Effect of Traumeel S®, a homeopathic formulation, on blood-induced inflammation in rats

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SUMMARY. Objective: to evaluate the activity of Traumeel S® (TRS), a homeopathic formulation containing *Arnica montana* and other plant extracts and minerals on an animal model of traumatic inflammation. Design: TRS and individual components thereof were administered locally to rats 1 h before hind-paw injection with 0.1 ml of homologous blood and the development of oedema was measured over five hours. In each experiment, a control group was treated with saline Main outcome measures: Paw volume of each rat was measured before oedema and 1, 3, and 5 h after oedema induction. Serum levels of IL-6 were determined at hour 5. Results: The decrease of paw oedema, associated with the process of healing, was more rapid in rats treated with TRS ($P<0.05$ after 3 h and $P<0.01$ after 5 h). Similar effects were also induced by separate injection of most, but not all, TRS ingredients. The efficacy of complete mixture of TRS was higher than the combination of a selection of active components. TRS also reduced oedema development when administered after the oedema induction. The therapeutic effect of TRS was associated with a significant decrease of systemic interleukin-6 production. Conclusion: TRS seems to act by speeding up the healing process instead of blocking the development of oedema from the beginning. Moreover, its effect cannot be considered as the 'sum' of its active components and probably a synergistic interaction occurs to determine the final effect.

INTRODUCTION

The blossoms of *Arnica montana* have been used for therapeutic purposes because of their antiphlogistic and analgesic effects. More than 150 chemical substances have been found in Arnica blossoms and, among these, helenalin and its derivatives and flavonoids are the most important.[1] In vitro and in vivo studies have shown the anti-inflammatory and antimicrobial activity of these substances.[2–4] Homeopathic preparations of *Arnica montana*, often used in combination with other drugs, have been reported in clinical trial to have therapeutic effects in conditions such as trauma[5], childbirth[6,7], haematoma[8,9] and dental pain[10]. However, other studies have had negative results.[11–15] A preparation containing active principles from *Arnica montana* together with other plant extracts and minerals has been developed by Heel GmbH and called Traumeel S® (TRS). The anti-inflammatory effect of TRS is claimed to result from the activity of single components on the different phases of inflammation. For instance, it has been suggested[16] that *Aconitum*, *Chamomilla*, *Hamamelis* and *Hypericum* may reduce the pain associated with inflammation; *Aconitum*, *Arnica*, *Hamamelis*, *Hypericum*,

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Millefolium may have antihemorrahagic effects; *Arnica, Calendula, Echinacea, Symphytum* may accelerate the wound-healing; *Mercurius solubilis* may be on anti-inflammatory and anti-viral agent; *Hamamelis* may prevent the venous stasis; *Hepar sulfuris* may improve cellular respiration. The main indications of TRS are referred to different types of lesions and inflammatory processes involving muscles and joints, sprains and bruises. Its efficacy has been demonstrated in randomized, double-blind, placebo-controlled trials.[17–19] However, the mechanism by which this drug exerts its therapeutic effects remains to be clarified.

In a previous series of experiments, we tested TRS in vivo on acute and chronic experimental inflammatory conditions caused by intra-paw injection of carrageenan (carrageenan oedema) or Freund's complete adjuvant (adjuvant arthritis).[20,21] The results suggested that the local administration of TRS reduced oedema development and this inhibition was similar to the effect exerted by aspirin at dose of 30 mg/kg in the same experimental model. In adjuvant arthritis model, the i.p. administration of TRS every 2 days led to a significant reduction in acute local inflammation, without affecting the chronic arthritic process.

