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EFFECTS OF 6-[*p*-(4-PHENYLACETYLPIPERAZIN-1-YL)PHENYL]-4,5-DIHYDRO-3(2H)PYRIDAZINONE (CCI 17810) AND ASPIRIN ON PLATELET AGGREGATION AND ADHESIVENESS

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1 The effects of 6-[*p*-(4-phenylacetylpiperazin-1-yl)-phenyl]-4,5-dihydro-3(2H)pyridazinone (CCI 17810) on platelet aggregation and adhesiveness have been investigated and compared with those of aspirin.

2 *In vitro*, CCI 17810 was a potent inhibitor of the aggregation of human platelets induced by collagen, adenosine 5'-diphosphate (ADP) (primary response), thrombin and arachidonic acid, with EC_{50} values in the range 0.5 to 10 μg/ml. The second phase of the response to adrenaline was blocked by concentrations in the range 15 to 25 μg/ml. Platelets from rats, rabbits and dogs were as sensitive as human platelets to the effects of CCI 17810. Aspirin was nearly as effective as CCI 17810 against collagen, and adrenaline but about 10 times less active against arachidonic acid; it did not inhibit the primary response to ADP and was only a weak inhibitor of thrombin-induced aggregation.

3 In mice, single oral doses of CCI 17810 in the range 12.5 to 100 mg/kg inhibited collagen-induced thrombocytopenia. Arachidonic acid-induced mortality was markedly reduced by 10 mg/kg and possibly slightly reduced by 1 mg/kg. Aspirin was considerably less active than CCI 17810 in inhibiting collagen-induced thrombocytopenia but was almost as active as CCI 17810 in reducing arachidonic acid-induced mortality.

4 *In vitro*, CCI 17810 reduced the adhesiveness of human platelets to glass beads (retention of platelets in glass bead columns). Single oral doses of CCI 17810 in the range 25 to 200 mg/kg reduced mouse platelet adhesiveness; rat platelet adhesiveness was reduced by doses in the range 12.5 to 100 mg/kg. Aspirin (20 or 200 mg/kg) slightly increased mouse platelet adhesiveness.

Introduction

In the search for potential antithrombotic drugs we have tested a wide range of compounds for effects on platelet aggregation and adhesiveness. 6-[*p*-(4-Phenylacetylpiperazin-1-yl) phenyl] -4,5-dihydro-3(2H)pyridazinone (CCI 17810, Figure 1) is one of a series of piperazin-1-yl-phenyl pyridaziones with marked inhibitory activity (Lecker, Griffett, Kinnon, Kumar, Smith & Tomich, unpublished).

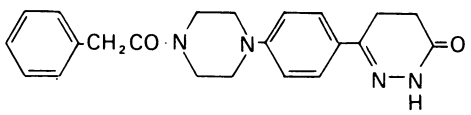

Figure 1 Structure of CCI 17810

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The aggregation of platelets by collagen, adenosine 5'-diphosphate (ADP), arachidonic acid, thrombin or adrenaline in the presence and absence of CCI 17810 was measured by a nephelometric technique (Born & Cross, 1963). Platelet-rich plasma (PRP) from several species was investigated.

Collagen- and arachidonic acid-induced platelet aggregation *in vivo* was studied by methods based on those of Nishizawa, Wynalda, Suydam, Sawa & Schultz (1972) and Kohler, Wooding & Ellenbogen (1976) respectively. An intravenous injection of either agent into mice results in the formation of platelet aggregates that lodge in the pulmonary circulation and cause respiratory distress. Nishizawa *et al.* (1972) showed that the platelet count was reduced by intravenous injections of collagen into mice but they used death as the indicator of platelet aggregation in their screen for platelet aggregation inhibitors. We used the percentage fall in platelet count 3 min after an intravenous injection of a non-lethal dose of collagen as our parameter and found the test eminently suitable for routine screening of new com-

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pounds for platelet inhibitory activity. The test is simple and rapid and requires only small amounts of test compound. We were less successful in detecting thrombocytopenia of a useful degree and duration in mice surviving arachidonic acid injections so for this agent we used mortality as our end-point.

Human and rat platelet adhesiveness (or, more correctly, platelet retention in glass bead columns) was measured by a conventional glass bead column technique (Hellem, 1960; Salzman, 1963). Insufficient blood to pass through columns was available from mice so we modified the method for use with small volumes of mouse blood. We were thus able to compare *ex vivo* effects on platelet adhesiveness and *in vivo* effects on platelet aggregation in the same species.

