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TELEOCIDIN, A TUMOR PROMOTER, IS A POTENT PLATELET-AGGREGATING AGENT

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SUMMARY

Teleocidin caused irreversible aggregation of human platelets. Divalent cations and fibrinogen were required for teleocidin-induced aggregation. Teleocidin-induced aggregation was inhibited by PGE_1, dbcAMP, theophylline , metabolic inhibitors such as iodoacetate and antimycin A, or diltiazem, indicating that it was dependent on platelet metabolism. Platelets from a patient with thrombasthemia did not respond to teleocidin. Although teleocidin was capable of triggering platelet secretion, this secretion was initiated with a lag phase after the onset of irreversible aggregation and inhibitors of platelet secretion such as aspirin and trifluoperazine slightly modified this aggregation. These observations suggest that teleocidin causes primary aggregation by direct action on platelet membrane in a similar manner as ADP.

INTRODUCTION

Teleocidin, an indole alkaloid, which was isolated from the mycellia of Streptomyces (1,2), seems to be a new type of tumor promoter (3). Although teleocidin is structurally unrelated to 12-O-tetradecanoylphorbol-13-acetate (TPA), teleocidin has many biological effects such as the induction of ornithine decarboxylase, the induction of HL-60 cell adhesion, the inhibition of terminal differentiation of Friend erythroleukemia cells (3), the induction of human lymphoblastoid cell aggregation (4), the inhibition of binding of epidermal growth factor (EGF) (5) and the mitogenic effects on lymphocytes (6) similar to those of TPA (4,5,6). These biological effects are thought to be mediated through the interactions of tumor-promoting agents with the cell membrane. Umezawa et al. (7) recently reported that teleocidin inhibited the binding of TPA to cell-surface receptors, suggesting that the action of both may be mediated by same or a similar receptor system.

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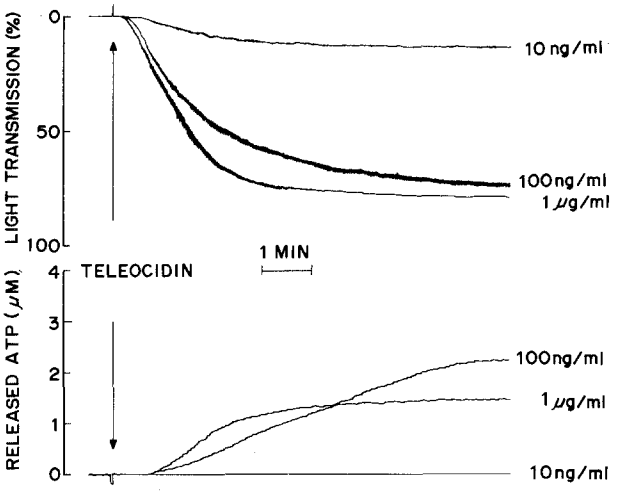

Fig. 1. Concentration-dependent aggregation and ATP secretion of platelets in PRP by teleocidin. An arrow indicates an addition of teleocidin at a final concentration of 10 ng/ml, 100 ng/ml or 1 μg/ml.

Submicromole concentrations of TPA caused irreversible platelet aggregation which was similar to ADP-induced platelet aggregation (8,9), but very little is known about the effect of teleocidin on platelets. We report here that teleocidin can induce platelet aggregation, the pattern of which is similar in many respects to that of ADP-induced platelet aggregation.

MATERIALS AND METHODS

Teleocidin isolated from the mycellia of Streptomyces was obtained from Fujisawa Pharmaceutical Industries, Ltd., Osaka, Japan (1,2). Teleocidin was dissolved in 50% ethanol (1mg/ml) and stored at -20°C. The stock solution was diluted with deionized water just before the use. The final concentration of ethanol in the experiments was less than 0.05%. Human fibrinogen (grade L) from Kabi, Stockholm, Sweden, was treated with diisopropylfluorophosphate (DFP, Sigma) as described by Mustard et al. (10) to inactivate any coagulant activity present in it.

