

Ig E-mediated immediate-type hypersensitivity to the pyrazolone drug propyphenazone

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Background: Propyphenazone (1,2-dihydro-1,5-dimethyl-4-(1-methylethyl)-2-phenyl-3H-pyrazol-3-one; PP) is a nonsteroidal anti-inflammatory drug frequently used as mild analgesic medicament. It belongs to the chemical group of pyrazolones. Severe adverse reactions to PP are frequent and have generally been regarded as pseudoallergic or intolerance reactions. Presently, there are no useful in vitro test systems available for the detection of antibodies directed against analgesic drugs. **Objective:** The purpose of this study was to unequivocally demonstrate that IgE-mediated Type I allergy is the main mechanism leading to immediate-type adverse reactions to the analgesic drug PP.

Methods: We investigated 53 young adult patients with adverse reactions to PP. All patients developed symptoms suggestive of IgE-mediated anaphylaxis within 30 minutes after intake of a painkiller containing PP. Patients were subjected to skin tests (prick test and intracutaneous test). In addition, a novel ELISA system was developed to prove the existence of specific IgE antibodies in patients' sera.

Results: In 44 of 53 (83%) patients, skin tests showed typical wheal and flare reactions. Significant amounts of PP-specific serum IgE was detected in 31 of 53 (58%) of the serum samples. Moreover, in 7 of 9 patients with skin test negative results, PP-specific IgE could be detected. The assay was PP-specific because only PP, but no other pyrazolone derivative (antipyrine, aminophenazone, or metamizol), was able to inhibit IgE-binding in the system.

Conclusion: Propyphenazone is a sensitizing agent in susceptible individuals and can elicit IgE-mediated anaphylaxis. By using skin tests and our ELISA system we were able to confirm Type I allergy in 51 of 53 (96%) patients in this study. (J Allergy Clin Immunol 2003;111:882-8.)

Key words: Adverse drug reaction, drug allergy, propyphenazone, IgE, anaphylaxis, ELISA

Abbreviations used

EDC:	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
HSA:	Human serum albumin
ICT:	Intracutaneous test
MALDI-TOF:	Matrix-assisted laser desorption/ionization-time of flight
NSAID:	Nonsteroidal anti-inflammatory drug
PP:	Propyphenazone
sNHS:	Sulfo-N-hydroxysuccinimide
SPT:	Skin prick test

Analgesic drugs can elicit immunologically mediated hypersensitivity reactions (allergy) of practically every type. On the other hand, nonsteroidal anti-inflammatory drugs (NSAIDs) can interfere with different pathways in the metabolism of inflammatory mediators (eg, blockade of cyclooxygenase), leading to pseudoallergic reactions in genetically predisposed individuals, especially asthmatic subjects.¹⁻⁴ In many cases skin tests are not useful to identify the mechanism of the reaction, and in vitro tests are often not available or not reliable.⁵ The diagnosis of drug allergy is frequently based on a characteristic case history and, if necessary and justified, provocation tests.

Pyrazolone derivatives, including antipyrine (phenazone), 4-aminopyrine (aminophenazone), metamizol (novalgin), and 4-isopropylpyrine (propyphenazone; PP; Fig 1, A) are analgesic substances known for a long time. PP is a typical representative of the so-called mild analgesics that are taken on demand. It is mainly used in combination with other NSAIDs in tablets, which can be obtained without prescription in many countries. PP is mentioned as elicitor of allergic/pseudoallergic reactions in several textbooks and in epidemiologic studies. Moreover, the drug has been described in casuistic reports to induce different types of allergic reactions.⁶⁻¹¹ However, there are no data available on the nature of the underlying immunologic mechanisms. The aim of this study was to investigate PP-induced adverse reactions. We have developed a novel and specific in vitro system to detect allergen-specific IgE antibodies in sera of patients with PP allergy. Our results show that PP is a relevant sensitizer and induces specific IgE antibodies. We conclude that adverse reactions to PP are frequently Type I allergic reactions.

