Brain-Specific Chemical Delivery Systems for β-Lactam Antibiotics. In Vitro and in Vivo Studies of Some Dihydropyridine and Dihydroisoquinoline Derivatives of Benzylpenicillin in Rats†

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Four chemical delivery systems (CDS's) based on a dihydropyridine ⇄ quaternary pyridinium salt redox system were used for the brain delivery of benzylpenicillin (BP). CDS's **5** and **9** are diesters of C1 and C2 diols in which one hydroxyl group is esterified by the benzylpenicillin-3-carboxylic group and the other by dihydrotrigonelline. CDS's **13a** and **17** are benzylpenicillin esters of amino alcohols in which the amine group is acylated by dihydrotrigonelline (**13a**) or by 1,2-dihydro-2-methyl-4-isoquinolinecarboxylic acid (**17**). In vitro relative stability studies showed that both CDS's and quaternary pyridinium salts were quite unstable in rat and rabbit blood or brain but much more stable in dog or human blood. Kinetic studies performed in rat brain homogenate demonstrated the facile enzymatic oxidation of the CDS's to the corresponding quaternary salts. Hydrolysis of the CDS's and the quaternary salts resulted in the release of benzylpenicillin. In biological media CDS **13a** also yielded a water addition product, the 6-hydroxy-1,4,5,6-tetrahydropyridine derivative. In vivo distribution studies were carried out in rats. After iv administration of equimolar doses of BP and CDS's, brain benzylpenicillin levels were found to be substantially higher and more prolonged in case of **5** and **9** than of BP itself. However, administration of **13a** and **17** resulted in lower brain benzylpenicillin levels due to the water addition reaction and a nonspecific brain delivery, respectively. The remarkable increase of BP levels as well as the prolonged effect after the administration of **5** and **9** is a result of an improved penetration through the blood-brain barrier of the lipophilic CDS's and a "lock-in" effect of the corresponding quaternary salts generated in situ.

Penicillins are among the most important antibiotics used in the management of central nervous system (CNS) infections. In the treatment of bacterial meningitis, penicillin G (benzylpenicillin) remains a drug of choice.[1] However, the penetration of penicillin into the CNS is poor, due mainly to the low lipid solubility of the drug, resulting in CSF/plasma ratio of 1–2%.[2] Although the penetration of penicillin in inflamed meninges is much increased,[3] large doses and frequent systemic administration must be employed to achieve effective CNS concentrations. Besides, penicillins in the CNS are relatively quickly actively transported back into the blood. It is therefore desirable to design penicillins that possess both higher lipid solubilities to improve the CNS penetration and slower CNS elimination rates.

The purpose of this investigation was to improve the distribution of penicillin with reversible chemical modifications. Therefore, a brain-specific drug delivery system[4-6] based on a dihydropyridine ⇄ quaternary pyridinium salt redox system was designed for benzylpenicillin. This chemical delivery system is analogous to the endogenous NADH ⇄ NAD^+ coenzyme system and has been demonstrated to accomplish brain-specific delivery in many cases.[7-12] Various chemical delivery systems (CDS's) of benzylpenicillin have been synthesized and proved to be more lipophilic than the parent compound.[13] In the present paper, several of these CDS's and the corresponding quaternary pyridinium salts were selected (Chart I) for the in vitro study of the relative stabilities in various biological media obtained from various sources, and the in vivo distribution studies in rat.

Results and Discussion

In Vitro Studies. In order to prove that a CDS system possesses the appropriate physicochemical properties for successful clinical application, it is necessary to determine the stability and the transformation pathways both for the