In the present paper, we report a series of studies aimed to improve the knowledge on therapeutic action of TRS by using a new model, intra-paw injection of a small amount of homologous blood. This mimics a traumatic blood extravasation, a condition usually treated with TRS. We also tested the activity of individual components of TRS and of a combination containing only the components showing anti-inflammatory activity. We also measured the serum level of interleukin-6 (IL-6), a cytokine which is known to be involved in inflammatory conditions.[22,23]

METHODS

Drugs

Traumeel S® was made available by Biologische Heilmittel Heel GmbH D-76532 Baden-Baden (lot Ch-B 207130 29 and Ch-B 010100 22) according to the German Homoeopathic Pharmacopoeia.[24] Starting from the crude extract, a series of 1:10 dilutions (indicated by D) in sterile physiological saline, were prepared and provided in 2.2-ml glass vials. The potentizing of the single constituents of TRS is conducted mechanically by an upward/downward movement of 50 mm distance conducted 10 times. TRS was prepared by adding the following decimal dilutions in a final volume of 2.2 ml of physiological saline:

Arnica montana D2, 2.2 µl; *Calendula officinalis* D2, 2.2 µl; *Hamamelis virginiana* D2, 0.22 µl; *Achillea millefolium* D3, 2.2 µl; *Atropa belladonna* D2 ana, 2.2 µl; *Aconitum napellum* D3, 1.32 µl; *Hepar sulfuris* D8, 2.2 µl; *Symphytum* D8, 2.2 µl; *Mercurius solubilis* D8, 1.1 µl; *Bellis perennis* D2, 1.1 µl; *Chamomilla* D3, 2.2 µl; *Echinacea angustifolia* D2, 0.55 µl; *Echinacea purpurea* D2, 0.55 µl; *Hypericum* D2, 0.66 µl.

Individual components of TRS were provided by the manufacturer at a dilution identical to the dilution present in the whole complex. For instance, individual Arnica montana D2 was prepared by diluting 2.2 µl of *Arnica montana* D2 in a final volume of 2.2 ml of physiological saline. Moreover, Heel provided the physiological saline that was used as control and a preparation containing only the single components that demonstrated anti-inflammatory activity in the blood-oedema model. This combination contained *Arnica montana, Hamamelis virginiana, Achillea millefolium, Atropa belladonna, Aconitum napellum* and *Mercurius solubilis* at the same dilution as in TRS.

Animals

The experiments were carried out on 416 male Sprague–Dawley rats purchased from Harlan, Italy. The animals, weighing 150–175 g, were kept under standardized conditions on standard diet and water ad libitum. Authorization for animal experiments was obtained by the Italian Ministry of Health.

Blood-induced oedema

At the time of the oedema induction, one rat was sacrificed and blood was collected by cardiac puncture using a heparin containing syringe and stored in a tube until use.

Animals were randomly assigned to different treatment groups and treated subcutaneously in the right hind paw with 0.1 ml of saline or TRS or its single components either 1 h before oedema induction or 30 min after oedema induction.

Oedema was induced by injecting the plantar surface of the right hind paw with 0.1 ml of the previously collected blood. This procedure triggers a slight inflammatory process which simulates the reactions following haematoma formation. No transfusion cross-reactions occur, because all the rats are syngenic.

Paw volume was measured with a water plethysmometer (7150 Ugo Basile, Italy) just before treatment (time 0) and 1, 2, 3 and 5 h after blood injection.[25] The precision of the test was determined by repeating the measurement of the same paw volume 60 times. In these conditions, the coefficient variation (CV) of paw volume was 3.93%. The oedema was assayed as foot increase volume with respect to the foot volume measured on time 0 minus the injected volume of blood (0.1 ml).

The average of paw swelling in the groups of treated animals was compared with that obtained in the group of control animals and the percentage of inhibition was calculated.

