IMMUNOLOGIC CROSS-REACTIVITIES OF THREE DIVERSE PENICILLINS

MICHAEL W. BRANDRISS, JAMES W. SMITH AND
HARRY G. STEINMAN.

*U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health,
National Institute of Allergy and Infectious Diseases, Laboratory of Clinical Investigations,
Bethesda, Maryland.*

RECENT investigations have demonstrated the importance of the benzylpenicilloyl group as an antigenic determinant in benzylpenicillin (PG) hypersensitivity (Levine and Ovary, 1961) (Fig. 1). It has been suggested that this antigen is formed by the conjugation to protein of the

highly reactive intermediate, benzylpenicillenic acid (Fig. 1, B). A number of conventional penicillins derived from 6-aminopenicillanic acid (6-APA) form penicillenic acid at varying rates and all those that have been tested cross-react immunologically with PG.

FIG. 1.—Structures of benzylpenicillin (PG), 6-aminopenicillanic acid (6-APA) and cephalothin (CPTH) and pathways of formation of the conjugate of PG. Reaction "B" has been demonstrated (Levine, 1961). Reaction "A", a direct transamidation involving the β -lactam ring, is proposed as an alternative through which 6-APA and CPTH as well as PG may become antigenic.

In our investigations two unconventional penicillins that do not undergo the penicillenic acid rearrangement *in vitro* have been studied. One of these, unsubstituted 6-aminopenicillanic acid, does not possess an acyl side-chain radical and hence cannot undergo the oxazalone rearrangement (Fig. 1). The other, 7-(thiophene-2-acetamido)-cephalosporanic acid, cephalothin (CPTH), is derived from 7-aminocephalosporanic acid, which possesses a 6-membered S containing ring (Fig. 1). The penicillin-like congeners derived from this nucleus are much more acid stable than the compounds derived from 6-aminopenicillanic acid (which possesses a 5-membered S containing ring) and do not form detectable amounts of penicillenic acid (Brandriss, Denny, Huber and Steinman, 1962). It, therefore, appeared of interest to investigate the immunologic activities, if any, of these two unusual penicillins.

Methods and Materials

New Zealand white rabbits were sensitized by injections of the penicillin pre-incubated with rabbit serum according to the procedure

used by Levine and Ovary (1961). The presence of antibody was determined by a number of procedures. The results of the hemagglutination and passive cutaneous anaphylaxis (PCA) studies are described here. The other data are to be published in a more detailed report.

The test antigens used for detecting PCA reacting antibodies were made by conjugating the individual penicillin with human γ -globulin (HGG) at an alkaline pH.

Results

The results of the hemagglutination studies are summarized in Table I. Sera from rabbits injected with PG regularly agglutinated CPTH coated cells as well as PG-coated cells. There was marked variation between individual sera in the degree of cross-reactivity. Sera from rabbits injected with 6-APA agglutinated PG coated cells in high titer but gave very weak cross-reaction with CPTH-coated cells. Sera from rabbits injected with CPTH gave strong reactions with CPTH coated cells but weak or negative reactions with PG-coated cells.

TABLE I

Hemagglutination Titers* of Rabbit Sera against Rabbit Erythrocytes incubated with Benzylpenicillin or Cephalothin

Rabbits sensitized with:	Rabbit No.	Cells incubated with:	
		PG	CPTH
Penicillin-G (PG)	1	128	64
	2	64	64
	3	128	16
	4	128	4
	5	64	64
	6	32	64
	7	8	64
6-Aminopenicillanic Acid (6-APA)	1	256	2
	2	256	4
	3	512	8
	4	256	2
Cephalothin (CPTH)	1	2	64
	2	16	512
	3	0	256
	4	0	512
	5	4	1024

*Titers are recorded as the reciprocal of the highest serum dilution that produced agglutination.

The results of the PCA studies are summarized in Table II. Antibenzylpenicillin sera benzylpenicilloyl-globulin conjugate was used regularly gave strong PCA reactions when the as the eliciting antigen. There were strong cross-reactions with the 6-APA conjugate and moderate to strong reactions with the cephalothin conjugate. Anti-6-aminopenicillanic sera gave strong PCA reactions whether the homologous antigen or the benzylpenicilloyl antigen was used to elicit the reaction. Weak cross-reactivity with the cephalothin conjugate was seen. Cephalothin sensitized animals yielded sera which gave the PCA reaction equally as well with benzylpenicilloyl antigen as with the homologous antigen but reacted weakly with the 6-APA conjugate.

Discussion

It had been demonstrated previously that the injection of 6-APA into rabbits caused the formation of antibody that agglutinated benzylpenicillin-coated red cells (Chisholm, English and MacLean, 1961). The present report demonstrates that cephalothin is also antigenic and that both 6-APA and CPTH cause the for-

mation of PCA-reacting as well as hemagglutinating antibodies that cross-react with the benzylpenicillin antigen. Since these two penicillins do not undergo a penicillenic type rearrangement, it is suggested that they become antigenic through a direct reaction with protein via a transamidation reaction involving the β -lactam ring. Such a direct combination with protein may also occur as a simultaneous competitive reaction even with those penicillins capable of the penicillenic-type re-arrangement (Fig. 1, A).

