

Cephalosporin chemical reactivity and its immunological implications

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Purpose of review

The aim of this article is to analyze the chemical reactivity of cephalosporins resulting in the epitope responsible for recognition by IgE antibodies and to establish the basis of the allergenicity.

Recent findings

Increasing evidence supports the role of cephalosporins in IgE hypersensitivity reactions. Third and fourth generation cephalosporins appear to be more involved in specific IgE reactions and often no cross-reactivity with traditional benzyl penicillin determinants exists. In some instances selective responses to unique cephalosporins occur and in others common side-chain similarities exist.

Summary

Lack of knowledge of the exact chemical structure of cephalosporin antigenic determinants has hindered clinical interpretation of allergic reactions to these drugs and hampered understanding of the specific recognition by IgE molecules of these determinants. Data indicate that R₂ is not present in the final conjugate and that recognition by IgE antibodies is mainly directed to the R₁ acyl side chain and to the β -lactam fragment that remains linked to the carrier protein in the cephalosporin conjugation process.

Keywords

beta-lactams, cephalosporins, cephalosporoyl chemical structure, drug allergy

Introduction

Allergic reactions to cephalosporins can be induced by the β -lactam ring structure common to all antibiotics from this family or by specific recognition to cephalosporin determinants. Although no standardized diagnostic tests are currently available to clinicians for use in allergy to β -lactams, cephalosporins are nevertheless widely prescribed in clinical practice for treatment of different bacterial infections and as prophylactic agents in surgery.

Lack of understanding of the exact chemical structure of cephalosporin antigenic determinants has hampered adequate evaluation of allergic reactions to these antibiotics, study of the specific molecular recognition by specific IgE antibodies, and hence development of standardized in-vitro and in-vivo diagnostic tests.

This review provides a detailed analysis of the chemical structures generated by cephalosporins and furnishes information concerning the possible final determinant involved in the specific IgE response.

The chemical structure of β -lactam antibiotics

The basic structure of both penicillins and cephalosporins consists of a four-member β -lactam ring. In penicillins this ring is condensed with a five-member sulphur ring (the thiazolidine ring), and in cephalosporins with a six-member ring (the dihydrothiazine ring) (Fig. 1). Enzymatic conversion by expandase, the deacetoxy cephalosporin C synthetase, converts the five-member thiazolidine ring of penicillins into a larger six-member ring, the dihydrothiazine ring [1]. Substitution at the R₁ and R₂ side chains has yielded many different chemical structures resulting in several antibiotic agents. These enable cephalosporins to penetrate the bacterial capsule and exert their antibacterial properties.

Classification of cephalosporins

Cephalosporins can be classified according to different criteria, such as their metabolism and stability to the action of β -lactamases [2], the substitution of the R₂ side chain [3], their pharmacokinetic properties [4,5], or their microbial properties, mainly related to their antibacterial spectrum [3]. Group I cephalosporins include molecules with the greatest activity against Gram positive bacteria; group II have the greatest activity against Gram negative

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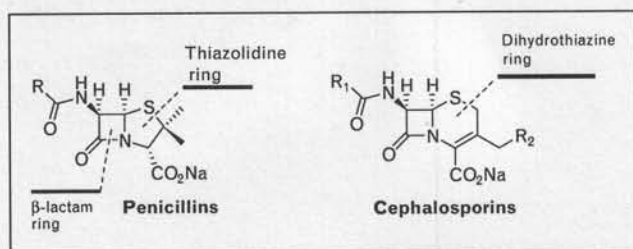
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Abbreviation

RAST radioallergosorbent test

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Figure 1. Penicillin and cephalosporin chemical structures



bacteria; group III against *Pseudomonas aeruginosa* and group IV against anaerobic bacteria [6]. From the clinical viewpoint, the most useful classification divides cephalosporins according to their historical development plus their common microbiologic and structural characteristics. Within this classification, according to different generations of cephalosporins [7–10], besides the well-known first, second and third generation cephalosporins, we now include the fourth generation for which newly developed molecules are continually being added [11–16].