The effects of aspirin, a known inhibitor of platelet aggregation and possibly antithrombotic in man, were determined for comparison with those of CCI 17810.

Methods

Platelet aggregation in vitro

Blood was collected from ante-cubital veins of male or female human volunteers who had not ingested aspirin for a week; blood was also collected from the central ear arteries of female New Zealand White rabbits (3.5 to 4.5 kg body weight) or jugular veins of male or female beagle dogs (8 to 14 kg); anaesthetized male Sprague Dawley rats (CFY; 300 to 400 g) were exsanguinated by direct cardiac puncture. The blood was prevented from coagulating with 1 volume of 3.8% w/v tri-sodium citrate dihydrate to 9 volumes of blood. PRPs were prepared by differential centrifugation at room temperature. Human and rabbit blood was spun at 600 *g* for 6 or 4 min respectively. Dog blood was spun at 400 *g* for two periods of 5 min and at 900 *g* for 5 min; platelets and plasma were removed after each spin and the three were combined. Rat blood was spun at 600 *g* for 5 min and 900 *g* for 5 min; the PRP was removed after completion of both spins and then diluted with platelet-poor plasma obtained by further centrifugation of the blood at 1400 *g* for 10 min. The platelet counts ($\times 10^3$/mm^3) in the PRPs used for aggregation studies were in the ranges 210 to 480 for man, 450 to 600 for rabbits, 220 to 560 for dogs and 930 to 1030 (after dilution) for rats.

Platelet aggregation was studied by a nephelometric technique (Born & Cross, 1963) in a 4-channel aggregometer (E.G. Tomich) or in single-channel aggregometers constructed from EEL colorimeters; optical density changes were monitored on Servoscribe recorders. For most experiments PRP-saline-inhibitor mixtures (0.9 ml PRP plus 0.1 ml saline/inhibitor) were incubated in a 37°C water bath for 2 min and then in the aggregometer (again at 37°C), with stirring, for 1 min before the aggregating agent was added. For a few experiments the incubation time was increased to 30 min. Collagen was used in amounts (0.5 to 4 μg/ml for human PRP or 2 to 20 μg/ml for rabbit or dog PRP) sufficient to induce a response that was 80 to 90% of the maximum obtainable. Arachidonic acid was used at a final concentration of 1 mM for human PRP and 0.2 mM for rabbit PRP. A single-phase readily reversible response was induced in human PRP by 0.5 to 2 μM ADP and in rat PRP by 1 to 4 μM ADP. Thrombin induced a single-phase reversible response in human PRP at a final concentration of 0.2 to 0.4 u/ml; clotting occurred with higher concentrations. In human PRP adrenaline induced a two-phase response at a final concentration of 10 to 100 μM. Platelet aggregation was not inhibited by 0.025% Tween 80 in saline or by 1% $NaHCO_3$. Activities of inhibitors are expressed as EC_{50} values i.e. concentrations required to inhibit the aggregation responses (maximum decrease in optical density) by 50%. Values were determined graphically from log concentration versus % inhibition plots for 3 to 5 concentrations of inhibitor. EC_{50} values were difficult to obtain for the second phase of the response to adrenaline because inhibition was usually all-or-none; hence activities against this aggregating agent are expressed as the minimum concentration required to abolish the response.

Platelet aggregation in vivo

Collagen-induced thrombocytopenia in mice Platelet aggregation *in vivo* was induced by intravenous injections of collagen (Hormon-Chemie) into male CRH mice weighing 30 to 35 g. Blood for platelet counts (Williamson, 1967) was obtained by cardiac puncture under ether anaesthesia and dipotassium ethylenediamine-tetraacetic acid (K_2EDTA) added to prevent coagulation. Preliminary investigations showed that the thrombocytopenic response was dose-related (Figure 2) and lasted for about 20 min (Figure 3). The response 1 min after the injection was greater than that at 3 min but the latter was chosen for inhibition studies because it was not always possible to obtain a blood sample exactly 1 min after the collagen injection. For determination of inhibitory activity, test compounds or vehicle (10% gum acacia) were administered orally to fasted mice in a dose-volume of 0.1 ml/10 g 1.5 h (or more for time-response experiments) before an intravenous injection of collagen, 1 mg/kg. The percentage fall in platelet count induced by collagen was calculated by reference to the platelet count in blood samples from untreated mice.

