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Immunomodulatory action of propolis. VI. Influence of a water soluble derivative on complement activity in vivo

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Abstract

The water soluble derivative (WSD) of propolis in a dose of 150 mg/kg was administered intravenously (i.v.), intraperitoneally (i.p.) and orally (p.o.) to mice. The alteration of serum alternative pathway (AP) complement level was observed. The WSD also influenced the process of acute inflammation provoked by zymosan in mice. The effect was strongly dependent on the route of WSD administration.

Keywords: Water-soluble derivative; Alternative pathway activation; Paw oedema

1. Introduction

A water soluble derivative (WSD) of propolis was found to possess an anticomplementary activity in vitro. Previous results indicated that its mode of action was complex and supposed that more than one complement components were involved (Ivanovska et al., 1993). Probably not all phenomena observed in vitro may take place after in vivo application of WSD. Having in view, firstly, that through alternative complement activation many non-specific host defense reactions are triggered, and secondly, that mice have been used to study the protective and adjuvant effect of WSD, in the present work the WSD effect on complement serum activity after administration through different routes to mice was studied. In order to produce a clinically useful medicine on the base of natural propolis we attempted to elucidate the complex mode of action of the WSD. The consequences, after the change of alternative pathway (AP) level, have been evaluated using, as a model, zymosan-induced paw oedema in mice. This is believed to be a complement-mediated reaction closely connected with AP activation.

2. Materials and methods

2.1. WSD treatment

The solution of WSD was freshly prepared in saline. Female or male mice (strain ICR) 7–10 weeks old, 18–20 g b.w., were obtained from a

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Table 1
Alternative pathway activity in mouse serum after treatment with WSD

Route of WSD application	AP activity (U/ml)			
	0.5[a]	1	3	24
Control	38.1 ± 3.8	36.0 ± 3.0	40.0 ± 5.4	40.0 ± 6.2
i.v.	45.5 ± 5.2[b] (+19.4)[c]	83.5 ± 8.2** (+139)	56.5 ± 6.0* (+41.2)	39.6 ± 4.0 (−1.0)
i.p.	50.0 ± 8.0* (+31.2)	92.0 ± 8.0** (+155)	60.0 ± 5.4* (+50.0)	58.0 ± 6.0 (+45.0)
p.o.	39.4 ± 3.8 (+2.6)	36.4 ± 5.0 (0.0)	49.0 ± 6.0 (+22.5)	46.7 ± 5.0 (+16.7)

[a]Time after WSD treatment.
[b]Mean ± S.D. of 5 individual sera.
[c]Values in parentheses represent the percent of activation or inhibition of AP, calculated to the control. Significant from the control;
*$P < 0.5$; **$P < 0.01$; Student's t-test.

local breeder. The animals were injected i.v., i.p. and p.o. at a dose of 150 mg/kg.

2.2. AP complement assay

At each interval 5 animals were bled and the AP activity in individual sera was determined towards rabbit erythrocytes (RaE) by a microtitre assay (Klerx et al., 1983). Data are expressed in units/ml (Takada et al., 1978).

2.3. Zymosan-induced paw oedema

In these experiments zymosan, as a 2% suspension in sterile saline (0.025 ml), was injected into the pad of the right hind paw. The left hind paw was treated with 0.025 ml of saline. The animals were sacrificed with ether at different times, both hind paws were removed and weighed. Control animals received the solvent under the same experimental conditions.

3. Results

The in vivo effect of WSD in mouse serum showed that after i.v. and i.p. application there was a rapid increase of AP activity in the first hour, followed by a decrease and return to control level after 24 h. If WSD was given p.o., insignificant elevation of AP activity was observed (Table 1).

Using the same dose and routes, WSD was ad-

Table 2
Effect of WSD in zymosan-induced paw oedema

Route of WSD application	Time (h)[a]	Change in paw oedema			
		0.5 h[b]	2 h	4 h	24 h
Control	—	26.0 ± 1.8[c]	44.0 ± 2.4	78.2 ± 3.0	30.0 ± 2.8
i.v.	0.25	19.5 ± 1.4** (−25)	26.8 ± 1.6*** (−39)	38.6 ± 1.2 (−25)	9.4 ± 2.2*** (−69)
i.p.	0.5	23.4 ± 2.0 (−10)	32.1 ± 1.2*** (−27)	60.2 ± 1.0*** (−23)	20.7 ± 1.4*** (−42)
p.o.	0.5	29.3 ± 1.2* (+10)	48.8 ± 1.8* (+10)	70.3 ± 2.2* (−10)	15.2 ± 1.8*** (−50)
p.o.	18	16.9 ± 1.1*** (−35)	14.9 ± 2.5*** (−66)	23.4 ± 1.6*** (−70)	21.9 ± 1.6*** (−28)

[a]Time of WSD application (150 mg/kg) prior to zymosan injection.'
[b]Time after zymosan injection.
[c]Each value represents the mean difference between the weight (mg) of zymosan and saline treated paws ± S.D. The percentage of inhibition calculated in comparison to the respective control is shown in parentheses.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, Student's t-test; $n = 6$/group.

ministered to mice prior to zymosan-induced paw oedema. The i.v. and i.p. injection caused a moderate reduction at the all stages of oedema formation (Table 2). Better inhibition was achieved after p.o. application of WSD 18 h before the phlogistic agent.

4. Discussion and conclusions

Propolis has been used for various medical purposes since antiquity, mainly as an anti-inflammatory agent and wound healing promoter (Filho and Carvalho, 1990). In more recent times, there has been a considerable interest in the therapeutic properties of propolis; its anti-infectious and immunomodulatory actions have been demonstrated (Ghisalberty, 1979; Greenaway et al., 1987). Our previous studies revealed that WSD, consisting of polyphenolic type of compounds, appeared to be a strong host defense stimulator (Dimov et al., 1992). The preventive ability of WSD against infections caused by gram-negative bacteria is believed to be mediated through an influence on some major immune mechanisms, including complement activity. The results of this study showed that in mice the interaction of WSD with complement enhanced the functional serum AP activity in a route-dependent manner. The peak of the oedema formation, according to our preliminary experiments and to other authors (Tarayre et al., 1989), is at the 6th hour after zymosan treatment. As to the complement change caused by WSD, the oedema maximum coincided with the phase of AP decrease. At the 4th hour, when the measurement was done, the active phase of oedema process falls in the period of enhanced AP activity.

The activation of AP results in the generation of anaphylatoxins (C3a, C5a and C3b), needed for many immune reactions (Muller-Eberhard, 1976; Schorlemer et al., 1981). According to our previous data, the best immunomodulating effect was observed after repeated i.p. treatment, which corresponds to WSD potency in altering complement activity. In the present experiments a significant reduction of acute inflammation was achieved after oral application of WSD. That is the case when serum AP level was not changed. Evidently, the route and the time of application were critical for the final effect.

The use of single constituents will allow more precise interpretation of data but the additive effect of the numerous compounds will be lost. The lack of side effects after WSD administration should not be neglected. On the other hand the complex content may also result in antagonistic effects. The present results support such a possibility. When WSD was given p.o., stronger inhibition was detected but with an initial increase of oedema volume. The various constituents have different metabolic cycles and dependently on the route of WSD application will not express their action simultaneously.

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