The R_1 side chain in first and second generation cephalosporins was made following experience with penicillins and includes the thiazolyl and phenylglycyl side chains. The R_1 side chain of the third and fourth generation cephalosporins has a common structure: the aminothiazole-oxime moiety plus some added carboxylic acid derivative salts (ceftazidime, cefixime) which enable penetration through the cell of Gram negative bacteria. A wider variation of substitutions exists at the R_2 position. Some substitutions contain positively-charged amino groups which affect the pharmacokinetics and antibacterial effect of the cephalosporin.

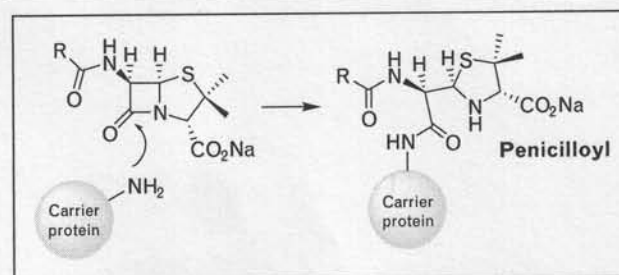
Immunologically, cephalosporins can be classified according to their nucleophilic properties that enable binding to the protein, with subsequent formation of the epitope. The chemical structure resulting after this conjugation process depends on the chemical properties of the cephalosporins involved in each case.

Immunochemistry: uncertainty about the antigenic determinant

The immunological behavior of β -lactam antibiotics is determined by their intrinsic chemical reactivity, which is related to the ability of the carbonyl group to act as an alkylating agent with the amino groups of proteins.

In penicillins, due to the chemical structure resulting from the condensation of the β -lactam with the thiazolidine ring, the high tension within the β -lactam ring results in increased chemical reactivity. In cephalosporins, the heterocycles are formed by four and six carbon

Figure 2. The penicillin conjugation to carrier proteins



The penicilloyl determinant formation as a stable, isolable, well-defined chemical structure.

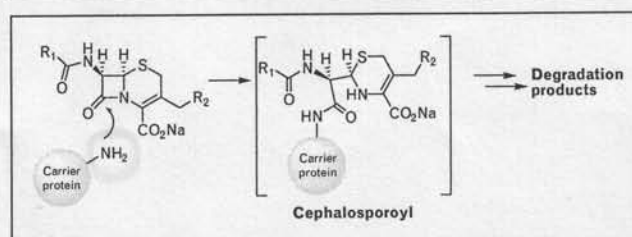
atoms, resulting in lower tension in the β -lactam ring. Haptenization of proteins by penicillins is therefore quicker and more efficient than by cephalosporins (Fig. 2). The conjugate formed by penicillins, benzyl penicilloyl, results in a chemical structure which is stable enough to enable purification and characterization by classical spectroscopic techniques.

In cephalosporins, the lower reactivity of the β -lactam ring slows haptenization. The R_2 chemical structure can modulate this reactivity depending on the capacity of the side chain to polarize the electronic binding (Fig. 3). In ceftizoxime, cefrodaxime, cefadroxil, cephalixin and cephadrine the 3' position is occupied by a hydrogen atom, a methoxy or a methyl group, with no effect on the kinetic hydrolysis of the β -lactam ring (Fig. 4).