Table I. Pseudo-First-Order Rate Constants (K) and Half-Lives ($t_{1/2}$) for the Disappearance of Quaternary Salts **4**, **8**, **12a**, and **16** from Various Biological Media[a]

medium	compd	$K \times 10^{-2}$, min^{-1}	$t_{1/2}$, min
rat whole blood	**4**	unstable[b]	unstable
	8	43.5 ± 3.8	1.6 ± 0.1
	12a	36.2 ± 1.0	1.9 ± 0.1
	16	11.5 ± 0.4	6.0 ± 0.2
rat 10% brain homogenate	**4**	94.9 ± 10.6	0.6 ± 0.1
	8	2.5 ± 0.2	28.2 ± 1.7
	12a	0.8 ± 0.0	92.1 ± 4.0
	16	1.0 ± 0.0	73.4 ± 1.7
rabbit whole blood	**4**	unstable	unstable
	8	33.0 ± 2.0	2.0 ± 0.1
	12a	23.0 ± 0.6	3.0 ± 0.1
rabbit 10% brain homogenate	**4**	136.6 ± 20.1	0.5 ± 0.1
	8	4.3 ± 0.5	16.7 ± 2.1
	12a	3.0 ± 0.2	23.4 ± 1.8
dog whole blood	**4**	unstable	unstable
	8	6.1 ± 0.2	11.5 ± 0.5
	12a	4.8 ± 0.1	14.5 ± 0.4
	16	5.1 ± 0.2	13.5 ± 0.5
human whole blood	**4**	unstable	unstable
	8	10.5 ± 1.0	6.7 ± 0.6
	12a	7.8 ± 0.8	9.1 ± 1.0

[a] Data show mean ± SE of three determinations performed in different animal samples. [b] Half-life > 0.5 min.

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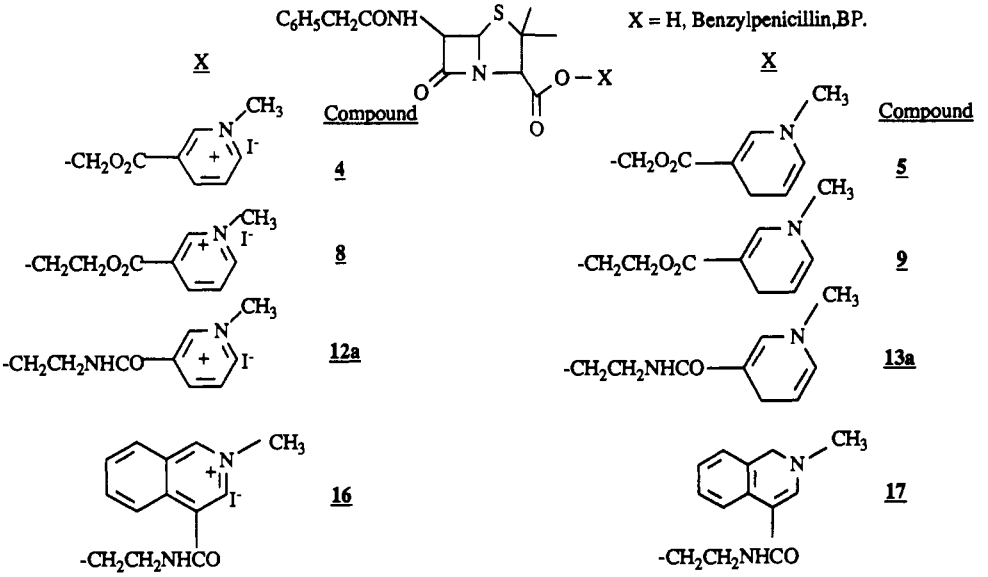
Chart I



CDS and for the corresponding quaternary pyridinium salt in biological media. The chemical delivery system concept requires an oxidation step in which the CDS present in the central nervous system is chemically transformed to a quaternary pyridinium salt form which is "locked in" because of its polar character. Subsequently, enzymatic hydrolysis to the parent compound results in specific and sustained central drug delivery. Therefore, stability studies for CDS's and their corresponding quaternary pyridinium salts in various blood and brain homogenates were carried out.