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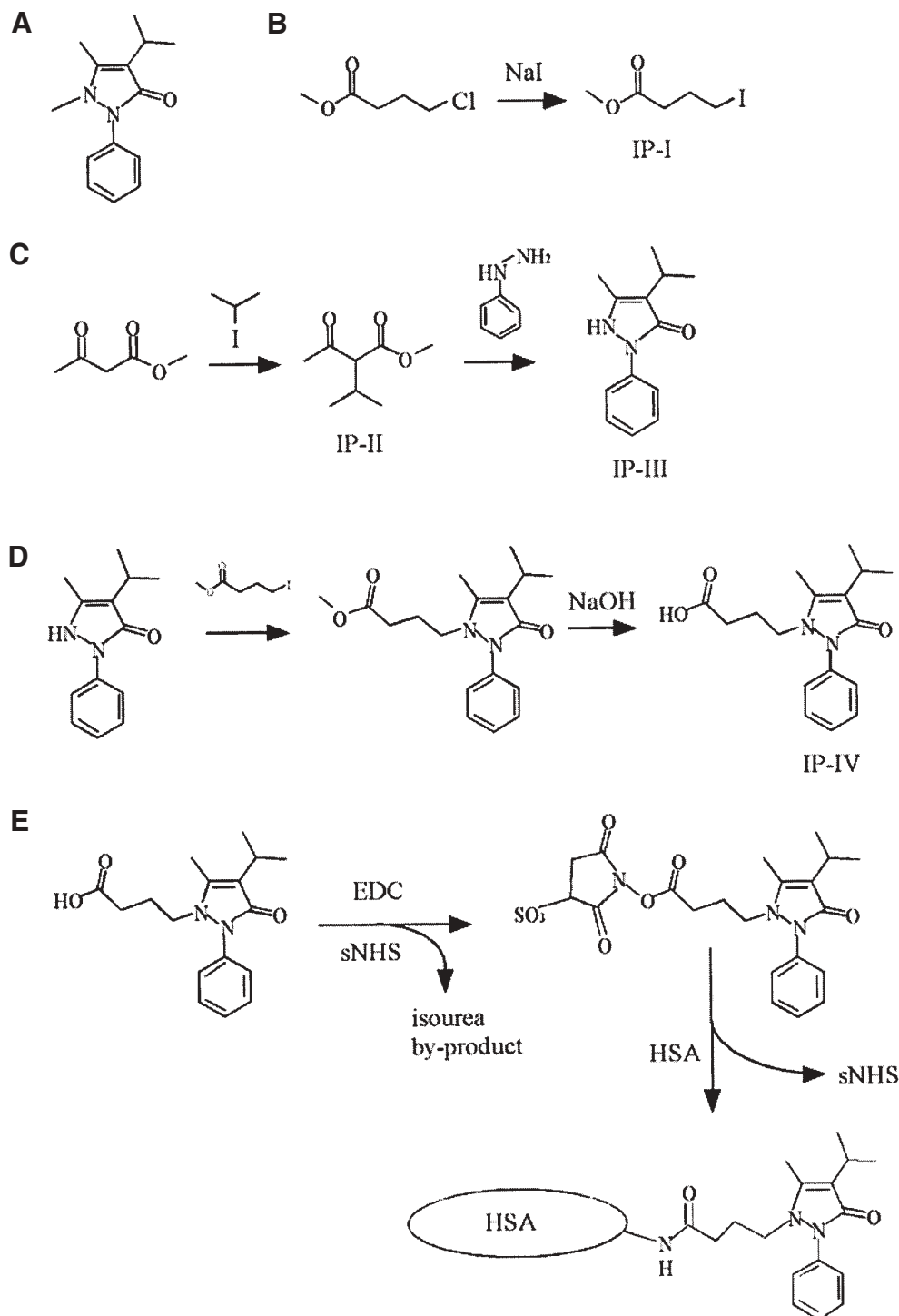


FIG 1. Chemical structure of PP (**A**) organochemical reaction scheme for the preparation of the PP-HSA conjugate (**B** through **E**). Synthesis of the spacer molecule (**B**) and the core of PP (**C**). Chemical linkage of the spacer to the core of PP and hydrolysis resulting in PP derivative (**D**) and chemical coupling of the PP derivative to HSA (**E**).

