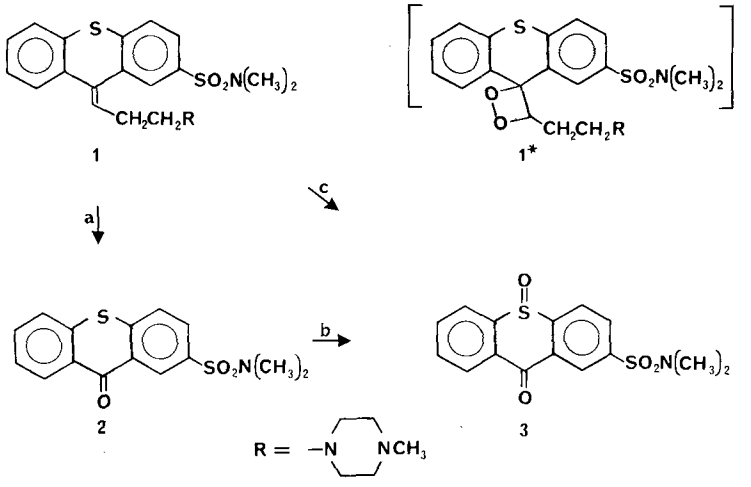


The chemical structures of thiothixene (**1**) and its chemical (**3**) and photochemical (**2**) oxidation products. Reaction conditions: a) O_2-hν, b) m-Cl-$C_6H_4CO_3H$, CH_2Cl_2, 0 °C, c) $KMnO_4$, acetate buffer pH 5.5.

reasonably confident that permanganate oxidation of **1** was resulting in formation of the thioxanthone sulfoxide **3.** This assumption was based upon known phenothiazine chemistry[9,10] and upon the pH profile for the oxidation reported by Mjörndal and Oreland, where optimal fluorescence was obtained between pH 5 and 8. At high pH side chain oxidation would be incomplete with permanganate, whereas at low pH random overoxidation could occur.

We confirmed this hypothesis by utilizing the thioxanthone **2** as a precursor for the quantitative generation of the sulfoxide **3** (meta-chloroperbenzoic acid, methylene chloride, 0 °C). The sulfoxide thus generated demonstrated a fluorescence spectrum (emission, 310 nm excitation) identical to that produced upon permanganate oxidation of **1.** We are currently investigating selective methods for the direct generation of **3** from **1** in organic extracts of plasma.

1 Acknowledgments. The authors wish to acknowledge the facilities and helpful suggestions provided by Drs R.N. Adams and G.L. Grunewald of the University of Kansas. This work was supported by UPHS grant GM 1341.

2 J.F. Muren and B.M. Bloom, J. med. Chem. *13*, 17 (1970).
3 N.M. Hasty and D.R. Kearns, J. Am. chem. Soc. *95*, 3880 (1973).
4 A.P. Schaap and G.R. Faler, J. Am. chem. Soc. *95*, 3381 (1973).
5 J.C. Dalton and F.C. Montgomery, J. Am. chem. Soc. *96*, 6230 (1974).
6 T. Iwaoka, H. Kokubun and M. Koizumi, Bull. chem. Soc. Japan *44*, 3466 (1971).
7 T. Mjörndal and L. Oreland, Acta pharmac. *29*, 295 (1971).
8 W.L. Pacha, Experientia *25*, 103 (1969).
9 T.J. Mellinger and C.E. Keeler, Analyt. Chem. *36*, 1840 (1964).
10 S.P. Massie, Chem. Rev. *54*, 797 (1954).

Inhibitory effect of tiaramide on ADP-induced aggregation in rabbit platelets[1]

S. Takano, T. Aikawa[2], T. Oishi[2], M. Goto[2], O. Tamura[2] and T. Suzuki

Department of Pharmacology, Fukushima Medical College, Fukushima 960 (Japan), 15 November 1977

Summary. Tiaramide in 10^{-4} or 10^{-5} M depressed the ADP-induced aggregation of rabbit platelets using the turbidimetric method. In modified Chandler's loop method, tiaramide in the same concentration accelerated the restoration of the time course of disaggregation.

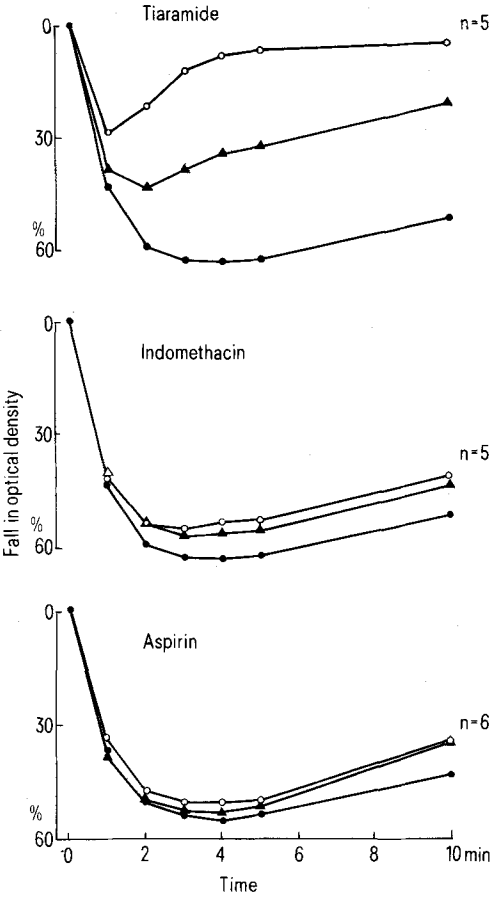
Many non-steroidal anti-inflammatory drugs inhibit the release of platelet constituents normally induced by collagen and thrombin[3,4], but do not inhibit the primary platelet aggregation induced by ADP. Tiaramide (4-[5-chloro-2-oxo-3-benzothiazolinyl-acetyl]-1-piperazine ethanol hydrochloride) was synthesized as a water-soluble analgesic and anti-inflammatory drug[5]. The present paper describes the inhibitory effect of tiaramide on ADP-induced aggregation of rabbit platelets and compares the potency among tiaramide, indomethacin and aspirin, using turbidimetric and modified Chandler's loop method[6].