As shown in Table I, the stabilities of the quaternary pyridinium salts were determined by measuring the pseudo-first-order rate constant (K, min^{-1}) and half-life ($t_{1/2}$, min) of the disappearance of the compound from various sources of blood and brain homogenate. The hydrolysis process of all pyridinium salts was much facilitated by the presence of enzymes (e.g., esterase) in biological media. The hydrolysis of **4** is considered to be a two-step process, giving first the unstable hydroxymethyl ester of benzylpenicillin, which then spontaneously decomposes into benzylpenicillin. The hydrolysis of **4** in all media examined was very fast, and the stability in blood was too low to be determined. The resulting parent compound, benzylpenicillin, was detected in each medium by microbioassay at completion of the reaction: quantitative conversion was shown.

The stability of the diesters was increased by increasing the bridge between the two ester linkages by one methylene unit. In rat or rabbit 10% brain homogenate, the half-lives of **8** were more than 30 times those of **4**. Although **8** was not stable either in rat or rabbit blood, its half-life was 4–10 times longer in dog or human blood. The first hydrolysis product of **8** was the hydroxyethyl ester of benzylpenicillin (**6**), which is more stable than the hydroxymethyl product. Slow hydrolysis of **6** in a second step released benzylpenicillin. The half-lives of **6** in rat, rabbit, and human blood were 0.5, 0.8, and 8.2 min, respectively, while in rat and rabbit 10% brain homogenate, they were 58.4 and 26.6 min, respectively. Since the hydrolysis of **6** is a rate-determining step, its lipophilicity as well as bactericidal activity can be considered to affect the specific "lock-in" and sustained effect of the CDS's in brain. In the case of **12a** and **16**, the amide linkage attached to the pyridinium ring side is much more stable than the ester linkage attached to the penicillin side.[13] Therefore, the hydrolysis of **12a** and **16** can be considered a one-step reaction, resulting in the release of benzylpenicillin. As shown in Table I, the stabilities of amide–esters **12a** and **16** were higher than those of the diol diesters **4** and **8** in all kinds of media [e.g., in 10% rat brain homogenate, the half-lives of **12a** and **16** were 92.1 and 73.4 min, much longer than those of **4** and **8** (0.6 and 28.2 min, respectively)]. It was reported that esterases obtained from rodent sources such as rat and mouse showed a higher degree of hydrolytic activity compared to esterases from dog and human sources.[14] The results of this study confirm these observations.

In buffer solutions, as described previously, at a pH range of 5–9, CDS's **5**, **9**, and **13a** showed acid- and base-catalyzed reactions. The acid-catalyzed reactions resulted in the water addition compounds, whereas base-catalyzed hydrolysis resulted in the release of benzylpenicillin (e.g., **5**, **13a**, or **17**) or (hydroxyalkyl)penicillin (e.g., **9**). The acid-catalyzed water addition reaction was prohibited by using a dihydroisoquinoline instead of a dihydropyridine carrier (e.g., **17**). According to the brain chemical delivery system concept, the lipophilic CDS's are expected to be oxidized to their corresponding quaternary pyridinium salts in the CNS as well as in the periphery. Therefore, ideal conditions for the CDS's would be as follows: (1) the oxidation of CDS occurs before hydrolysis, and (2) the rates of hydrolysis of the quaternary pyridinium salts are slow in order to achieve a sustained delivery. However, in the case of the benzylpenicillin chemical delivery system, the stability of both the CDS's and the corresponding quaternary pyridinium salts toward hydrolysis seemed to be relatively low.

As shown in Table II, **5** was the least stable CDS of the series, being very unstable in rat and rabbit blood ($t_{1/2}$ < 1 min). However, a significant increase of its stability in dog blood ($t_{1/2}$ = 3.3 ± 0.2 min) or human blood ($t_{1/2}$ = 15.9 ± 1.8 min) was observed. Since the pyridinium salt **4** was even less stable (Table I) than **5** in most of the media examined, it was not expected to accumulate in the media, and consequently the hydrolysis product, benzylpenicillin,

