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COMMUNICATIONS

Anti-complement activity of oleanolic acid: an inhibitor of C_3-convertase of the classical complement pathway

ARUNA KAPIL, SHALINI SHARMA, *Pharmacology Division, Regional Research Laboratory, Jammu Tawi 180 001, India*

Abstract—Oleanolic acid is a naturally occurring compound, isolated from *Luffa cylindrica*, which inhibits the in-vitro immunohaemolysis of antibody-coated sheep erythrocytes by guinea-pig serum. In further experiments this reduced immunohaemolysis was found to be due to inhibition of the C_3-convertase of the classical complement pathway. The threshold concentration for inhibition of C_3-convertase was 100 $\mu g\,mL^{-1}$. However, higher concentrations of oleanolic acid showed constant inhibitory effects on immunohaemolysis. Oleanolic acid also exhibited weak inhibitory effects on individual components of the complement system.

Oleanolic acid is a triterpene (3β-hydroxyolean-12-en-28-oic acid), occurring in a large number of plants in the free state as well as its acetate and as glycosides in many saponins. It has been isolated from seeds of *Luffa cylindrica* (Barua & Bose 1960) and found to possess anti-inflammatory and anti-arthritic activity in a variety of experimental models (Singh et al 1992). Since complement is one of the major mediators of the inflammatory response (Dias da Silva & Lepow 1967; Goldstein 1988), it was assumed that inhibition of complement activity would be expected to inhibit inflammatory models in which complement activation is involved. The release of anaphylatoxins on the activation of the complement system results in inflammation or allergic reactions by increasing vasopermeability and oedema formation (Williams & Jose 1981), by releasing lysosomal enzymes (Becker et al 1974) and stimulating leucocyte chemotaxis (Ward et al 1979). The pro-inflammatory role of complement activation is demonstrated in experimental models, such as the Arthus reaction, an inflammatory reaction characterized by a localization of an antigen-antibody-complement (Ag-Ab-C) in the wall of affected vessels (Sell 1980). Oleanolic acid seems to be a selective inhibitor of complement activation both for further experimental research and for possible therapeutic use.

We now report the anti-complementary activity of oleanolic acid and ibuprofen and demonstrate that inhibitory action on complement activation involves inhibition of C_3-convertase of the classical complement pathway.

Materials and methods

Oleanolic acid was obtained from the NPC Division of the laboratory and ibuprofen from Boots Co., Bombay, India. Healthy and hygenically maintained male guinea-pigs, 150–200 g, were obtained from the Animal House of this Institute.

Haemolytic assay. The in-vitro haemolysing effects of oleanolic acid and ibuprofen via classical complement pathway and alternate pathway were determined spectrophotometrically (Kapil & Moza 1992). The source of complement was fresh pooled guinea-pig serum as well as human serum. For the classical complement pathway, the veronal saline buffer (25 mM, pH 7·4, containing 0·15 mM Ca^{2+}) was used as a diluent in the complement assay. Sensitized sheep erythrocytes were incubated with complement-incubated oleanolic acid and for the alternate pathway, veronal buffer containing 0·02 M Mg^{2+} and 8 mM ethylene glycol bis(2-aminoethyl) tetra-acetic acid was used as a diluent in the complement assay. Rabbit normal erythrocytes were incubated with oleanolic acid-incubated guinea-pig serum. The degree of haemolysis produced by released haemoglobin in the supernatant after sedimentation of the remaining erythrocytes was determined spectrophotometrically at 413 nm. The anti-complement activities of oleanolic acid and ibuprofen were also tested using fresh human serum as a source of complement.

C_3-Convertase activity. The method of Bitter-Suermann et al (1970) was followed for determination of C_3-convertase activity. Briefly, C_3-depleted guinea-pig serum (by pre-incubation with 3 mg zymosan mL^{-1} guinea-pig serum for 1 h at 37°C) with and without oleanolic acid (100 μg/0·1 mL diluent buffer) was incubated with sheep sensitized erythrocytes for 30 min at 37°C. The resulting C_3-convertase-bearing erythrocytes were washed and incubated with purified guinea-pig C_3-protein at 37°C for 1 h. After a final incubation, the degree of haemolysis in the supernatant was determined spectrophotometrically at 413 nm and inhibition was calculated. Ibuprofen was also tested simultaneously.

Purification of C_3-complement protein. C_3-Complement protein was purified as described by Kabat & Mayer (1948) and Bitter-Suermann et al (1970). The effect of different concentrations of oleanolic acid on the classical complement pathway as well as on C_3-convertase was also determined. Simultaneously, standard drug and controls were also run.