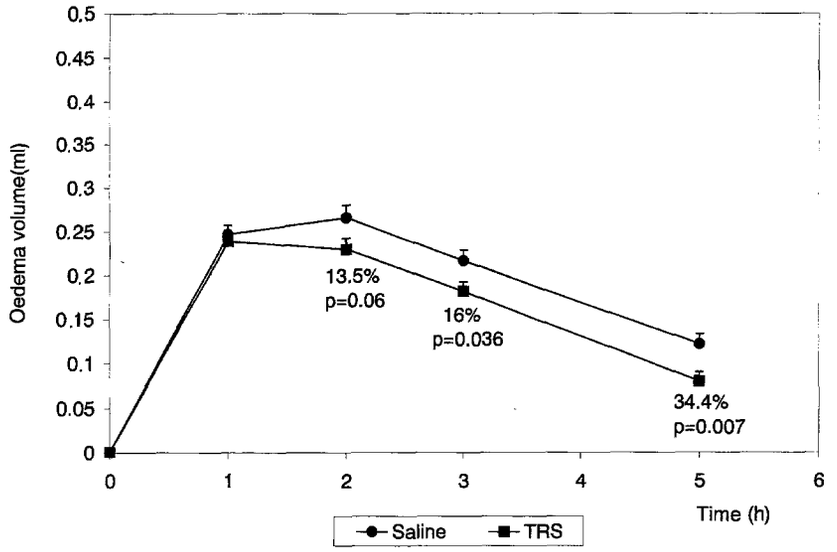

Fig. 1 Time-course of oedema volume (ml) in rats receiving in hind paw 0.1 ml of saline or TRS 1 h before oedema induction. Data represent the mean ± sem of 14 separate experiments. In each experiment, 8–9 animals were treated with TRS and the same number of animals were treated with saline as control. A total of 96 rats in either TRS treated and control groups were utilized in this series of experiments. Percentage of inhibition and statistical analysis (Student's *t*-test) are reported.

Serum IL-6 bioassay

Five hours after blood injection, rats were sacrificed and blood was collected by cardiac puncture in order to evaluate the serum level of IL-6.

Blood was centrifuged and the sera were stored at –20°C until testing for IL-6 activity. To measure IL-6 activity, the murine IL-6-dependent hybridoma cell line 7TD1 was used.[23] Briefly, cells were cultured in 96-well flat, bottom microtitre plates (2×10^4 cells/well) with serial dilutions of the sera for three days (37°C, 8% CO_2). Cells proliferation was determined by hexosaminidase reaction.[26] 60 µl of 7.5 mM hexosaminidase in 0.1 M citrate buffer pH 5.0 containing 0.5% Triton-X-100 were added to cells and the plates were then incubated at 37°C in 100% humidity for 4 h. The color reaction was developed and enzyme activity blocked by addition of 90 µl of 0.1 M glycine buffer pH 10.4, per well. Extinction was measured in a microplate reader (Reader 400, SLT Labs Instruments) at 405 nm using as reference 620 nm. The standard used in this assay was a human recombinant interleukin-6 (Boehringer, Mannheim, Germany).

Statistical analysis

The statistical evaluation of the data was carried out by applying the Student's *t*-test for unpaired data.

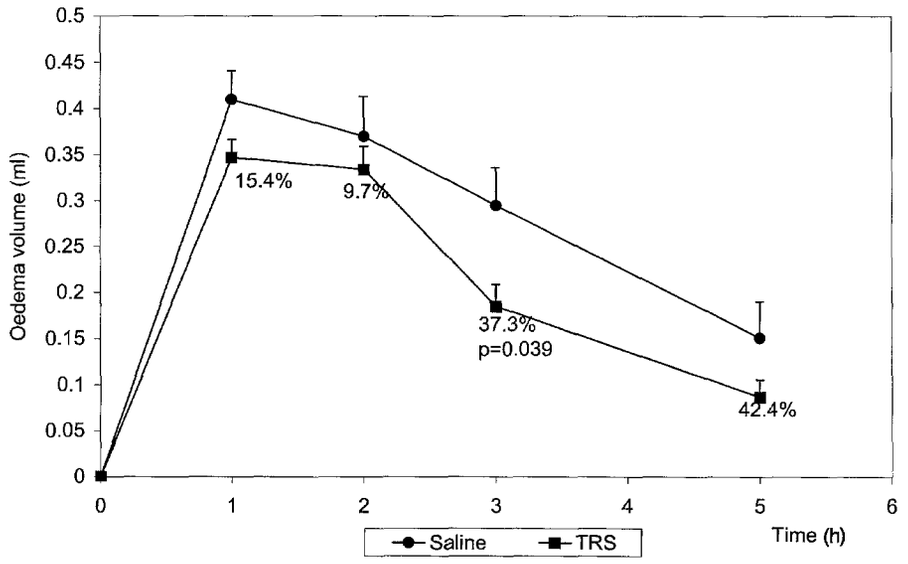

Fig. 2 Time course of oedema volume in rats receiving in hind paw 0.1 ml of saline or TRS 30 min after oedema induction. Data represent the mean ± sem of a single experiment, n=7 animals in each treatment group. Percentage of inhibition and statistical analysis (Student's *t*-test) are reported.