Arachidonic acid-induced mortality in mice Arachidonic acid was administered intravenously in a dose

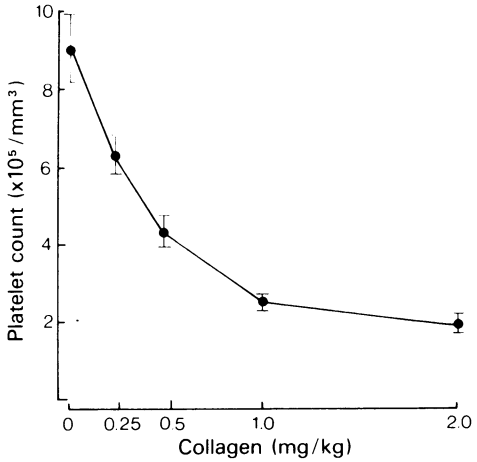

Figure 2 Platelet counts 3 min after single intravenous injections of collagen to mice. Each point is the mean for 8 mice; vertical lines show s.e.mean.

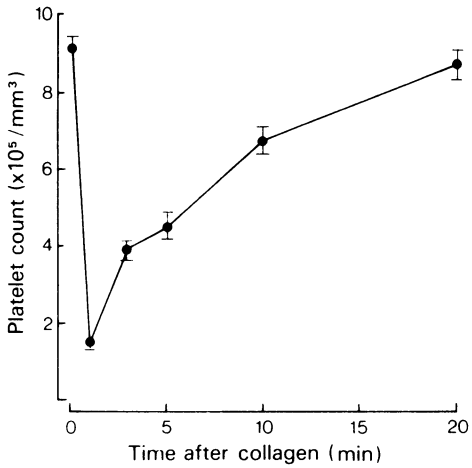

Figure 3 Platelet counts at various times after single intravenous injections of collagen (1 mg/kg) to mice. Each point is the mean for 8 mice; vertical lines show s.e. mean.

volume of 0.1 ml/10 g body weight. Preliminary experiments showed that the percentage mortality was dose-related over the range 20 to 60 mg/kg and that a dose of 60 mg/kg killed 90 to 100% of the mice within 2 to 3 min. The cause of death appeared to be respiratory arrest; non-lethal doses induced respiratory distress for up to 10 min. For determination of inhibitory activity, test compounds or vehicle were administered orally to groups of 10 fasted mice in a dose volume of 0.1 ml/10 g 1.5 or 18 h before an intravenous injection of arachidonic acid, 60 mg/kg. Per cent mortalities in each group were noted.

Platelet adhesiveness in vitro *and* ex vivo

Platelet adhesiveness in human and rat blood was determined by a glass bead column technique, and in mouse blood by a modification that involved mixing blood and beads in a polystyrene vial. Human volunteers (male or female) were bled from an antecubital vein, and anaesthetized rats (male CFY weighing 200 to 300 g for *in vitro* experiments and 120 to 150 g for *ex vivo* experiments) and mice (male CRH weighing 30 to 35 g) were exsanguinated by direct cardiac puncture. For humans and rats, native blood in a 2 ml disposable syringe was forced mechanically up a vertical polythene tube containing 1.5 g of 0.5 mm diameter glass beads and the blood was expelled at constant speed into a polystyrene vial containing 2 mg K_2EDTA. The contact time was 21 to 23 s and the collection time (for 0.6 ml blood) 30 s. A control sample of blood was merely anticoagulated with K_2EDTA. Platelet counts were determined electronically (Williamson, 1967) on the blood before and after passage through the column; the difference, expressed as a percentage of the first count, was termed the adhesiveness. For the mouse platelet adhesiveness test, 0.5 ml of blood was transferred to a polystyrene vial containing 0.1 g of glass beads and gently mixed under standardized conditions for 1 min, when the blood was immediately decanted into a second vial containing 2 mg K_2EDTA. Platelet counts were determined on this blood and on a sample that had been added directly to K_2EDTA. The difference between the counts, expressed as a percentage of the latter, was termed the adhesiveness.

For *in vitro* experiments on human blood, test compounds were added to the blood 3 min before it was passed through the column of beads. For *ex vivo* experiments, fasted rats or mice were dosed orally with test compound or vehicle in dose volumes of 0.2 ml/100 g (rats) or 0.1 ml/10 g (mice) 1.5 h (or more for time-response experiments) before the test.