Materials and methods. Blood from a sodium pentobarbital (30 mg/kg, i.m.) anaesthetized rabbit was extracted from the carotid artery into an injection-syringe containing 1/10 vol. of trisodium citrate (3.8% w/v). Platelet-rich plasma (PRP) was prepared by collecting the upper part of the supernatant at 140× g after 12 min centrifugation at 4 °C. Platelet concentration was adjusted at 60×10^4/cmm^3. After 20 min incubation with the anti-inflammatory drug, ADP was added to PRP and the aggregation was measured in an Evans aggregometer (37 °C). In the loop method, a 25-cm long polyvinyl tube with an inner diameter of 4 mm was used. PRP was mixed individually with the anti-inflammatory drug in the tube rotating at 16 rpm for 20 min on a turntable, with an angle of 23 degrees. After adding ADP, the aggregation and disaggregation was observed for 15 min at 25 °C.

Effects of anti-inflammatory drugs on disaggregation phase after addition of ADP using modified Chandler's loop method

Drugs (M)	5 min				10 min				15 min				No. of experiments
	N	S	A	P	N	S	A	P	N	S	A	P	
* 0.9% NaCl				5				5				5	5
Indomethacin 10^{-4}				5			1	4			1	4	5
Indomethacin 10^{-5}			1	4			2	3	2		1	2	5
Tiaramide 10^{-4}	2	2	1		5				5				5
Tiaramide 10^{-5}	1		2	2	3	1		1	4			1	5
** 0.9% NaCl			4	2			5	1			5	1	6
Aspirin 10^{-4}			2	4			4	2			4	2	6
Aspirin 10^{-5}			3	3			5	1		1	4	1	6

The arabic figures in the table indicate numbers of the state of aggregated platelets at every 5 min after ADP. Each state is represented according to M.J. Silver's classification as normal state (N), snow storm (S), aggregates (A), and plug (P). * and ** are different preparations.



Effects of tiaramide, indomethacin and aspirin on ADP-induced aggregation of rabbit platelets using Evans aggregometer. ADP (5×10^{-6} M) was added at 0 in time to the PRP already incubated for 20 min with the drug. The degree of aggregation was expressed as a percentage of the OD of PPP (platelet-poor plasma). Solid circles: 0.9% NaCl; open circle: the drug of 10^{-4} M; triangles: the drug of 10^{-5}M.

Results and discussion. ADP in 5×10^{-6} M caused more than 50% aggregation and disaggregation within 4 min using turbidimetric method. The figure shows the significant inhibitory effect of tiaramide on ADP-induced aggregation in comparison with those of indomethacin and aspirin. In control experiments, the maximal degree of aggregation was 63.2±6.11% (n=5, at 4 min after ADP), but incubation with 10^{-4} or 10^{-5} M of tiaramide decreased the maximal degree to 28.9±2.82% (n=5, $p<0.01$, at 1 min) or 43.0±5.96% (n=5, $p<0.05$, at 2 min) respectively, immediately followed by disaggregation. In the loop method, the PRP incubated with 0.9% NaCl produced snow storm or aggregates[6] at 33.2±2.53 or 41.1±3.53 sec respectively after ADP in 11 preparations. All 3 drugs did not influence the time taken to induce these phases. But when the courses of platelet dispersion after aggregation were compared, tiaramide in 10^{-4} M apparently accelerated restoration to normal state in all 5 PRP and tiaramide in 10^{-5} M in 4 PRP, although the control PRP still formed a plug at 15 min after ADP (table, the upper part). Indomethacin and aspirin did not produce a significant effect on the aggregation. The measurement of pH of PRP after aggregation-experiment demonstrated that there was no difference in pH between control and test PRP. All 3 drugs significantly inhibited collagen-induced aggregation as assayed by the turbidimetric method, and tiaramide was also most effective[7]. Both methods demonstrated that tiaramide was most active in inhibiting the ADP-induced aggregation of 3 drugs.

1 Acknowledgment. We are very grateful to Dr T. Takashima, Fujisawa Pharmaceutical Co. Ltd, for gifts of the pure tiaramide.

2 T. Aikawa, T. Oishi, M. Goto and O. Tamura are students of Fukushima Medical College.

3 J.R. O'Brien, Lancet *1*, 894 (1968).
4 G. Evans, M.A. Packham, E.E. Nishizawa, T.F. Mustard and E.A. Murphy, J. exp. Med. *128*, 877 (1968).
5 T. Takashima, Arzneimittel-Forsch. *22*, 711 (1972).
6 M.J. Silver, Am. J. Physiol. *218*, 384 (1970).
7 S. Takano, unpublished data.