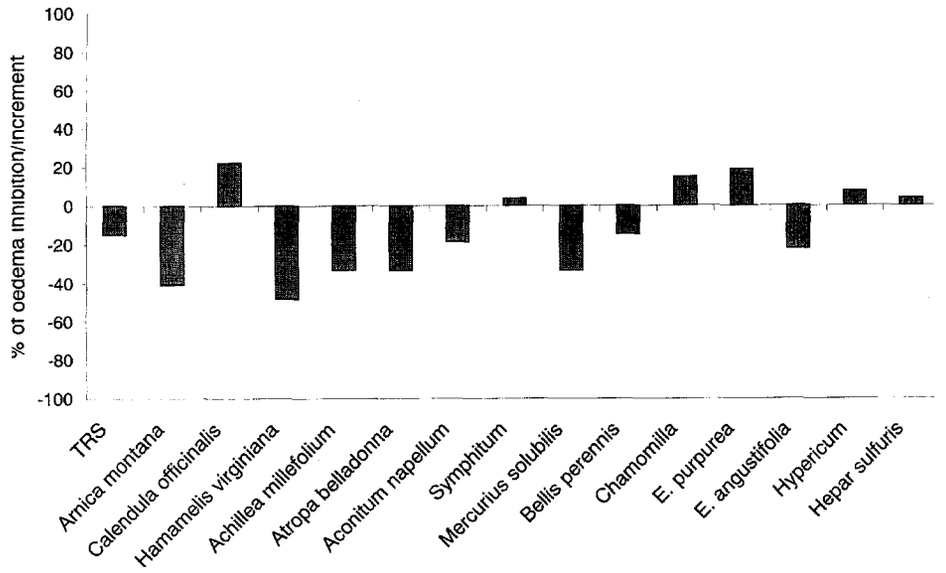

Fig. 3 Percentage of oedema inhibition or increment at the second hour in comparison to saline-treated controls of the single components contained in TRS. All substances are administered 1 h before oedema induction. Data represent the mean of two separate experiments (n = 14–21 animals in each treatment group).

RESULTS

The effect of TRS on oedema development following local blood injection documents a time-dependent oedema reduction. In fact, the initial increase of paw volume (first hour) was almost identical in rats injected with TRS as in control group (Fig. 1). Then, the paw volume of TRS-treated rats started to decrease, while that of control groups continued to increase and peaked at the second hour. Thus, the decrease of paw oedema, associated with the process of healing was more rapid in TRS treated rats, so that, 3 and 5 h after the injection of blood, the local inflammation was significantly lower in treated animals ($P<0.05$ and $P<0.01$ respectively). In this series of experiments, TRS was injected locally 1 h before blood. Similar results were obtained also in an experiment where TRS was injected locally 30 min after blood (Fig. 2).

In this case, the animals treated with TRS developed since the beginning a lower oedema reaction that was significant 3 h after oedema induction ($P<0.05$).

On the same experimental model, we tested the single components of TRS to identify the most active substances which are responsible for such a oedema reduction. Due to the high number of components to be analysed, this experiment could be performed only in two separate trial for each single component. In all experiments TRS and saline as control were included. In Figure 3, the percentages of oedema increment or inhibition 2 h after blood induction by single TRS components are reported. It can be noted that not all of the single substances contained in TRS exerted the same effect on blood-induced oedema. In particular, inhibitory effects were exerted by *Arnica montana*, *Hamamelis virginiana* and *Atropa belladonna*, while *Achillea millefolium*, *Aconitum napellum*, *Mercurius solubilis*, *Echinacea purpurea*, *Chamomilla* and *Calendula* seemed to have, when used alone, a slight pro-inflammatory effect; other components did not influence the development of the oedema.