Chemicals

For inducing platelet aggregation *in vitro* adenosine-5'-diphosphate (ADP, sodium salt, Sigma), adrenaline bitartrate (Calbiochem), and topical thrombin (Parke Davis) were used as solutions in Tris-buffered saline (pH 7.4) and arachidonic acid (Sigma, 99% pure) was dissolved in ethanol; Collagenreagent 'Horm' (Hormon-Chemie, München) was used undiluted for rabbit and dog PRP but was diluted with buffer provided (pH 2.8) for human PRP. Aggregating agents were used in volumes of 1 to 20 μl (not more than 5 μl for arachidonic acid) added to 1.0 ml of PRP-inhibitor mixture. For inducing platelet aggregation *in vivo*, Collagenreagent 'Horm' was diluted 1 to 10 with saline: arachidonic acid was dis-

solved in ethanol and the solution (60 mg/ml) was diluted to the required concentration with 100 mM sodium carbonate. For *in vitro* experiments CCI 17810 (micronized batch EPKB 7/4) was suspended in 0.5% v/v Tween 80 and diluted with saline (0.9% w/v NaCl solution) to give a 0.5 mg/ml suspension in 0.025% Tween 80/saline. For *in vivo* and *ex vivo* experiments it was administered orally as a suspension in 10% w/v aqueous gum acacia. Aspirin (0.5 or 2 mg/ml) was dissolved in 1% w/v $NaHCO_3$ for *in vitro* experiments and suspended in 10% aqueous gum acacia for oral administration.

Results

Platelet aggregation in vitro

The effects of CCI 17810 on the aggregation of human platelets by collagen, ADP, thrombin, arachidonic acid and adrenaline are illustrated in Figure 4. Mean EC_{50} values for several species and aggregating agents are in Tables 1 and 2; the values for aspirin are included for comparison. CCI 17810 had similar activities against collagen, ADP (1st phase), arachidonic acid and thrombin, but was somewhat less active against the 2nd phase of the response to adrenaline: species differences were negligible. In some experiments with human PRP the effects of incubating the PRP with CCI 17810 for the usual 3 min before addition of collagen were compared with the effects after 30 min incubation. The effects after 30 min (EC_{50} value = 2.2 ± 1.0) were slightly greater than after 3 min (EC_{50} value = 4.4 ± 2.3) but the difference was not significant (paired *t* test on 4 observations).

Aspirin was nearly as active as CCI 17810 against collagen and adrenaline (2nd phase) but was about 10 times less active than CCI 17810 against arachidonic acid; it did not inhibit the 1st phase of the response to ADP and gave very variable results with thrombin. The effects of thrombin were never inhibited by more than about 40%, even with concentrations as high as 500 μg/ml.

Platelet aggregation in vivo

Collagen-induced aggregation in mice The inhibitory effects on collagen-induced thrombocytopenia of various doses of CCI 17810 administered orally to mice 1.5 h before the test are shown in Table 3. The

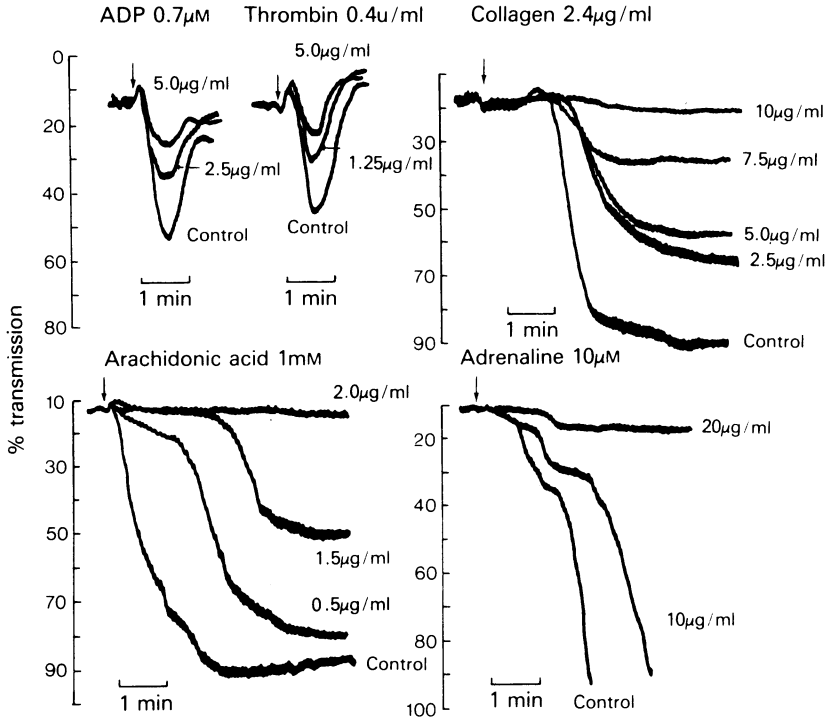

Figure 4 Inhibition of human platelet aggregation by CCI 17810. CCI 17810 (0.5 mg/ml in 0.025% Tween 80/saline) was incubated with 0.9 ml PRP at 37°C for 3 min before the aggregating agent was added. The volume of the PRP-inhibitor mixture was adjusted to 1.0 ml with saline. Concentrations of aggregating agents and of CCI 17810 are final concentrations in PRP. A downward deflection of the pen indicates platelet aggregation; tracings have been superimposed.

