelets perform their functions is adhesion to the injured vessel wall, which may be regarded as the crucial first step of the homeostatic process. Acquired or genetic

defects for platelet adhesion may seriously compromise the homeostatic process, while an unnecessary increase in platelet adhesiveness may result in an increased risk of vascular disorders. Since *Traumeel*®S has been used to treat traumatic conditions where the hemorrhagic events are highly probable and since helenalin lactones from *Arnica montana* have been found to inhibit platelet aggregation [7], it was of interest to explore platelet function in the presence of *Traumeel*®S.

The adhesion of human platelets to fibrinogen-coated surfaces was measured using a calorimetric method based on the membrane enzyme acid phosphatase [8]. The p-nitrophenol produced by the reaction was measured with a microplate reader at 405 nm against a platelet-free blank. The percentage of adherent cells was calculated on the basis of a standard curve obtained with a known number of platelets.

3. Results

The therapeutic administration of *Traumeel*®S led to a significant reduction in acute local inflammation (first phase of adjuvant arthritis) in comparison to the controls (Figure 1). Since this effect occurred during the first 2-7 days in the hind paw where the Mb was injected and not during the generalized arthritic reaction of immunological origin, it suggests a symptomatic action on local inflammation rather than a capacity to modulate the whole arthritic process. This prompted us to use the conventional models for acute inflammation.

The effects of *Traumeel*®S on carrageenan edema are reported in Figure 2. It can be noted that systemic administration of *Traumeel*®S (i.m.) did not reduce edema development. When the drug was injected into the hind paw one hour before carrageenan, it appeared to reduce edema volume by up to 15% (p=0.05). This inhibition by *Traumeel*®S is similar to the effect exerted by aspirin at a dose of 30 mg/kg in the same experimental model. The local injection of *Traumeel*®S at the same time and half an hour after edema induction did not influence the

EXPERIMENTAL MODELS	ACTION OF TRAUMEEL®S
Neutrophil adhesion and superoxide anion production	↔
Platelet adhesion	↔
Carrageenan-induced edema	↓ by injection in loco 1h before
Adjuvant arthritis	First acute phase
	Second chronic phase

↔ no significant effect; ↓ inhibition

Superoxide anion production reflects the ability of the cells to mount an oxidative metabolic response, necessary to kill microorganisms.
 Neutrophil adhesion reflects the ability of the cells to express and activate membrane adhesion molecules, a prerequisite for chemotaxis and phagocytosis.
 Platelet adhesion is the crucial first step of the hemostatic process which is strictly interlinked to inflammatory reactions.
 Carrageenan-induced edema is a model of acute inflammation.
 Adjuvant arthritis is a model of chronic inflammation.

Tab. 1: Traumeel®S activity on experimental models *in vitro* and *in vivo*.

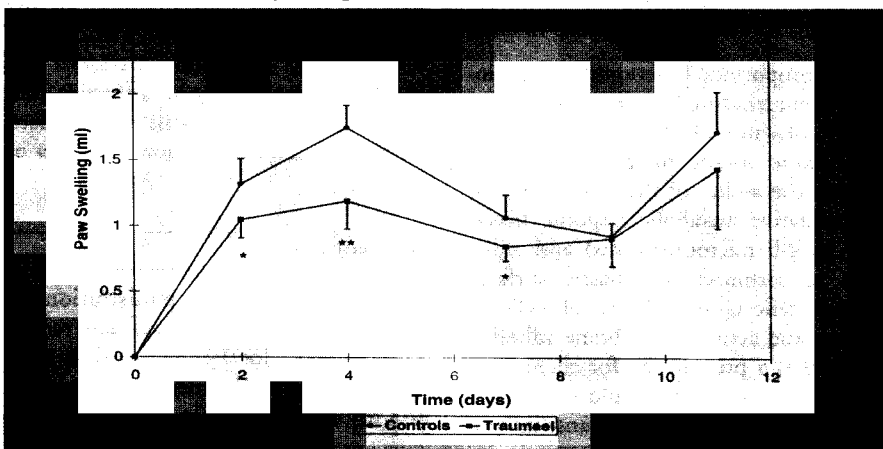


Fig. 1: Acute phase of adjuvant arthritis (AA): injected paw swelling of control rats and rats receiving 0.15 ml of Traumeel®S i.p. every two days from day 2 to day 28 after AA induced by 0.3 mg/rat of *Mycobacterium butyricum* (Mb) in paraffin oil. Data represent mean ± S.D. of 10 animals. **P<0.001, *P<0/01 (Student-t test).

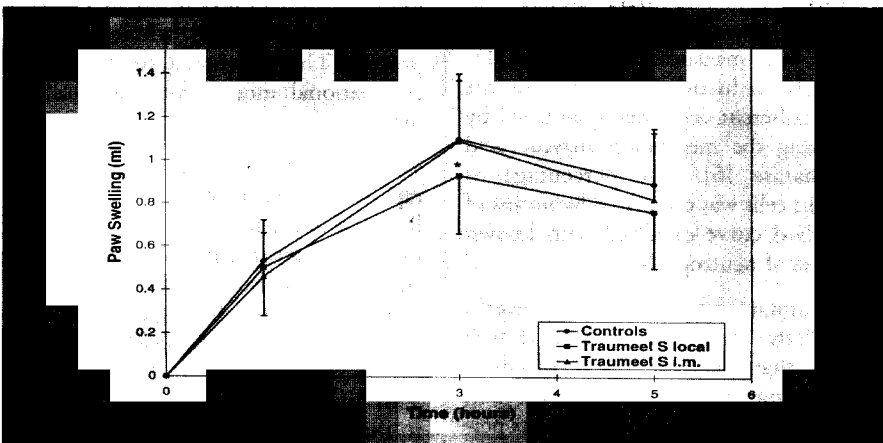


Fig. 2: Carrageenan-induced edema: Paw swelling of control rats (n=24), rats receiving in hind paw 0.2 ml of Traumeel®S 1 hour before carrageenan (n=24) and rats receiving two injections i.m. of 0.2 ml of Traumeel®S half an hour before and after edema induction (n=8). The results are mean ± S.D. of three separate experiments. *P<0.05 (Student-t test).

development of inflammation (data not shown).