Since the antigens derived from the two other penicillins cross-reacted strongly with benzylpenicillin, they presumably contain structures related to the benzylpenicilloyl group which has been shown to be the major determinant group in benzylpenicillin hypersensitivity. The unsubstituted 6-aminopenicillanic acid molecule produced an antigen which differed in specificity from the substituted molecule, benzylpenicillin, as measured by cross-reactions with cephalothin. The relation of the cross-reactivity between CPTH and PG observed in hyperimmunized rabbits to potential cross-reactions in humans remains to be clarified.

TABLE II

Passive Cutaneous Anaphylaxis titers* of Rabbit sera with Benzylpenicillin (BPO-HGG), 6-aminopenicillanic acid (6-APA-HGG) or Cephalothin (CPTH-HGG).

Rabbits sensitized with:	Rabbit No.	Eliciting antigen		
		BPO-HGG	6-APA-HGG	CPTH-HGG
Benzylpenicillin	1	20,480	2,560	1,280
	2	10,240	5,120	320
	3	2,560	640	<40
	4	2,560	—	40
	5	1,280	640	320
	6	2,560	320	320
	7	1,280	160	160
6-Aminopenicillanic acid (6-APA)	1	1,280	5,120	80
	2	5,120	2,560	> 40
	3	5,120	5,120	—
	4	2,560	2,560	40
Cephalothin (CPTH)	1	320	40	320
	2	1,280	< 40	2,560
	3	1,280	—	1,280
	4	1,280	320	1,280
	5	1,280	< 80	1,280

*Titers are recorded as the reciprocal of the highest serum dilution that produced a positive PCA reaction.

Summary

Cephalothin and 6-aminopenicillanic acid were antigenic in rabbits despite their inability to undergo a penicillenic acid-type rearrangement *in vitro*. Both hemagglutinating- and PCA-reacting antibodies formed in response to injection with these antigens cross-reacted with the benzyl-penicillin antigen.

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TOXICITY OF THE PENICILLINS

G. T. STEWART

Queen Mary's Hospital for Children, Carshalton

THE absence of direct toxicity is one of the most remarkable properties of the penicillin molecule; that is to say, the penicillin molecule as we know it and as we should use it. If you try hard enough, you can make it toxic by altering it or abusing it, as I shall try to show later. With this reservation, one can say that penicillin G and the main therapeutic derivatives of 6-aminopenicillanic acid (6-APA) at present in use are virtually non-toxic. By this I mean that these drugs can be given in doses very much larger than those of any comparable biologically-active substance to some animals and to man without disturbing organic function or causing any signs or symptoms suggestive of damage to essential tissue (Table I). In mice, rats and dogs, penicillin G, the phenoxypenicillins, methicillin, the isoxazoles and ampicillin are all tolerated intravenously in doses of 2 g./kg. or more, and in twice that dosage subcutaneously or orally. With some derivatives (e.g. ampicillin) it is difficult to establish a toxic dose within the limits of solubility. Extremely high doses (5 g./kg.) may cause convulsions if given intravenously: this would be equivalent to about half a kilogram by injection or a kilogram by mouth to man! At about half this dose level, the isoxazoles

cause transient hypotension but in other respects there is no interference with vital functions. The toxic level in man has never been established but it is known that methicillin and penicillin G can be given intravenously in doses of 20 g. per day for weeks on end, and that ampicillin and the isoxazoles can be given in doses of at least 4 g./day (80 mg./kg. day) without any signs of immediate or delayed toxicity. This means that concentrations of penicillin G and methicillin of 50 mg.% can circulate harmlessly in the blood and tissues; in other words, that penicillin is no more toxic than glucose or urea, and much less toxic than many other physiological substances. When such large doses are being given, it is best to use the sodium salt as the potassium cation can be toxic: 15 mega units of penicillin G supplies 25 mEq. of K⁺ which may cause cardiac dilation, especially if the heart is already damaged, as in bacterial endocarditis, which is the main disease requiring such high doses. Even the sodium cation may rise if there is any renal impairment and it should be remembered that normal doses of methicillin yield 5-10 mEq. of Na⁺. Some among you may recall that, in the early days, the toxicity of successively purified batches of crude peni-

TABLE I
Tolerance of Various Species to Penicillins

Drug	Mouse	Dose tolerated (mg./kg./day) parenterally				Man
		Rat	Guinea Pig	Rabbit*	Dog	
Penicillin G	3,000	3,500	5	500	500	1,000
Methicillin	3,000	4,000	10	500	250	400
Ampicillin	5,000	5,000				100
Cloxacillin	2,000	500	10	200		80

* More sensitive to oral dosage.