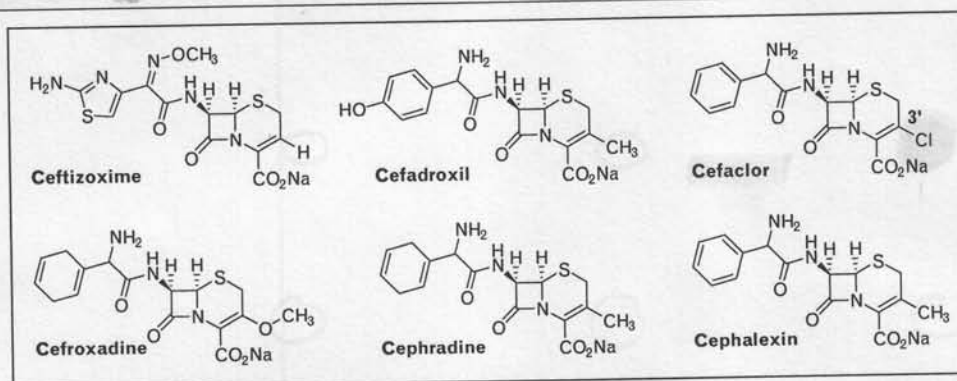
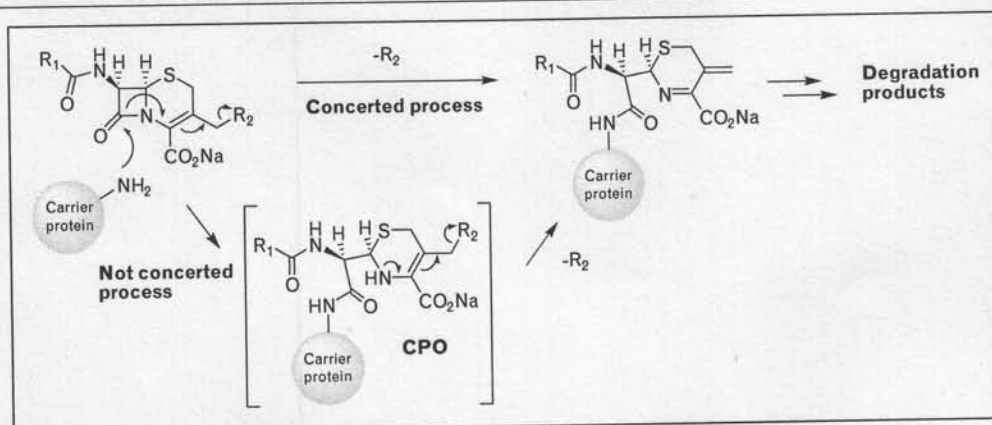
Cefaclor has a chlorine atom at the 3' position, the high electronegative value of which facilitates the opening of the β -lactam ring by induction.

The R_2 side chain at the 3' position may act as a leaving group, as occurs in the majority of clinically relevant cephalosporins, thus increasing the reactivity of the β -lactam group (Fig. 5). This substitution not only affects the binding of cephalosporins to penicillin binding proteins but it also has an electronic effect on the chemical reactivity of the β -lactam carbonyl group, which is related to its antibiotic activity.

Figure 3. The cephalosporins conjugation to carrier proteins



The intermediate cephalosporoyl is not a stable, isolable and well-characterized structure.

Figure 4. Chemical structures of cephalosporins with R₂ side chains that cannot work as leaving groupsFigure 5. The two proposed mechanisms for the cephalosporins conjugation thorough the β -lactam ring opening

Concerted versus non-concerted pathways. CPO, cephalosporoyl.

Evidence for the R₂ departure with the opening of the β -lactam ring

Several studies deal with the elimination of R₂ by hydrolysis, aminolysis and hydrazinolysis of the cephalosporins [17,18]. Theoretical and experimental evidence suggests that the opening is a concerted process with the subsequent expulsion of the R₂ [19–23]. No evidence has been shown for cephalosporoyl formation when R₂ acts as a good leaving group, like acetoxy or pyridinium. In other cases the expulsion of the R₂ group is not a concerted process with the opening of the β -lactam ring, although this process may also occur in the presence of certain enzymes.

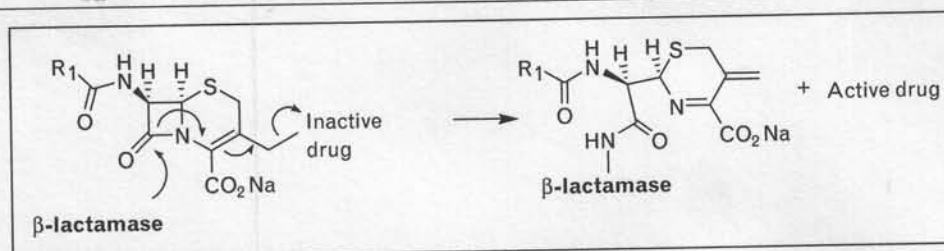
Kinetic studies combined with absorption and nuclear magnetic resonance spectroscopy have shown the structure of the opening of the β -lactam ring: cephalosporoyl [17,18]. Either in a concerted fashion or in stages, the opening of the β -lactam ring leads to elimination of the R₂ when this is configured as a leaving group. The process is well documented chemically and this property has been used as a strategy to obtain cephalosporins that

can apply in a double action way [24–28]. When the R₂ is conformed as the inactive form of the drug, the action of the β -lactam in the cephalosporin implies the release of the drug *in situ* (Fig. 6).