Venous blood was collected into a plastic syringe containing $^{1}/_{10}$ volumes of 3.8% sodium citrate from human donors who had taken no drugs for at least one week. Platelet-rich plasma (PRP) was prepared by centrifuging the citrated blood at 300 g for 6 min at room temperature. PRP was centrifuged at 1000 g for 15 min and the platelet pellet was resuspended in washing buffer (pH 6.5)(11) with 0.35% albumin and apyrase. The washing procedure was repeated three times and the final platelet pellet was resuspended in a Tyrode's solution (pH 7.4) (which contained 2 mM calcium and 1 mM magnesium) with 0.35% albumin to achieve a final platelet count of about 300,000/μl. Platelet counts were obtained using a Thrombocounter C (Coulter Electronics. Fla., USA). Platelet aggregation and ATP secretion studies were performed using a Chrono-log lumi-aggregometer (Chrono-log Corp., Pa., USA). Platelets were loaded with serotonin by preincubation of PRP with [^{14}C] 5-hydroxytryptamine creatinine sulphate for 40 min at 37°C. After the addition of teleocidin, the reaction was stopped

Table 1. Effect of teleocidin on ^{14}C-serotonin release

minutes after addition of teleocidin (100ng/ml)	^{14}C-serotonin release (%)	extent of aggregation (%)
1	0.9 ± 0.5	49
2	13.3 ± 0.6	81
4	22.4 ± 1.1	97
6	33.7 ± 1.7	100

^{14}C-serotonin release study and aggregation study were carried out in aggregometer cuvettes at the same time. ^{14}C-serotonin release is expressed as a percentage of the total radioactivity incorporated by the platelets. Values of ^{14}C-serotonin release are given means ± S.E. for three experiments. Values of the extent of aggregation which are expressed as percentages of the aggregation height at the indicated time to the maximal aggregation are means for three experiments.

at the specified time by the addition of glutaraldehyde at a final concentration of 0.2%. After centrifugation, aliquots of the supernatant were counted.

RESULTS

Concentration-dependent platelet aggregation induced by teleocidin is illustrated in Fig. 1. Platelets in PRP aggregated weekly in response to teleocidin at a final concentration of 10 ng/ml without the release of detectable amounts of ATP. Teleocidin at concentrations of 10 ng/ml and 1 μg/ml induced irreversible platelet aggregation accompanied by the release of ATP. The release of ATP, however, began about 1 min after the addition of teleocidin. The pattern of teleocidin-induced aggregation was not associated with a transient decrease in light transmisstion and a narrowing of oscillations, suggesting that teleocidin-induced aggregation could occur to some extent without significant shape change.

Time course of the release of ^{14}C-serotonin is shown in Table 1. Only about 1% release of ^{14}C-serotonin was detected 1 min after the addition of teleocidin (100 ng/ml), at which the extent of aggregation already attained to about 50% of maximal aggregation. Thereafter the release of ^{14}C-serotonin increased in a time-dependent manner. The pattern of the release of ^{14}C-serotonin seemed to be similar to that of ATP.

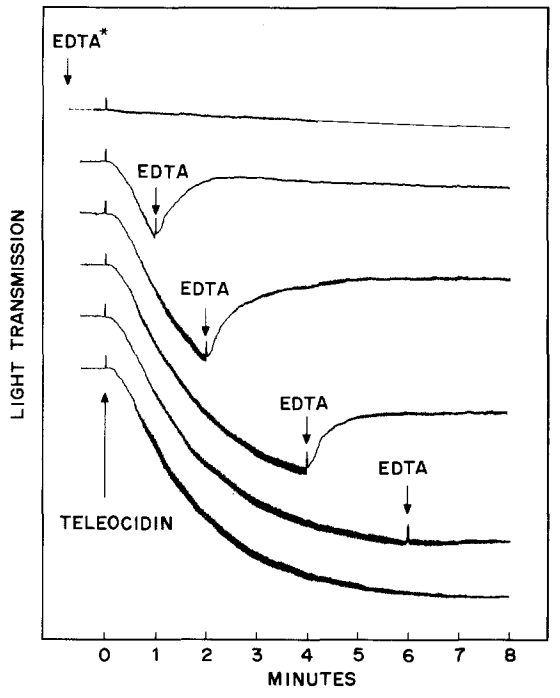

Fig. 2. Effect of EDTA on teleocidin-induced platelet aggregation. Arrows indicate additions of teleocidin (100 ng/ml) and EDTA (5 mM). EDTA* was added 2 min before the addition of teleocidin.

EDTA (5mM) added to PRP 2 min before the addition of teleocidin (100 ng/ml) completely prevented teleocidin-induced aggregation (Fig. 2). When EDTA was added after the addition of teleocidin, aggregation was also arrested. After completion of aggregation, the addition of EDTA had little effect.