Table 1 Inhibition of human platelet aggregation by CCI 17810 and aspirin

Aggregating agent	*EC_{50} value μg/ml PRP*	
	CCI 17810	*Aspirin*
Collagen	4.0 ± 1.3 (7)	10.1 ± 3.4 (5)
ADP (1st phase)	5.6 ± 0.7 (5)	inactive at 500
Arachidonic acid	1.3 ± 0.6 (5)	15.9 ± 3.4
Thrombin	2.9 ± 1.0 (6)	EC_{50} not obtainable, max. inhib. about 40%
Adrenaline (2nd phase)*	15–25 (4)	20–50 (4)

EC_{50} values (mean ± s.e) were determined after 3 min incubation of test compound with PRP at 37°C; (*n*) = number of experiments.
*For adrenaline, activities are minimum concentrations required to abolish the 2nd phase of the response.

Table 2 Inhibition of aggregation of platelets from several species by CCI 17810 and aspirin

Aggregating Agent	*Species*	*EC_{50} value μg/ml PRP*	
		CCI 17810	*Aspirin*
Collagen	Man	4.0 ± 1.3 (7)	10.1 ± 3.4 (5)
	Dog	2.6 ± 0.7 (4)	13.4 ± 2.1 (4)
	Rabbit	1.4 ± 0.4 (3)	4.5 ± 0.4 (3)
ADP (1st phase)	Man	5.6 ± 0.7 (5)	inactive at 500 (3)
	Dog	3.8 ± 0.9 (4)	inactive at 500 (3)
	Rat	7.6 ± 1.3 (6)	inactive at 500 (3)
Arachidonic acid	Man	1.3 ± 0.6 (5)	15.9 ± 3.4 (6)
	Rabbit	4.5 ± 1.7 (4)	6.1 ± 1.3 (3)

EC_{50} (mean ± s.e.) were determined after 3 min incubation of test compound with PRP at 37°C; (*n*) = number of experiments.

Table 3 Effects of single oral doses of CCI 17810 on collagen-induced thrombocytopenia in mice

CCI 17810 (mg/kg)	*Collagen* (mg/kg)	*Experiment 1*		*Experiment 2*		*Mean % reduction in PC*	*% reduction of control response**
		PC	*% reduction in PC*	*PC*	*% reduction in PC*		
0	0	838 ± 38		899 ± 37			
0	1	371 ± 37	56	353 ± 17	61	59	
12.5	1	603 ± 46	28	524 ± 24	42	35	41
25	1	557 ± 47	34	547 ± 20	39	37	37
50	1	640 ± 48	24	645 ± 25	28	26	56
100	1	734 ± 33	12	670 ± 34	25	19	68

CCI 17810 was administered orally 1.5 h before the test. Collagen was injected i.v. 3 min before the mice were bled.
PC = mean platelet count ($\times 10^3/mm^3$) ± s.e. for groups of 4 mice.
* Control response = thrombocytopenic response to collagen in control mice, i.e. 59%.

effects were dose-related over the range 25 to 100 mg/kg but 12.5 mg/kg appeared to be as active as 25 mg/kg. Time-response relationships for 25 and 100 mg/kg are shown in Figure 5; the peak effect for each dose was at 1.5 h. Thereafter the effect of the lower dose diminished steadily but was still just significant 18 h after dosing; with 100 mg/kg inhibition at 18 h was greater (though not significantly so) than at 3 or 6 h (cf. also effects on platelet adhesiveness).

Aspirin was considerably less active than CCI 17810 in reducing collagen-induced thrombocytopenia in mice and the results were less reproducible

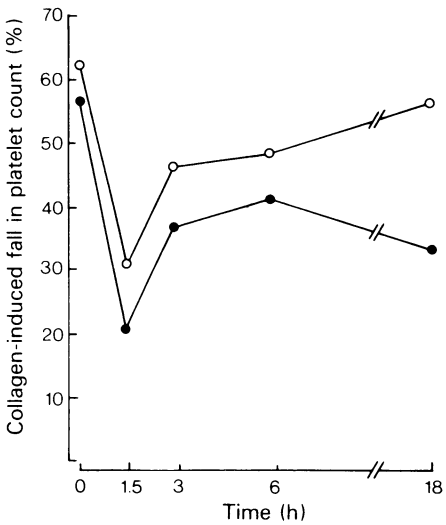

Figure 5 Effects of single oral doses of CCI 17810 on collagen-induced thrombocytopenia in mice: (○) 25 mg/kg; (●) 100 mg/kg. Blood samples for platelet counts were taken 3 min after an intravenous injection of collagen (1 mg/kg). Values were calculated from the results of 2 (100 mg/kg) or 3 (25 mg/kg) experiments each with 4 mice per group (see Table 3 for method of calculation).