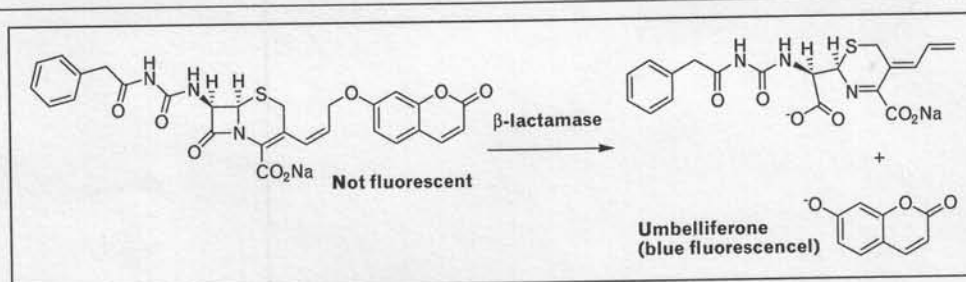
Beta-lactamases are used as biological markers for the identification of pathogenic bacteria resistant to β -lactam antibiotics. Based upon the ability of the R₂ to act as a leaving group [29], cephalosporins can also be used as sensors to monitor processes or biological interactions (Fig. 7).

Consequently, the initial product of aminolysis of cephalosporins (cephalosporoyl) is unstable, probably being degraded with the rupture of the dihydrothiazine group [30,31]. Apart from evidence of the R₂ side chain extrusion, when this may act as a leaving group, no evidence is yet available for the resulting chemical structure and it has not been possible to isolate and characterize the aminolysis products resulting from the scission of the dihydrothiazine moiety of cephalosporins (Fig. 8) [32].

Figure 6. Dual-action cephalosporins



Cephalosporins exert their biological activity by covalent binding to bacterial enzymes, opening of the β -lactam ring is accompanied by liberation of the 3'-substituent if that substituent can function as a leaving group.

Figure 7. Cleavage of the β -lactam ring of a cephalosporin

Cleavage triggers spontaneous elimination of any leaving group previously attached to the 3'-position.

The addition of water to the exocyclic double bond of structure 1 (Fig. 8) to generate structure 2 (Fig. 8) was recently described and this precursor is responsible for the degradation products of the dihydrothiazine ring [33].