METHODS

Patients

Fifty-three patients (33 women and 20 men) were enrolled in the study. Their mean age was 31.3 years. The patients took an anal-

gesic tablet containing PP on demand for headache, toothache, or pain associated with menstruation. The dose of PP was between 150 and 300 mg per tablet. In all cases the medicaments contained other substances in addition (Table I). None of the patients had any accompanying medication except contraceptives. In all cases the

TABLE I. Clinical and diagnostic characterization of studied patients

Patient	Age	Sex	Drug comp.	Onset*	Clinical symptoms	Interval†	SPT	ICT	ELISA‡	IgE (kU/L)
1	44	F	PP,P,C	30 min	OAS/prur/urt/ang/tachy	4 mo	Pos	—	Neg	132
2	16	F	PP,P, E	3 min	OAS/prur/urt/ang/vom	7 days	Pos	—	Neg	229
3	34	M	PP,P,C,E	10 min	Prur/urt/ang/asth/shock	1 mo	Pos	—	Pos	1004
4	27	M	PP,P, C	30 min	Urt/ang/shock	2 wk	Neg	Neg	Neg	91
5	38	M	PP,P, C	30 min	OAS/urt/ang/asth	2 wk	Pos	—	Neg	87
6	44	M	PP,P, C	5 min	Prur/shock	1 wk	Pos	—	Pos	1757
7	21	F	PP,P,C,E	20 min	Prur/ang/asth	1 wk	Neg	Pos	Pos	1045
8	41	F	PP,P, C	2 min	OAS/urt/ang/asth/ shock	10 days	Pos	—	Pos	87
9	25	F	PP,P, C	30 min	Prur/urt/ang	2 y	Neg	Pos	Pos	1818
10	28	F	PP,P, C	20 min	Prur	2 y	Neg	Pos	Neg	22
11	39	F	PP,P, C	10 min	Prur/urt/asth	3 wk	Pos	—	Neg	38
12	36	M	PP,P, Co	30 min	OAS/prur/tachy/asth	4 mo	Neg	Neg	Pos	98
13	24	F	PP,P, Co	10 min	Prur/urt/asth	1 mo	Neg	Pos	Pos	32
14	22	F	PP,P, C	15 min	Prur/urt/asth/shock	4 mo	Neg	Neg	Pos	189
15	33	F	PP,P,C,E	30 min	Prur/ang/shock	4 wk	Pos	—	Neg	299
16	63	F	PP,P, E	15 min	Prur/ang/shock	1 y	Neg	Pos	Pos	357
17	24	M	PP,P, E	10 min	OAS/prur/ang	8 mo	Neg	Pos	Pos	51
18	32	F	PP,P, C	30 min	Urt/ang/asth/shock	3 y	Neg	Neg	Neg	72
19	33	M	PP,P, C	20 min	Prur/tachy	2 y	Pos	—	Pos	171
20	39	F	PP,P, C	30 min	Vom/asthma/diarrhea	7 y	Neg	Pos	Neg	12
21	42	M	PP,P, C	10 min	OAS/asth	10 y	Neg	Pos	Neg	79
22	37	M	PP,P,C,E	10 min	OAS/asth	6 wk	Neg	Pos	Neg	31
23	45	F	PP,P, C	10 min	Prur/urt/cramps	15 y	Neg	Pos	Neg	40
24	37	F	PP,P, C	10 min	Prur/urt/ang/tachy/ shock	3 mo	Neg	Neg	Pos	149
25	32	M	PP,P, E	10 min	Prur/ang/asth/tachy	3 wk	Pos	—	Pos	24
26	25	F	PP,P, C	5 min	Prur/urt/ang/asth	1 mo	Pos	—	Pos	>2000
27	27	M	PP,P, C	15 min	Prur/ang	2 y	Neg	Pos	Neg	226
28	35	F	PP,P, C	10 min	Prur/ang/urt	2 y	Neg	Pos	Neg	91
29	20	F	PP,P, C	20 min	Prur/urt/asth/ rhinitis	4 wk	Neg	Pos	Pos	33
30	39	M	PP,P, C, E	10 min	Prur/ang/shock	2 wk	Pos	—	Pos	308
31	35	F	PP,P, C	3 min	Prur/ang	2 y	Neg	Pos	Pos	44
32	19	M	PP,P, C	10 min	Prur/rhinitis/asth	6 mo	Pos	—	Pos	34
33	21	F	PP,P, C	30 min	Prur/urt	3 days	Neg	Neg	Pos	593
34	38	F	PP,P, C	30 min	Prur/ang/shock	10 days	Neg	Pos	Pos	391
35	43	F	PP,P, C	5 min	Urt/asth/shock	10 y	Neg	Pos	Neg	732
36	20	M	PP,P, C	1 min	OAS/rhinitis/asth/ang/shock	1 y	Pos	—	Pos	56
37	35	F	PP,P, C	20 min	Prur/urt	5 y	Neg	Pos	Neg	116
38	21	M	PP,P, C	20 min	Prur/urt/rhinitis/asth	2 days	Neg	Neg	Pos	42
39	26	M	PP,P, C	10 min	Prur/urt/asth	2 y	Neg	Neg	Pos	104
40	34	F	PP,P, C	1 min	OAS/prur	15 y	Neg	Pos	Pos	41
41	24	M	PP,P, C	10 min	Prur/urt/ang/tachy	2 wk	Pos	—	Pos	443
42	30	F	PP,P, C	5 min	Prur/urt/ang/rhinitis	1 y	Pos	—	Neg	35
43	20	F	PP,P, C	10 min	OAS/ang/shock	3 mo	Pos	—	Pos	230
44	36	M	PP,P, E	10 min	OAS/prur/ang/asth	2 wk	Pos	—	Pos	376
45	42	F	PP,P, C	5 min	Prur/urt/ang/shock	3 y	Neg	Pos	Neg	170
46	43	F	PP,P, C	10 min	OAS/prur/urt/shock	2 wk	Pos	—	Pos	165
47	21	M	PP,P, C	30 min	Prur/asth	1 y	Neg	Pos	Pos	660
48	20	F	PP,P, C	30 min	Prur/urt	1 wk	Neg	Pos	Neg	60
49	31	F	PP,P, C	30 min	Prur/ang	4 y	Neg	Pos	Neg	632
50	29	F	PP,P, C	15 min	Prur/urt/ang	1 wk	Neg	Pos	Neg	33
51	16	F	PP,P, E	30 min	Prur/urt/asth	1 y	Neg	Pos	Neg	31
52	18	F	PP,P, C	5 min	OAS/prur/asth/shock	2 wk	Neg	Neg	Pos	557
53	37	M	PP,P, C	15 min	Prur/asth/shock	2 wk	Pos	—	Pos	806