(Table 4); i.e., it was totally inactive in experiment 1 but had slight to moderate inhibitory effects in experiments 2 and 3.

Arachidonic acid-induced mortality in mice Preliminary experiments using only a few mice per group suggested that CCI 17810 and aspirin were potent inhibitors of arachidonic acid-induced mortality in mice. The results of experiments with low doses (10 or 1 mg/kg) of CCI 17810 and aspirin administered to groups of 10 mice 1.5 or 18 h before an intravenous injection of arachidonic acid (60 mg/kg) are given in Table 5. At 10 mg/kg orally both compounds reduced mortality at 1.5 h, but not at 18 h after dosing; at 1.5 h, 1 mg/kg of CCI 17810 was marginally active but aspirin was inactive. Mice that lived suffered a short period of respiratory distress.

Platelet adhesiveness in vitro

CCI 17810 and aspirin were tested *in vitro* at a final concentration of 200 μg/ml for effects on human platelet adhesiveness. CCI 17810 reduced adhesiveness by 35 ± 5.0% (mean ± s.e. for 6 experiments). Aspirin was inactive in 5 experiments and increased adhesiveness by 40% in a 6th experiment.

Platelet adhesiveness ex vivo

Effects in mice Single oral doses of CCI 17810 (25, 50, 100 or 200 mg/kg) administered to mice 1.5 h before the test reduced platelet adhesiveness in a dose-related manner (Table 6); platelet counts were not affected. The effects of adhesiveness of the 10 mg/kg dose were maximal at 1.5 h and were still significant at 3, 6 and 18 h (Figure 6). In fact, as in the aggregation test, 18 h effects were somewhat more pronounced than those at 6 h. Aspirin (20 or 200 mg/kg) increased adhesiveness slightly but not significantly (Table 7).

Table 4 Effects of single oral doses of aspirin on collagen-induced thrombocytopenia in mice

		Experiment 1		*Experiment 2*		*Experiment 3*			
Aspirin (mg/kg)	*Collagen* (mg/kg)	*PC*	*% reduction in PC*	*PC*	*% reduction in PC*	*PC*	*% reduction in PC*	*Mean % reduction in PC*	*% reduction of control response**
0	0	991 ± 57		913 ± 67		886 ± 42			
0	1	403 ± 59	59	356 ± 70	61	367 ± 23	59	60	
5	1	339 ± 21	66	396 ± 21	57	398 ± 38	55	59	2
20	1	363 ± 43	63	397 ± 43	57	474 ± 27	47	56	7
80	1	393 ± 41	60	461 ± 17	50	455 ± 24	49	53	12
320	1	328 ± 17	67	490 ± 41	46	484 ± 17	45	53	12

Aspirin was administered orally 1.5 h before the test. Collagen was injected i.v. 3 min before the mice were bled. PC = mean platelet count ($\times 10^3/mm^3$) ± s.e for groups of 4 mice.

* Control response = thrombocytopenic response to collagen in control mice, i.e. 60%.

Table 5 Effects of single oral doses of CI 17810 or aspirin on arachidonic acid-induced mortality in mice

Experiment	*Treatment* *Compound*	*Dose* (mg/kg)	*Time (h) before test*	*Arachidonic acid induced mortality* (%)
	Vehicle			90
1	CCI 17810	10	1.5	0
	Aspirin	10	1.5	20
	Vehicle			100
2	CCI 17810	1	1.5	70
	Aspirin	1	1.5	100
	Vehicle			100
3	CCI 17810	10	18	100
	Aspirin	10	18	100

Groups of 10 mice were injected intravenously with arachidonic acid 60 mg/kg.