The *in vitro* experiments showed that Traumeel®S neither stimulated nor inhibited the functions of the neutrophils such as superoxide anion production and adhesion.

Furthermore, Traumeel®S did not affect either positively or negatively the platelet adhesion stimulated by two natural agonists, ADP and thrombin.

A summary of Traumeel®S activity on experimental models *in vitro* and *in vivo* is shown in Table 1.

4. Discussion

The most significant finding from these investigations is that Traumeel®S does not appear to act as a conventional anti-inflammatory drug since none of the cellular functions tested *in vitro* showed a significant inhibition caused by the drug. Traumeel®S did reduce acute inflammation in the test animals. Traumeel®S, at the highest concentration attainable in connective tissues by local injection, is not toxic to leukocytes and platelets. Thus, the normal defensive and homeostatic functions of these cells are preserved.

An important positive conclusion that can be drawn from all of the granulocyte experiments is that Traumeel®S seems safe for these types of cells and therefore does not inhibit the antimicrobial first defenses which the granulocytes represent. This point appears particularly significant in view of the possible use of the drug in immunocompromised patients or where the local tissue anatomy could lead to risk of infection (i.e., traumas with lacerations, burns, otolaryngeal diseases, etc.)

Moreover, the fact that the cell metabolism is not stimulated (primed) as with other drugs suggests that Traumeel®S does not have the potentially deleterious effects of immunostimulation agents like cytokines, endotoxins (lipopolysaccharides), and other bacterial components. The use of such agents is contraindicated in patients with inflammation of endogenous origin (in particular autoim-

munity). However, based on our data this does not appear to be a problem with *Traumeel®S*.

The *in vivo* studies confirm that *Traumeel®S* does not act in the same way as conventional anti-inflammatory drugs and suggest the need to find new and more appropriate models to investigate its mechanism of action. Since the adjuvant arthritis as a model for autoimmune disease is not significantly inhibited by *Traumeel®S*, the drug does not appear to have immunosuppressive or immunomodulatory effects, a conclusion which is consistent with its lack of effects on leukocyte functions *in vitro*. However, *Traumeel®S* is able to significantly inhibit the first phase of arthritis when the disease is induced by a weaker agent (Mb at lower concentration). In carrageenan-induced edema tests *Traumeel®S* reduces edema only when the injection is administered locally.

Traumeel®S appears to exert its therapeutic effects by interacting not with a specific cell type or biochemical mechanism among those studied thus far, but

by fine and complex regulation of acute local inflammation where it is known that neuropeptides released by sensitive nerve endings play a critical role [9]. Preliminary observation led us to hypothesize that *Traumeel®S* may affect neurogenic mechanisms of inflammation and further research is needed to investigate this possible mechanism of action.

References

- [1] Wijnsma R, Woerdenbag HJ, Busse W. *Zeitschrift für Phytotherapie*. 1995; 1:48-62.
- [2] Kubo I, Muroi H, Kubo A, Chaudhuri SK, Sanchez Y, Ogura T. Antimicrobial agents from *Heterotheca inuloides*. *Planta Medica*. 1994; 6:218-21.
- [3] Zell J, Connert WD, Mau J, Feuerstake G. *Fortschr. Med.* 1988; 106:96.
- [4] Thiel W, Borho B. *Biol. Med.* 1991; 20:506.

[5] Cuzzolin L, Conforti A, Adami A, Lussignoli S, Menestrina F, Del Soldato P, Benoni G. *Pharmacol. Res.* 1995; 31 (1):61-5.

[6] Bellavite P, Chirumbolo S, Mansoldo C, Gandini G, Dri P. *J. Leukocyte Biol.* 1992; 51:329-35.

[7] Schroder H, Losche W, Strobach H, Leven W, Willuhn G, Till U, Schror K. *Thromb. Res.* 1990; 57:839-45.

[8] Bellavite P, Andrioli G, Guzzo P, Chirumbolo S, Manzato F, Lechi C. *Anal. Biochem.* 1994; 216:444-50.

[9] Barnes JP, Belvisi MG, Rogers DF. *TIPS* 1990; 11:185-89.

For the authors:

Anita Conforti
Istituto di Farmacologia
Universita di Verona
Policlinico B. Roma
37134 Verona
Italy

NOTE: The formulas of *Traumeel®Tablets* and *Traumeel®Oral Drops*, as produced in the United States, contain the identical ingredients as *Traumeel®S*, used in this study. However, two ingredients, *Arnica montana, radix* and *Hypericum perforatum*, are at the 3X potency in the U.S. produced tablets and oral drops.

Traumeel®S tablets and drops are not available in the U.S.