P, Paracetamol; C, caffeine; E, ergotamine; Co, codeine; OAS, oral allergy syndrome; *prur*, pruritus; *urt*, urticaria; *ang*, angioedema; *asth*, bronchial asthma; *shock*, anaphylactic shock; *tachy*, tachycardia; *vom*, vomiting; *Pos*, positive; *Neg*, negative.

*Time interval between intake of the drug and the first symptoms.

†Time interval between allergic event and doctor's visit.

‡ELISA system using PP-HSA conjugate.

history reflected a typical systemic adverse reaction to the drug of the immediate type (Table I), ie, symptoms occurred within the first 30 minutes after intake of the medicament. Twenty-seven patients were treated for anaphylaxis by a called emergency doctor or needed hospitalization.

Skin tests

Skin tests were performed on the volar forearm skin by using a test set containing sterile solutions of chininum hydrochloride 1%, Na-salicylate 1%, Na-diethylbarbiturate, metamizol 0.5%, phenylbutazone 0.5%, propyphenazone 0.25%, and paracetamol 0.5%. In a first series skin prick tests (SPTs) were performed with these substances. As controls, SPTs with histamine (10 mg/mL) and 0.9% saline were performed. Wheal and flare reactions of half the size of the positive control or 3 mm larger than the negative control were considered positive. If no positive reaction occurred after 60 minutes, intracutaneous testing (ICT) was subsequently performed. ICT was performed by using 0.04 mL of the above mentioned solutions. Physiologic saline solution was used as negative control. Reactions were controlled after 30 and 60 minutes and after 24 hours. ICT reactions were considered positive if a wheal of more than 10-mm diameter was observed.⁵ The substances used are regularly used for the diagnostic setup of adverse reactions to NSAIDs. In general these substances do not induce unspecific irritant reactions in patients or normal control subjects; one exception is chinine, which sometimes induces erythematous reactions in skin sensitive individuals.