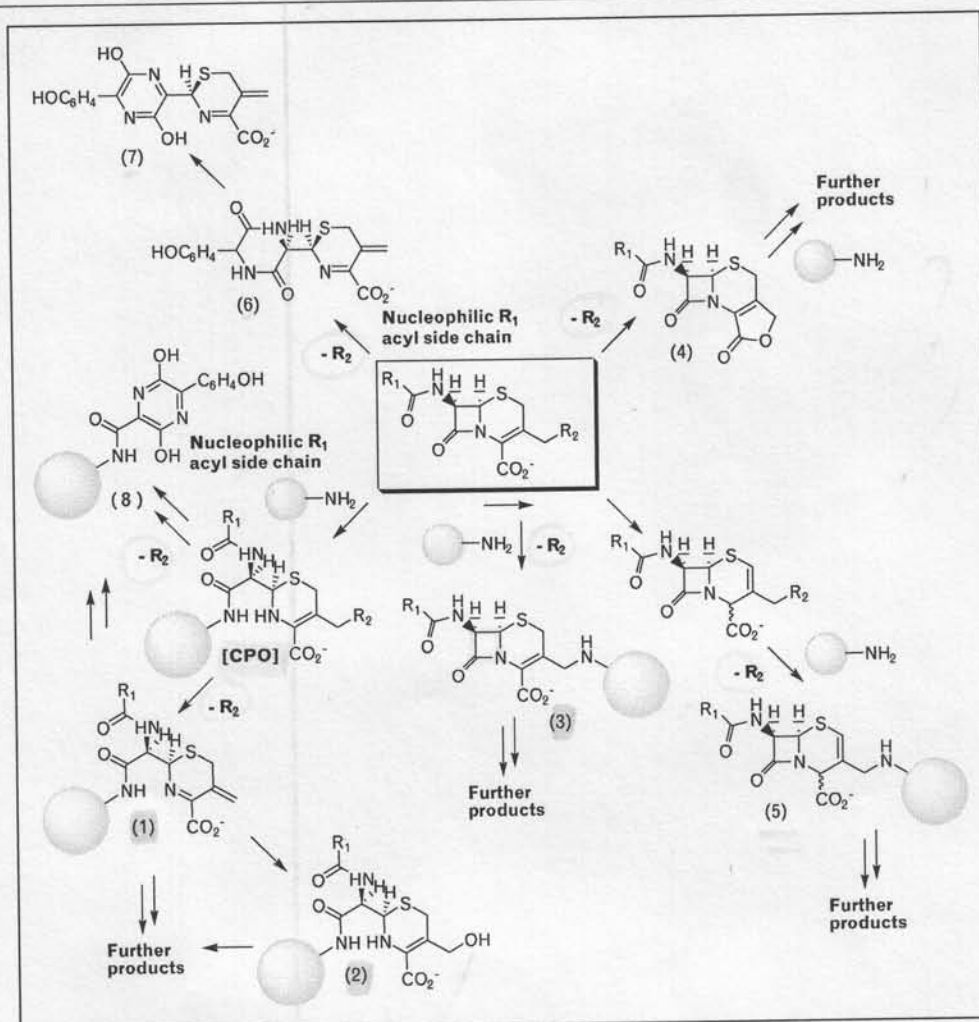
Several studies have described a high number of degradation products of the different cephalosporins, depending on the chemical structure of the particular cephalosporin and the reaction conditions, mainly the pH [34,35]. The ease with which the R_2 acetoxy group can be replaced by different nucleophiles and its reactivity with nitrogen nucleophiles has been described [36]. Cephalosporins with some R_2 side chains can thus undergo reactions with the amino groups of the carrier proteins, not only via the carbonyl of the β -lactam ring, but also via R_2 substitution, giving forms such as structure 3 (Fig. 8). This mechanism of action enables the cephalosporins to bind to a carrier protein conforming a hapten-carrier conjugate in which the β -lactam structure is intact and its capacity to be attached by a new nucleophile is decreased. These types of structures produce a new epitope in which the R_2 side chain is not present.

Despite the facility for substitution of the R_2 side chain by sulphur and nitrogen nucleophiles, oxygen nucleophiles do not react and no evidence exists for the direct formation of a lactone like structure 4 (Fig. 8). However, in water, a significant hydrolysis of the acetic ester to the corresponding alcohol, that lactonizes to structure 4, can

be observed. This derivative can undergo opening of the β -lactam ring by reaction with nucleophiles [31], facilitating its conjugation to several carriers and the conformation of a new epitope. The isomerization of the double bond to the 2,3 position results in equilibration of the reactivity of the two electrophilic centers of the molecule, with a reduction in the reactivity of the carbonyl group of the β -lactam ring and, consequently, the possible competence of the 3' position enabling the formation of conjugates with a form such as structure 5 (Fig. 8) [37].