Table 6 Effects of single oral doses of CCI 17810 on mouse platelet adhesiveness

CCI 17810 (mg/kg)	*Experiment 1* *PC*	*P.Ad.*	*% change in P.Ad.*	*Experiment 2* *PC*	*P.Ad.*	*% change in P.Ad.*	*Mean % change in P.Ad.*
0	993 ± 70	52 ± 6.1		850 ± 30	52 ± 5.0		
25	930 ± 66	41 ± 2.6	−21	868 ± 51	43 ± 5.0	−17	−19
50	933 ± 68	33 ± 1.1	−37	911 ± 15	45 ± 4.0	−13	−25
100	984 ± 100	35 ± 5.5	−33	877 ± 19	28 ± 2.6	−46	−40
200	986 ± 64	17 ± 3.7	−67	961 ± 59	24 ± 4.3	−54	−61

CCI 17810 was administered orally 1.5 h before the test. PC = mean platelet count ($\times 10^3$/mm^3) ± s.e. for groups of 4 mice. P.Ad. = % adhesive platelets; mean ± s.e.

Table 7 Effects of single oral doses of aspirin on mouse platelet adhesiveness

Aspirin (mg/kg)	*Experiment 1* *PC*	*P.Ad.*	*% change in P.Ad.*	*Experiment 2* *PC*	*P.Ad.*	*% change in P.Ad.*	*Mean % change in P.Ad.*
0	863 ± 69	43 ± 3.8		1007 ± 37	53 ± 5.1		
20	1055 ± 21	52 ± 1.7	+21	1009 ± 54	57 ± 2.7	+ 8	+15
200	941 ± 48	50 ± 5.8	+16	946 ± 84	63 ± 5.0	+19	+18

Aspirin was administered orally 1.5 h before the test. PC = mean platelet count ($\times 10^3$/mm^3) ± s.e. for groups of 4 mice. P.Ad. = % adhesive platelets; mean ± s.e.

Table 8 Effects of single oral doses of CCI 17810 on rat platelet adhesiveness

CCI 17810 (mg/kg)	*Experiment 1* *n*	*PC*	*P.Ad.*	*% change in P.Ad.*	*Experiment 2* *n*	*PC*	*P.Ad.*	*% change in P.Ad.*	*Mean % change in P.Ad.*
0	5	720 ± 28	77 ± 3.8		6	717 ± 8	84 ± 0.6		
12.5	5	754 ± 17	68 ± 7.0	−12	5	715 ± 20	49 ± 2.5	−42	−27
50	4	772 ± 12	51 ± 5.2	−34	6	693 ± 26	44 ± 1.4	−51	−43
200					5	698 ± 21	26 ± 5.5	−69	−69

CCI 17810 was administered orally 1.5 h before the test. *n* = number of rats per group. PC = mean platelet count ($\times 10^3$/mm^3) ± s.e. P.Ad. = % adhesive platelets; mean ± s.e.

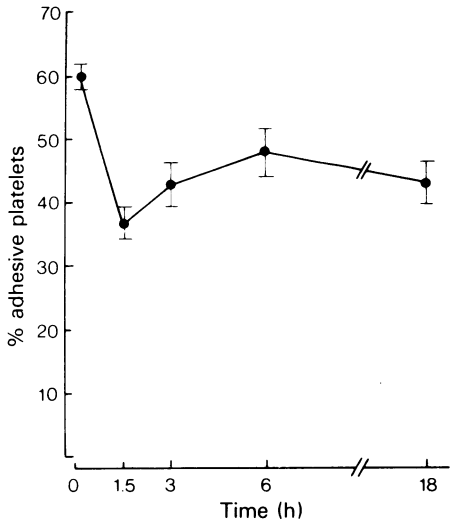

Figure 6 Effects of a single oral dose of CCI 17810 (100 mg/kg) on mouse platelet adhesiveness. Each point is the mean for 8 mice; vertical lines show s.e.mean.

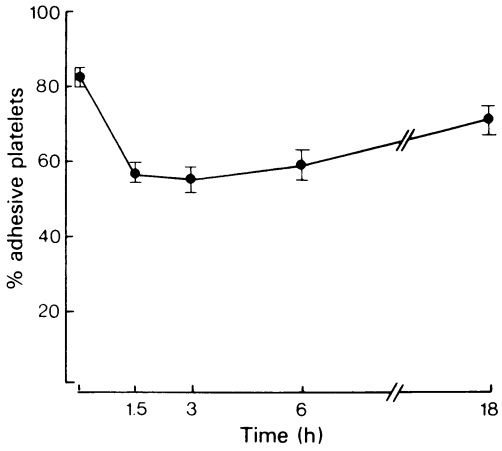

Figure 7 Effects of a single oral dose of CCI 17810 (50 mg/kg) on rat platelet adhesiveness. Each point is the mean for 8 rats; vertical lines show s.e. mean.