Preparation of propyphenazone-human serum albumin conjugates for serologic testing

To establish an ELISA system for the determination of PP-specific IgE we coupled PP to human serum albumin (HSA). To circumvent steric hindrance of antibody-PP-HSA interaction,¹² PP was linked to a spacer molecule with 4 carbon atoms via the nitrogen in position 1 of the pyrazolone moiety. As shown in Fig 1, *B*, the spacer molecule (methyl-4-iodobutyrate, intermediate product I; IP-I) was synthesized from methyl-4-chlorobutyrate with sodium iodide as follows; 170 g sodium iodide and 100 g methyl-4-chlorobutyrate were suspended in 600 mL dried acetone and boiled under reflux for 8 hours. The suspension was filtered, and the solution was diluted with water and extracted with distilled diethylether. The extract was washed with sodium thiosulfate and water, dried with sodium sulfate, and evaporated. The crude oil of IP-I was directly used for the next step. As shown in Fig 1, *C*, the core of PP (N-demethylpropyphenazone, IP-III) was synthesized from phenylhydrazine and methyl-2-isopropyl-3-oxobutyrate (IP-II), which was synthesized from methyl-3-oxobutyrate with 2-iodopropane, as follows; 45 g sodium hydride was washed 3 times with n-hexane under nitrogen and added to 600 mL distilled dry tetrahydrofuran. After dropwise addition of 110 mL methyl-3-oxobutyrate the mixture was boiled for 1 hour and cooled before careful addition of 150 mL 2-iodopropane. The solution was heated under reflux for 20 hours, then cooled, diluted in 1.2 L water, and extracted with 3 × 200 mL ethylacetate. The combined extracts were washed with water and brine, then dried with sodium sulfate, and evaporated. Distillation gave 60 g IP-II as a colorless oil. Then, 24 g IP-II and 1 mL acetic acid were added to 200 mL dried methanol. Then, 15 g phenylhydrazine was added dropwise. The solution was boiled under reflux for 1 hour, evaporated, and kept at 140°C for 1.5 hours. As shown in Fig 1, *D*, the spacer molecule was linked to N-demethylpropyphenazone, and the methyl ester was hydrolyzed leading to 4-(1,2-dihydro-5-methyl-4-(1-methylethyl)-3-oxo-2-phenyl-3H-pyrazolyl)-butyric acid (IP-IV), as follows; 10 g sodium hydride was washed with n-hexane 3 times under nitrogen and added to 300 mL

dimethyl sulfoxide. To clarify, the suspension was stirred at room temperature for 5 hours. Then, 33 g IP-III was dissolved in 200 mL dimethyl sulfoxide and added dropwise under cooling. Thereafter, 40 mL IP-I was added dropwise within 60 minutes. The solution was stirred for 24 hours at room temperature and diluted with 1.5 L water. After addition of acetic acid to pH 3, the solution was extracted with ethylacetate, washed with water, dried with sodium sulfate, and evaporated. The crude product was dissolved in 150 mL distilled dry tetrahydrofuran, 150 mL methanol, and 160 mL 5 mol/L NaOH, heated under reflux for 4 hours and stirred at room temperature for 12 hours. The solution was diluted with water, acidified with hydrochloric acid to pH 2, and extracted with ethylacetate. The apolar extract was extracted with sodium bicarbonate, and the combined aqueous extracts were acidified with acetic acid to pH 3.5 and reextracted with ethylacetate. The combined extracts were washed with water, dried with sodium sulfate, evaporated, and crystallized in methyl t-butylether/methylenchloride. The correct structure of IP-IV was confirmed by ¹H-nuclear magnetic resonance spectroscopy at 200 MHz (Bruker Avance DRX 200; Bruker-Biospin, Rheinstetten/Karlsruhe, Germany). Moreover, the resulting product was determined to be greater than 98.0% pure by reversed phase-HPLC (HP 1090; Hewlett-Packard, Palo Alto, Calif) by using a 4 × 250 mm LiChrospher C18 column with 5-μm particle size. Finally, this molecule reacted with HSA (Baxter-Immuno, Vienna, Austria) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and sulfo-N-hydroxysuccinimide (sNHS) to form a PP-HSA conjugate (HSA-PP, Fig 1, *E*). For determining the molecular ratio of PP per HSA molecule, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry of reduced and nonreduced HSA-PP was performed. For reduction of HSA-PP, 20 μg of the conjugate was incubated in the presence of 5 mmol/L dithiothreitol at 37°C for 1 hour. Then, 0.5 μL containing 0.1 to 1.0 μg of conjugate in 10 mg/mL gentisic acid (matrix), 40% acetonitrile, and 0.1% trifluoroacetic acid was applied to the target slide and dried in an airstream. Samples were analyzed with the Kompact MALDI-TOF IV mass spectrometer (Kratos Analytical, Manchester, UK) in the linear flight mode by using time delayed extraction optimized for molecular mass of 70,000. For calibrating the instrument, the molecular peaks of myoglobin and gentisic acid were used. By MALDI-TOF mass spectrometry, HSA-PP was determined to contain 9 ± 2 PP molecules per protein molecule.