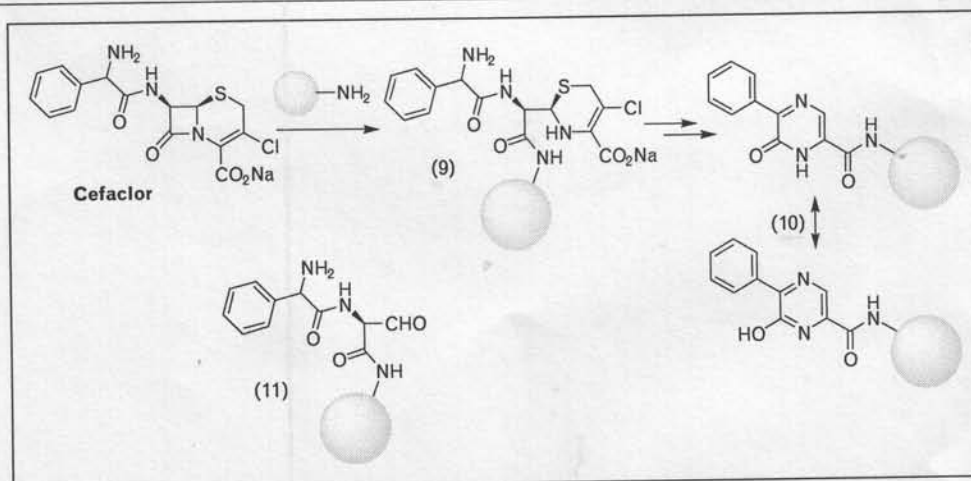
In cephalosporins with nucleophilic groups at R_1 , such as cephaloglycin, cefaclor, cephalixin, cefadroxil and cephadrine, autoaminolytic reactions may occur to yield the compound shown by structure 6 (Fig. 8), in which the intramolecular opening of the β -lactam ring is followed by R_2 exclusion, when this side chain can act as a good leaving group, for example in cephaloglycin. For cefaclor, six different fluorescent products have been identified with a form related to structure 7 (Fig. 8) [34]. When cefaclor reacts with nitrogen nucleophiles (Fig. 9) the intramolecular aminolysis competes with the intermolecular process and the intermediate cephalosporoyl structure 9 (Fig. 9) can react intramolecularly to yield a compound like that shown by structure 10 (Fig. 9) [32], and the hypothesis of the aldehyde of structure 11 (Fig. 9) as a key intermediate in the formation of a fluorescent pyrazinone like structure 10 seems the most plausible.

Figure 8. Possible pathways for the cephalosporin chemical reactivity with nucleophiles



CPO, cephalosporoyl.

Figure 9. Aminolysis of aminocephalosporins such as cefaclor giving a degradation product with a pyrazine nucleus

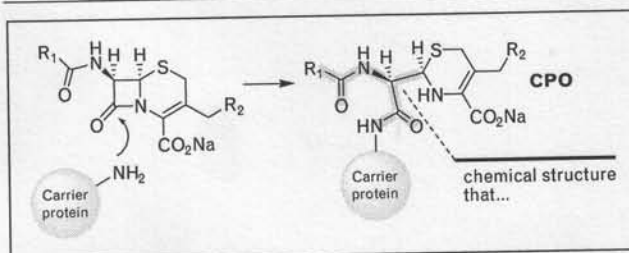


Approaches to the chemical identification of the epitope

Very little information exists about allergenicity of cephalosporins, especially regarding the structural differences between these compounds. Few studies have been undertaken to identify which part of the molecule is recognized by specific IgE antibodies. In fact, much of the data concerning the chemical nature of the allergenic determinant have been produced with IgG or other isotype human polyclonal antibodies [38]. In contrast to the many studies of the chemical behavior of penicillins, in which the chemical structure of the compounds involved has been well established, studies are lacking of the chemical behavior of cephalosporins, mainly their reactivity. Despite this lack of research, similar behavioral patterns have been assumed between penicillins and cephalosporins, without taking into account the fact that different cephalosporins may have different reactivity models, depending on the chemical structure of their side chains. Several hypotheses have been put forward in immunological and clinical papers, with the subsequent negative effect produced by interpretation of the data.

Efforts to identify hapten determinants have been undertaken, but the resulting data have proved speculative, resulting in concern regarding decision-taking [39–41]. Three models of response may occur in subjects who are allergic to cephalosporins: subjects who have cross-reactivity with determinants of the penicillins, subjects who are cross-reactive with different cephalosporins and subjects with reactivity to a single cephalosporin [42]. These results support previous findings concerning non-classic determinants and show that only a minority of subjects seem to recognize benzyl penicilloyl as a relevant determinant.