Results

Oleanolic acid showed a maximum pronounced inhibitory effect on the haemolytic activity of the complement system towards antibody-coated erythrocytes. Table 1 shows the decreased immunohaemolysis of sheep antibody-coated erythrocytes induced by fresh pooled guinea-pig serum (1 : 100) in the presence of oleanolic acid as well as in the presence of ibuprofen. Oleanolic acid showed 85% and ibuprofen showed a 64% inhibitory effect on the classical complement pathway, whereas via the alternate pathway no inhibitory effect was observed. With human serum, oleanolic acid as well as ibuprofen showed a marked inhibitory effect on the complement activity via the classical pathway (data not shown). When antibody-coated erythrocytes were incubated with guinea-pig serum-incubated oleanolic acid of different concentrations for 1 h at 37°C, a significant inhibitory effect was observed at a concentration of 100 $\mu g\,mL^{-1}$, but at higher concentrations it showed a constant inhibitory effect of immunohaemolysis.

To assess the specific mechanism of reaction of the whole

Correspondence: A. Kapil, Pharmacology Division, Regional Research Laboratory, Canal Road, Jammu Tawi 180 001, India.

Table 1. Effect of oleanolic acid and ibuprofen on classical and alternate complement pathways.

Compound	Anticomplement activity			
	Classical		Alternate	
	Absorption at 60 min	Inhibition (%)	Absorption at 30 min	Inhibition (%)
Control	0·992 ± 0·009	–	0·959 ± 0·012	–
Oleanolic acid	0·145 ± 0·002*	85·38	0·950 ± 0·015	0·93
Ibuprofen	0·350 ± 0·013*	64·72	0·947 ± 0·011	1·25

The data are the mean ± s.d. of triplicate determinations. *$P < 0·001$ compared with control.

complement system, we treated the effect of oleanolic acid on individual complement components (C1-C4) via the classical pathway. Very weak inhibitory effects were observed. However, when oleanolic acid was applied to check its effect on C_3-convertase a marked inhibitory effect was observed.

When sensitized sheep erythrocytes and C_3-convertase were incubated with C_3-protein-incubated oleanolic acid for 1 h at 37°C, a significant inhibitory effect was observed at a concentration of 100 $\mu g\,mL^{-1}$ oleanolic acid (Table 2). The degree of haemolysis by terminal complement components was determined.

Discussion

The inhibitory effect of oleanolic acid on guinea-pig serum results in an anti-inflammatory effect (Englberger et al 1988; Goldstein 1988) and anti-arthritic activity (Sell 1980). The inhibition of the classical pathway of complement activation was shown in-vitro by the decreased immunohaemolysis of antibody-coated sheep erythrocytes incubated with guinea-pig serum. Our studies demonstrate that this inhibitory effect of oleanolic acid on classical complement activity is mainly due to the inhibition of C_3-convertase, a serine protease which indicates that inhibition of serine proteases in general may be involved in anti-inflammatory activity. Like oleanolic acid, ibuprofen, a non-steroidal anti-inflammatory drug, was also tested, for comparison. When C_3-convertase is inhibited by oleanolic acid, pro-inflammatory anaphylactic peptides are not released, with the result that no inflammatory responses are observed.

The present study is in agreement with previous reports of Englberger et al (1988) on rosamarinic acid, and Kapil & Moza (1992) on boswellic acids, which showed that these acids inhibit complement C_3-convertase and may result in its anti-inflammatory activity in which complement activation is involved.

Oleanolic acid possesses anti-complement activity and is also reported to possess marked anti-inflammatory and anti-arthritic activity (Singh et al 1992). One possible mechanism, selective inhibition of classical C_3-convertase activity by oleanolic acid, would seem to offer potential for the therapy of inflammation and other conditions associated with complement activation.

Table 2. Inhibitory effect of different concentrations of oleanolic acid on C_3-convertase of the classical complement pathway.

Amount of oleanolic acid ($\mu g\,mL^{-1}$)	Anti-complement activity	
	Absorption at 60 min	Inhibition (%)
Control	1·350 ± 0·037	–
12·5	1·234 ± 0·026**	8.59
25	1·031 ± 0·021*	23·63
50	0·804 ± 0·017*	40·44
100	0·388 ± 0·014*	71·26
200	0·395 ± 0·010*	70·74

The data are the mean ± s.d. of triplicate determinations. *$P < 0·001$, **$P < 0·02$ compared with control.

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