Determination of PP-specific IgE by ELISA and immunoblotting

HSA-PP or HSA alone was coated onto ELISA plates (Maxisorb; NUNC, Roskilde, Denmark) in carbonate buffer (pH 9.6) at a concentration of 100 μg/mL (50 μL/well), overnight at 4°C. After blocking for 6 hours with 100 μL of 5% dry milk in PBS-0.05% Tween 20 (DMPT), 50 μL of patients' or control sera (1:2) was incubated overnight at 4°C. After washing, 100 μL of horse anti-human IgE (Allercoast EAST; Kallestad, Chaska, Minn) conjugated with alkaline phosphatase (1:2 in DMPT) was incubated overnight at 4°C. After washing, *para*-nitrophenylphosphate (1 mg/mL) was added for 2 hours at 37°C. Optical density (OD) was measured at 405 nm, with the wavelength of 550 nm as reference. Ten sera derived from nonallergic individuals with normal total IgE levels (UniCap; Pharmacia Diagnostic, Uppsala, Sweden) were used as controls. Five of these individuals were exposed to PP within the last month, ie, they had used analgesics containing PP, and 5 had not taken PP previously. Sera from patients with high total IgE levels (greater than 500 kU/L) and allergic to cat or house dust mite or pollen as well as 10 individuals with a history of an adverse reaction to the NSAID diclofenac were also tested for control purposes.

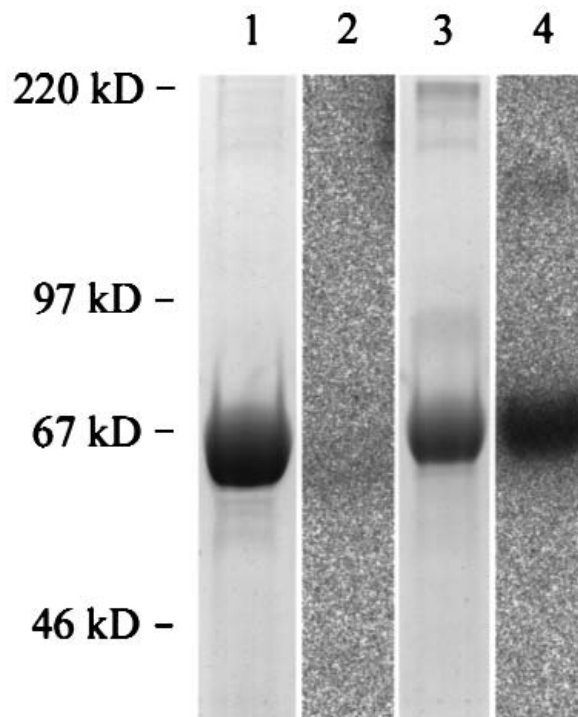


FIG 2. SDS-PAGE and IgE immunoblot analysis of PP-HSA conjugate; lanes 1 and 3 show a protein stain of HSA and HSA-PP conjugate, respectively. Lanes 2 and 4 show IgE immunoblots with a serum pool of individuals with PP allergy; patients' IgE do not bind to HSA (lane 2) but react significantly with HSA-PP conjugate (lane 4).

Furthermore, IgE reactivity to HSA-PP was analyzed by SDS-PAGE and IgE immunoblot analysis. Proteins were separated in 15% (w/v) SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Sera were diluted 1:10 in 25 mmol/L Tris-HCl pH 7.5, 0.15 mol/L NaCl, 0.5% (v/v) Tween 20, 0.5% (w/v) BSA, and 0.05% (w/v) Na-azide. Bound IgE was detected by using ^{125} I-rabbit anti-human IgE (RAST; Pharmacia-Upjohn, Uppsala, Sweden) and phosphorimaging and autoradiography. Ten individual serum samples and a serum pool of PP-allergic patients were tested; specificity was proved by including the control sera mentioned above. For inhibition experiments, sera of 10 individuals and a serum pool of patients with PP allergy (1:2) were preincubated overnight at 4°C with different concentrations of PP, antipyrine (Sigma, Vienna, Austria), metamizol (Sigma), aminophenazone (Sigma), or PBS alone as control. Microtiter plates used for this incubation were pretreated for 6 hours with DMPT at room temperature. The next day these preincubated serum samples were tested in the ELISA system described above.