Figure 10. Proposed skeleton that remains linked to the carrier protein after chemical degradation in cephalosporin conjugation to carrier proteins

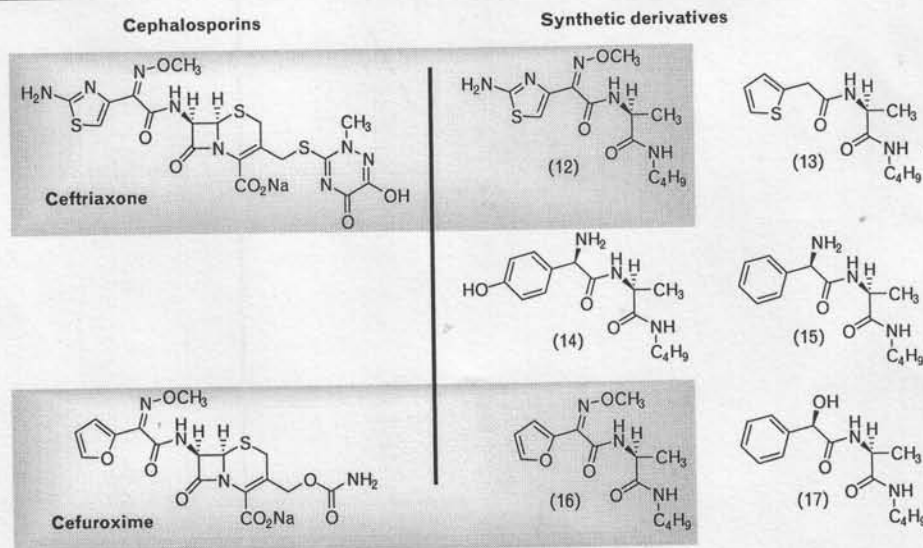


CPO, cephalosporoyl.

Evidence that the R_2 side chain is lost after the opening of the β -lactam ring by the carrier protein and that the resulting structure becomes unstable led to the hypothesis that the R_2 and the dihydrothiazine moiety become lost in the process of conjugation of the cephalosporins with the carrier protein. Thus, only the R_1 and part of the β -lactam ring remain bound to the carrier proteins and contribute to the chemical structure of the epitope recognized by IgE, becoming responsible for allergy to cephalosporins (Fig. 10).

The first attempt to define in detail the chemical structure of the antigenic determinant responsible for the immunological response in cephalosporins has recently been undertaken in conjunction with radioallergosorbent test (RAST) inhibition studies [43]. A number of monomeric N-acyl-L-alanyl butanamides have been synthesized (structures 12–17 in Fig. 11). These have a well-defined chemical composition and stereochemistry,

Figure 11. Synthesized chemicals comprising the haptenic structure that are recognized by ceftriaxone and cefuroxime, respectively



incorporating the complete R_1 side chain and the amino acid residue included in the β -lactam fragment of the relevant cephalosporins.

The RAST inhibition studies were in good concordance with skin test and direct RAST assays, indicating that the process of molecular recognition is mainly directed to the R_1 side chain and to the part of the β -lactam ring that remains bound to the carrier protein in the process of conjugation of cephalosporins. These data have enabled determination of the structural requirements necessary for recognition by IgE antibodies, resulting in the possibility of an in-vitro assay to detect and quantitate IgE antibodies.

Conclusion

The inherent chemical reactivity of cephalosporins implies that the opening of the β -lactam ring by nucleophilic reagents generates an intermediate cephalosporoyl which is chemically unstable and that suffers multiple fragmentation reactions. Despite the structural similarities with penicillins, those cephalosporins that have a good R_2 leaving group undergo the process of expulsion when they conjugate to carrier proteins by opening of the β -lactam ring. For these cephalosporins the unstable dihydrothiazine moiety is enough to undergo further degradation processes. As a result, conjugation of cephalosporins by the β -lactam ring leads to loss of the R_2 side chain and to fractionation of the dihydrothiazine ring and this does not form part of the epitope presented in the hapten-carrier conjugate. Only the R_1 side chain and a fragment of the β -lactam ring remain bound to the carrier protein, constituting the epitope resulting from these conjugates. The presence of an R_2 side chain that may act as a good leaving group is closely related to enhanced reactivity of the β -lactam ring for nucleophilic attack. The effect of the R_2 side chain on the conjugation of the carrier protein can be interpreted only from a kinetic perspective, such that an increase in the capacity of the R_2 as a leaving group results in increased reactivity for the attack of nucleophiles to the β -lactam ring, increasing the facility and kinetics of the conjugation process.

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