In the light of these data, we were interested to check the activity of a preparation containing only the substances that showed anti-inflammatory activity. As shown in Table 1, the effect of this combination ('active combination') was lower than the effect of the whole formulation of TRS on oedema development. In the same Table, the results on IL-6 serum levels 5 h after the oedema induction are shown. Previously, we observed that the serum levels of IL-6 were increased 21-fold during the oedema (from 4.5 to 95.3 U/ml serum). Assuming

Table 1 Effects of TRS and of active combination on oedema volume and serum IL-6 levels measured 5 h after oedema induction

Treatment	Oedema volume% (mean ± standard deviation)	IL-6 production % (mean ± standard deviation)
Saline	100	100
TRS	65.6 ± 11.8 (n=14)**	55 ± 11.16** (n=4)
Active combination	94.04 ± 20.92 (n=6)	64.8 ± 12.9* (n=4)

n=number of experiments
*$P<0.05$; **$P<0.01$ (Student's t-test).

as 100% the IL-6 production of animals treated with saline, decreases of 45% and 35.2% were observed in the TRS-treated group and in the group treated with active combination respectively. Both effects were significant in comparison to the control group.

DISCUSSION

TRS is widely used in humans and has shown therapeutic efficacy in traumatic and inflammatory conditions.[19,27] In order to investigate the TRS effect in a model that mimics a traumatic blood extravasation, a human pathology in which TRS is indicated, we developed a new model of inflammation obtained by injecting in rat hind paw 0.1 ml of whole blood. The results clearly showed a therapeutic effect of TRS in this model.

Our data indicate that the kinetics of the inflammatory phenomenon following haemorragic lesion is different when animals are treated locally with TRS. In particular, TRS seems to act by speeding up the healing process instead of blocking the development of oedema from the beginning.

The study of the single TRS components showed that a considerable therapeutic effect is exerted by *Arnica montana*, *Hamamelis virginiana*, *Achillea millefolium*, *Aconitum napellum*, *Atropa belladonna* and *Mercurius solubilis*, while other substances seemed to be pro-inflammatory or have no effect. These results prompted us to develop a combination including all the inhibitory substances with the aim of obtaining a more active preparation. However, the results of this attempt were negative, indicating that the effect of TRS is higher than the 'sum' of its active constituents. This may suggest that synergistic interactions between many components – including those which are inactive when tested alone – occur to determine the final effect.

On the other hand, we have shown that some components of TRS (e.g. *Calendula, Chamomilla, Echinacea purpurea*) increased oedema development. It appears likely that there is some competition between the active principles. Some components that are inactive on oedema development, or even increase its expression, may have other therapeutic properties (like analgesic or anti-haemorragic effects) that have not been considered in this experiment.

It has been reported that the active constituents of *Arnica montana* responsible for its antiphlogistic effect are sesquiterpene lactones such as helenalin and its derivatives.[1] Helenalin has potent pharmacological activity, possibly by interacting with cellular thiols[28] and/or by inhibiting transcription factor NF-KB, which is a central regulator of cytokine production.[4] However, these pharmacological actions occur at µmol/mmol concentrations, while *Arnica montana* contained in TRS is administered at much lower doses. In TRS, Arnica solution is a 1/1000 dilution of D2 which, in turn, is a 1/100 dilution of crude extract. In this study, animals received only 0.1 ml of TRS. Moreover, helenalin is an ingredient of Arnica flowers but in this homeopathic preparation extracts from the root, containing only traces of helenalin, are employed (personal communication from Biologische Heilmittel Heel). Finally, we have previously observed that TRS does not have any effect in vitro on cells of inflammatory processer slike neutrophils and platelet aggregation.[20]

Therefore, the putative action mechanism of this homeopathic preparation appears to be different from a direct inhibitory effect of Arnica compounds on cellular or biochemical functions. All these observations are consistent with a secondary or induced action (that remains to be identified) on the healing process.

Our preliminary data indicate a possible role of IL-6 in the TRS regulation of inflammatory process, but due to the complex nature of the cytokine network further work is necessary to clarify this point.

In conclusion, it is conceivable that TRS accelerates the tissue changes involved both in the formation and in the elimination of oedema, with a net beneficial effect.

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