Fig. 3 shows the requirement of fibrinogen for teleocidin-induced aggregation. Suspensions of washed platelets did not aggregate or aggregated only to a slight extent in response to teleocidin unless fibrinogen was present in the suspending medium.

To evaluate the mechanism of teleocidin, we studied the influence of agents that inhibit platelet aggregation (Table 2). PGE_1 (1 μM), dbcAMP (2 mM) or theophylline (6 mM) added to PRP prevented teleocidin-induced aggregation. Apyrase (2 mg/ml) or creatine phosphate plus creatine phosphokinase (CP/CPK, 3 mM; 15 units/ml) slightly decreased the extent of aggregation. Iodoacetate (0.5 mM) or antimycin A (2 μM) alone partially inhibited teleocidin-induced

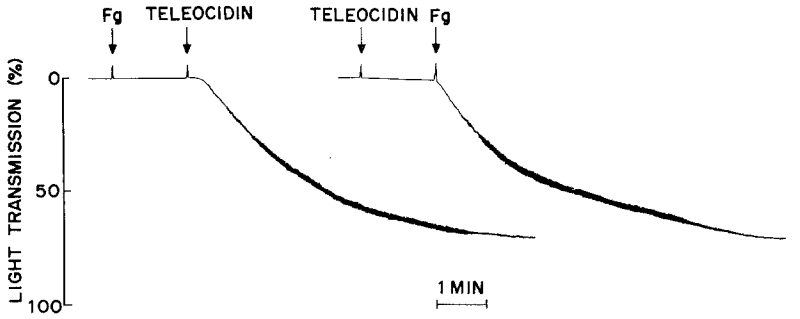

Fig. 3. Requirement of fibrinogen for teleocidin-induced platelet aggregation of washed platelets. Arrows indicate additions of Fg (fibrinogen, 500 μg/ml) and teleocidin (100 ng/ml).

aggregation but both together completely prevented aggregation. Diltiazem (0.5 mM), a calcium antagonist, inhibited aggregation.

As shown in Fig. 4, platelets in PRP from a thrombasthenic patient did not respond to teleocidin even at a concentration of 1 μg/ml.

In the presence of aspirin (100 μg/ml) or trifluoperazine (100 μM), a calmodulin inhibitor, the degree of aggregation induced by teleocidin (100 ng/ml) decreased to a slight extent. The release of ATP, however, was almost completely inhibited by aspirin or trifluoperazine.

Table 2. Effect of various inhibitors on teleocidin-induced platelet aggregation

inhibitor	preincubation (min)	aggregation
none	--	++++
PGE_1 (1μM)	2	0
dbcAMP (2mM)	5	0
theophylline (6mM)	30	+ ~ 0
apyrase (2 mg/ml)	3	+++
CP/CPK (3mM; 15 units/ml)	3	+++
diltiazem (0.5 mM)	5	0
iodoacetate (0.5 mM)	40	+
antimycin A (2 μM)	40	++
iodoacetate + antimycin A	40	0

After preincubation of PRP with inhibitor for the indicated period at 37°C, platelet aggregation induced by teleocidin (100 ng/ml) was initiated. Degree of aggregation without inhibitor is expressed as ++++.

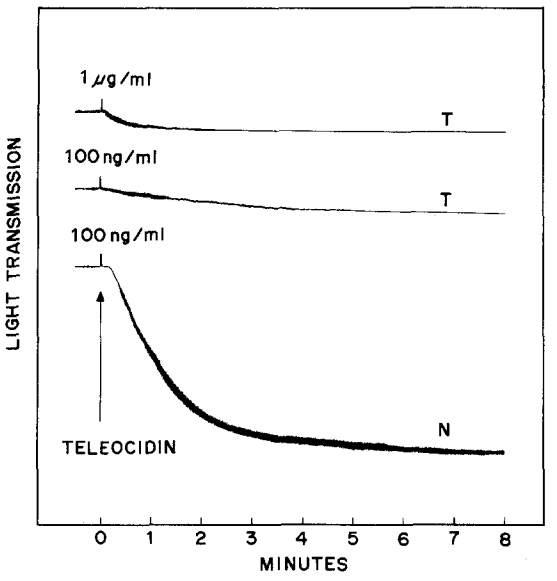

Fig. 4. Unresponsiveness of thrombasthenic platelets to teleocidin. T: PRP from a patient with thrombasthenia, N: PRP from a normal donor. An arrow indicates an addition of teleocidin.