RESULTS

Skin testing, ELISA, and immunoblotting

Table I depicts the summarized data of all patients. Twenty of 53 (38%) patients had positive results in SPT with PP. Of the remaining 33 patients with SPT-negative results, 24 had positive results in ICT with PP. In total, 44 of 53 (83%) of our patients could be diagnosed by skin testing. One patient had a positive SPT result with paracetamol and PP; one patient reacted in SPT with phenylbutazone and PP. Nine patients (patients 4, 12, 14,

18, 24, 33, 38, 39, 52) had negative results in skin test with PP and all other test substances used. In ELISA, with 3 SD above values of the mean of the negative controls as a cutoff, 31 of 53 (58%) patients had positive results with HSA-PP (OD: 0.070 to 2.126). None of the negative control subjects showed significant reactivity in the system (OD: 0.044 to 0.066). In 7 of 9 patients with negative skin test results PP-specific serum IgE could be detected with the ELISA system. In only 2 of 53 (4%) patients with a case history suggestive of Type I allergy against PP, the hypothesis of IgE-mediated anaphylaxis could not be confirmed. Ten individual sera (not shown) and a serum pool of individuals with PP-allergy (Fig 2) were also positive in immunoblots, ie, they showed an IgE-binding band to the HSA-PP conjugate. All control sera derived from atopic individuals, nonallergic individuals, and patients with adverse reactions to diclofenac were negative in ELISA as well as in IgE immunoblots (not shown).

Preincubation of 10 individual sera and a serum pool with different concentrations of PP inhibited IgE binding to PP in a dose-dependent manner (Fig 3). However, preincubation with antipyrine, metamizol, or aminophenazone (4-aminopyrine) did not influence IgE binding to PP significantly.

DISCUSSION

Adverse reactions to NSAIDs are frequent.^{13,14} A part of these reactions seems to be immunologically elicited; however, the proof of this mechanism is in many cases not possible. Skin tests are frequently not sensitive (or not suited to reflect the pathomechanism), and useful laboratory tests are not available. Case histories of patients with adverse reactions to PP are highly suggestive of Type I allergy. Here we demonstrate that immediate-type adverse reactions to PP can be classified as Type I allergy and are mediated by specific IgE antibodies.

PP is an analgesic substance widely used as painkiller, mostly orally and in combination with other NSAIDs. Although PP is mentioned as anaphylaxis-inducing drug in several textbooks and in a few clinical studies,^{6-7,10,11} this mechanism has not been unequivocally proved. Therefore, we have collected 53 cases of adverse reactions to PP-containing drugs between 1998 and 2001 in our clinic. In all patients the case report was clear; they took the tablets orally because of acute pain situations such as headache, toothache, or pain associated with menstruation. Oral allergy syndrome, ranging from itching at the oral mucosa to swelling of the tongue or larynx, was frequently observed as the first symptom. Subsequently in all our patients systemic symptoms developed. The reactions characteristically started with pruritus at the palms and feet, followed by urticaria and angioedema, in some cases culminating in anaphylaxis. Twenty-eight of 53 (53%) of the patients received emergency treatment and were then referred to our Allergy Clinic for clarification and diagnosis. The other patients came *sua sponte* after the event. By SPT with PP it was possible to elicit typical wheal and flare reactions in 38%