Effects in rats Single oral doses of CCI 17810 (12.5, 50 or 200 mg/kg) reduced platelet adhesiveness in a dose-related manner (Table 8). The effects of 50 mg/kg at various times after dosing are shown in Figure 7: the reductions in adhesiveness were similar 1.5, 3 and 6 h after dosing and were less, but still significant, 18 h after dosing.

Discussion

CCI 17810 is a potent inhibitor of platelet aggregation induced by collagen, ADP (1st phase), arachidonic acid, adrenaline (2nd phase) and thrombin. This wide spectrum of activity, particularly the inhibition of ADP-induced aggregation, suggests that CCI 17810 is not aspirin-like in action. Aspirin inhibits the release of ADP (Ball, Fulwood, Ireland & Yates, 1969) but not the effects of ADP *per se*. Its mechanism of action is believed to involve inhibition of cyclo-oxygenase, (Smith & Willis, 1971) the enzyme that converts arachidonic acid to the prostaglandin endoperoxide precursors of thromboxane A_2. The release of arachidonic acid from phospholipids, a process catalysed by phospholipase A_2, is stimulated by many aggregating agents including collagen, ADP and high concentrations of adrenaline and thrombin. However, the latter agent can act independently of the arachidonic acid pathway as shown by the weak effect of aspirin on primary aggregation induced by thrombin (O'Brien, 1968; cf. also Table 1 this publication). Also, the fact that thrombin-induced aggregation can occur in the presence of the ADP-inactivating system, creatine phosphate-creatine phosphokinase, suggests that it is not dependent on released ADP (Packham, Kinlough-Rathbone, Reimers, Scott & Mustard, 1977). If, as proposed by Hornstra (1979) the aggregation response occurring upon platelet-blood vessel wall interaction is for the greater part mediated by thrombin, then possession of inhibitory activity against thrombin-induced aggregation would be particularly important in a potential anti-thrombotic drug.

The reduction of platelet adhesiveness in glass bead systems by CCI 17810 but not by aspirin, accords with the results in the ADP-induced aggregation test. ADP released from red cells is believed to initiate platelet retention (adhesiveness plus aggregation) in bead columns (Hellem, 1960; Gaarder, Jonsen, Laland, Hellem & Owren, 1961) and presumably when blood and beads are mixed together as in our mouse test. It is possible that CCI 17810 reduces adhesiveness by stabilizing red cell membranes but a more likely explanation is that it inhibits the effects of the released ADP. Since the adhesiveness test was carried out in the absence of anticoagulants, it was possible that in experiments where CCI 17810 and blood were incubated together before the mixture was passed through columns of beads, thrombin might have been formed before completion of the test: inhibition of thrombin-induced aggregation might well have been implicated. Such an explanation is less likely in the *ex vivo* experiments where blood and beads were mixed immediately after cardiac puncture. CCI 17810 does not prolong the clotting time of blood (personal observation).

The *in vivo* and *ex vivo* effects of CCI 17810 indicate that the compound is orally absorbed and that it has a reasonable duration of action. Collagen and arachidonic acid aggregate platelets *in vivo* and

like aggregation induced *in vitro*, such aggregation is markedly inhibited by CCI 17810. The weak effects of aspirin against collagen-induced aggregation *in vivo* are a little surprising since *in vitro* it is nearly as active as CCI 17810. However, factors other than cyclo-oxygenase activation are involved in collagen-induced aggregation, particularly *in vivo*. For example, the activation of clotting factors (e.g. Factor XI on the platelet membrane) by collagen, and hence the involvement of thrombin, must assume more importance *in vivo* than *in vitro* as anticoagulant is present in the latter system. Compared to CCI 17810, aspirin is a poor inhibitor of thrombin-induced platelet aggregation.

The time-course for inhibition of collagen-induced thrombocytopenia (Figure 5) and reduction of platelet adhesiveness (Figure 6) by a high dose (100 mg/kg) of CCI 17810 administered to mice is difficult to interpret without knowledge of blood levels and metabolism. Perhaps sufficient quantities of an inhibitory metabolite are formed after high doses but not low doses of CCI 17810. Enhancement of the effect at later times is not observed in rats.

The role of platelets in thrombogenesis and the antithrombotic properties of platelet inhibitory drugs have been reviewed recently (Blakely, 1978; Claggett & Collins, 1978; Roden & Kendall, 1978; Tsu, 1978). A platelet inhibitory compound with the wide spectrum of activities described for CCI 17810 might well be expected to have antithrombotic properties. Its effects on experimental arterial thrombosis in rats have been determined (Lecker & Kumar, 1980).

E.G. Tomich died February 4th, 1980.

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