DISCUSSION

The results presented here show that teleocidin in minute amounts cause irreversible platelet aggregation without significant shape change. Although teleocidin was capable of triggering release reaction as demonstrated by ATP secretion (Fig. 1) and ^{14}C-serotonin release (Table 1), these release reactions took place with a lag phase after the onset of irreversible aggregation, suggesting that teleocidin-induced release reaction is secondary to aggregation, namely, so-called " aggregation-induced secretion ". TPA, a well known tumor promoter, which has an entirely different chemical structure from that of teleocidin, has been shown to cause irreversible platelet aggregation (8,9) without shape change (9). Zucker et al. (8) noted a marked similarity in the response of platelets to TPA and ADP. TPA also causes release reaction. White et al. (12) reported that TPA-induced release reaction occured before the onset of aggregation. Zucker et al. also reported TPA-induced release of serotonin and adenine nucleotides. However, the rate of release reaction they observed was much slower and the magnitude of it was much less.

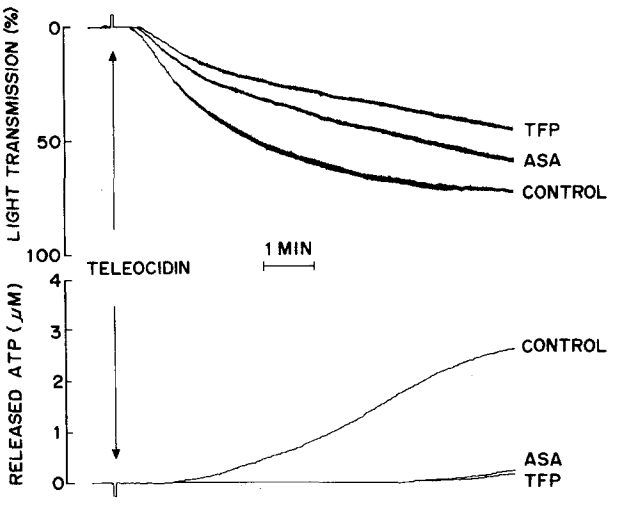

Fig. 5. Effect of aspirin and trifluoperazine on teleocidin-induced platelet aggregation and ATP secretion. After PRP was preincubated for 5 min with no addition, with ASA (aspirin, 100 μg/ml) or with TFP (trifluoperazine, 100 μM), teleocidin (100 ng/ml) was added.

The essential role of fibrinogen in teleocidin-induced aggregation is evidenced by the observation (Fig. 3) that suspensions of washed platelets did not aggregate with teleocidin unless fibrinogen was added. Although many stimuli can trigger platelet aggregation, ADP is clearly known to require fibrinogen as a cofactor (13). It is known that ADP-induced aggregation is mediated by the binding of fibrinogen to the specific receptor (14,15,16). So it is attractive to consider the hypothesis that fibrinogen binding sites on platelet membrane become available with teleocidin.

The inhibition of teleocidin-induced aggregation by raising the intracellular level of cAMP or by depleting metabolic ATP (Table 2) indicates that teleocidin-induced aggregation is dependent on platelet metabolism and is true aggregation rather than agglutination.

The failure of apyrase or CP/CPK to substantially inhibit teleocidin-induced aggregation indicates that released ADP seems not to have an essential role in this aggregation, but rather to have a synergistic effect on it.

Aspirin and trifluoperazine slightly decreased the extent of aggregation with inhibition of ATP release. This result coincided with the above mentioned

result that release reaction took place with a lag phase after the onset of irreversible aggregation, again suggesting that teleocidin induces primary aggragation independent of release reaction. Nishikawa et al. (17) recently reported that the inhibition of the first phase of ADP-induced aggregation by W7, a calmodulin inhibitor, was weaker than that of second.

These results show that there is a number of similarities between teleocidin-induced aggregation and ADP-induced aggregation in spite of some differences in the response of platelets to the two compounds. Interpretation of the details of the platelet response to teleocidin remains uncertain. However, the similarity in the effect of two structurally unrelated tumor promoters, teleocidin and TPA, will give us some insight of the mechanism of tumor promoter action on platelet.

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