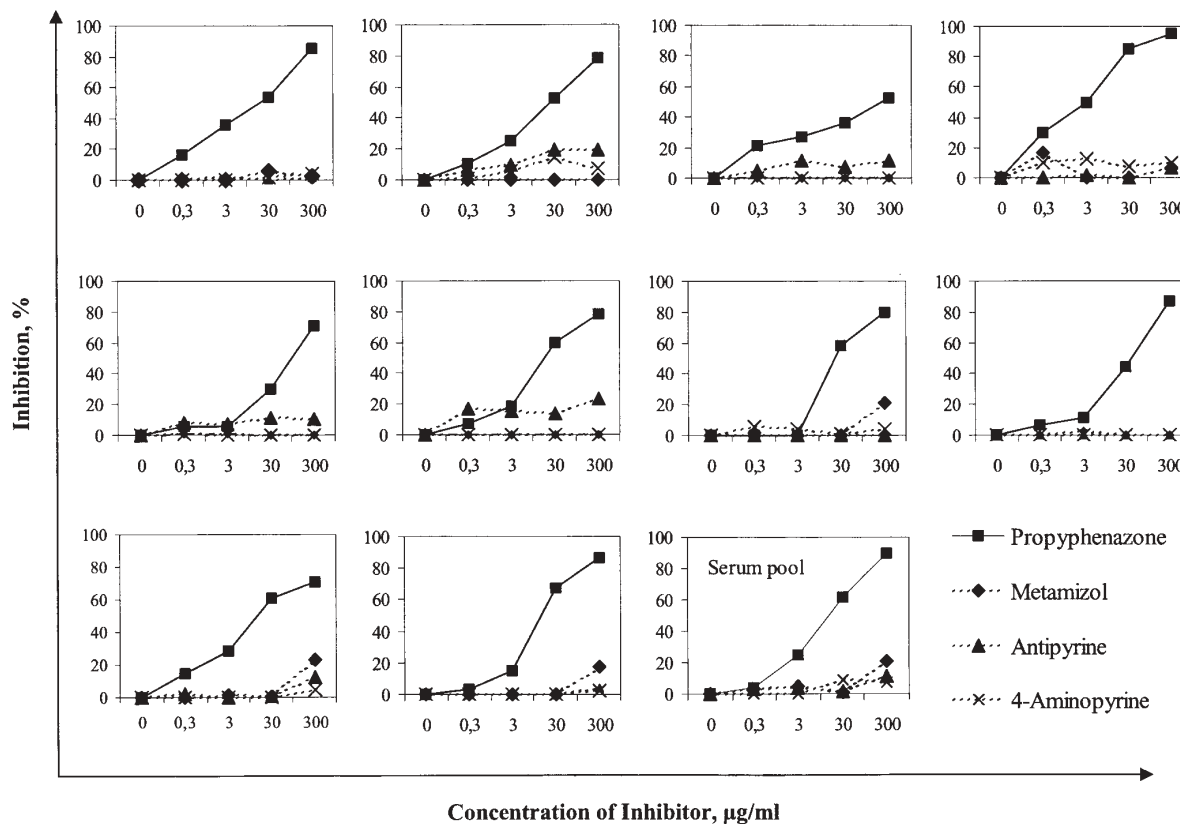


FIG 3. Inhibition of IgE binding to HSA-PP. Ten individual sera from patients with PP allergy and a serum pool were preincubated with serial concentrations of PP, metamizol, antipyrine, and 4-aminopyrine in PBS and then tested in PP ELISA.

of the patients. Only when SPT result was negative, the diagnostic procedure was continued with ICT. Seventy-three percent of the remaining patients had reactions with this method of higher sensitivity. In total, 44 of 53 (83%) individuals with PP allergy could be identified by using skin tests. In general, skin testing with drugs is neither a sensitive (many false negatives) nor a highly specific (false positives by irritant reactions) method.¹⁵ Our results show that in case of PP allergy, SPT and ICT are valuable methods for diagnosis. The skin tests showed typical wheal and flare reactions within minutes, indicating IgE recognition of the unmetabolized molecule. Twenty-six of 53 (49%) patients had elevated total IgE levels (Table I). This might indicate an inclination of atopic individuals toward developing PP allergy. To prove the existence of PP-specific serum IgE, we established an ELISA system by coupling PP to HSA. By using a spacer molecule, we took into consideration that the epitope could be influenced by the coupling process.¹² With our ELISA system we were able to detect specific IgE in 31 of 53 (58%) individuals with PP allergy. Moreover, ELISA enabled us to detect specific IgE in 7 of 9 patients with negative skin test results. In total, combining skin tests and serology, 51 of 53 (96%) of our patients could be identified as PP-allergic.

In one publication a common IgE-binding epitope for

pyrazolone drugs was described.¹² In contrast, our data suggest a highly specific recognition of PP, indicating a role for the propyl residue at position 4. Antipyrine (the core structure of pyrazolone drugs), metamizol (Novalgin), and aminophenazone differ from PP only at position 4 (Fig 1, A). None of these molecules inhibited IgE binding to PP in our ELISA system. The knowledge of the epitopes of allergy-inducing drugs can possibly help to develop new derivatives with a lower allergy risk.

So far, adverse reactions to PP were commonly believed to be intolerance/pseudoallergic reactions. We have identified PP as an allergen that induces IgE production and leads to systemic allergic reactions in certain individuals. These events are not rare, usually happen to healthy individuals, and are therefore not included in epidemiologic studies focused on hospitalized patients.^{4,7,11}

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