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Table II. Pseudo-First-Order Rate Constants (K) and Half-Lives ($t_{1/2}$) for Disappearance of CDS's 5, 10, 13a, and 17 from Various Biological Media[a]

medium	compd	$K \times 10^{-2}$, min^{-1}	$t_{1/2}$, min
rat whole blood	5	unstable	unstable
	9	unstable	unstable
	13a	271.2 ± 8.3	0.3 ± 0.0[c,d]
	17	108.3 ± 3.6	0.6 ± 0.0[c]
rat 10% brain homogenate	5	135.7 ± 10.7	0.6 ± 0.0[c]
	9	29.6 ± 1.4	2.4 ± 0.1[c]
	13a	12.5 ± 1.7	5.7 ± 0.7[c,d]
	17	2.5 ± 0.3	28.3 ± 3.0[c]
rabbit whole blood	5	94.9 ± 20.7	0.8 ± 0.2
	9	90.3 ± 17.5	0.8 ± 0.2[c]
	13a	55.3 ± 1.7	1.3 ± 0.0[c,d]
rabbit 10% brain	5	16.6 ± 1.6	4.3 ± 0.4
	9	17.4 ± 1.2	4.0 ± 0.3[c]
	13a	15.2 ± 1.0	4.6 ± 0.3[c,d]
dog whole blood	5	21.3 ± 1.1	3.6 ± 0.2
	9	16.8 ± 0.8	4.1 ± 0.2[c]
	13a	12.3 ± 0.6	5.7 ± 0.3[c,d]
	17	6.2 ± 0.4	11.4 ± 0.8[c]
human whole blood	5	4.5 ± 0.6	15.9 ± 1.8
	9	4.3 ± 0.3	16.2 ± 1.1[c]
	13a	3.7 ± 0.4	19.4 ± 2.1[c,d]

[a] Data show mean ± SE of three determinations performed in different animal samples. [b] Half-life < 0.5 min. [c] Quaternary salt was found in the medium. [d] Water addition product was found in the medium.

was the major product observed. In rat 10% brain homogenate, the rate of disappearance of CDS 5 ($t_{1/2}$ = 0.6 ± 0.0 min) was almost the same as that of its oxidation product 4 ($t_{1/2}$ = 0.6 ± 0.1 min); however, 4 could be observed in this medium, indicating that the oxidation rate of 5 in rat brain homogenate is probably much faster than its hydrolysis. Although accurate kinetic studies were not able to be carried out due to the low stability of 5, the oxidation reaction of CDS 5 in brain tissue was clarified.

CDS 9 ($t_{1/2}$ = 2.4 ± 0.1 min) was much less stable in biological media than the corresponding pyridinium salt ($t_{1/2}$ = 28.2 ± 1.7 min); therefore, the incubation of 9 in biological media resulted in concomitant formation of the corresponding oxidation product 8, which subsequently followed the described transformation pathway to release first the relatively stable (hydroxyethyl)penicillin, as described before, and then benzylpenicillin, the parent compound.

The rate of disappearance of 9 in blood was almost the same as in the case of 5. However, in 10% rat brain homogenate, its stability was about 4 times higher than that of 5. Figure 1 is an example of the disappearance of 9 as well as the appearance of oxidation and hydrolysis byproducts, 8 and 6, by using 10% rat brain homogenate as a testing medium. As shown, the dihydropyridine CDS 9 disappeared rather quickly via oxidative and hydrolytic pathways (K = 0.2973 min^{-1}, $t_{1/2}$ = 2.3 min), and in the meantime, the oxidation product 8 and hydrolysis product 6 appeared and increased in concentration. The disappearance of 8 from 10 to 30 min showed a pseudo-first-order fashion with a rate constant K of 0.0227 min^{-1} ($t_{1/2}$ = 30.5 min) and was accompanied by an increased concentration of 6. The much faster rate of appearance of 6 (from 0.25 to 2 min) in comparison with the disappearance of 8 indicates that 8 was not the only source of the formation of 6. The decomposition of 9 therefore was considered as implying both oxidation and hydrolysis reactions. The hydrolysis by product 6 was then further hydrolyzed to benzylpenicillin at a slower rate (K = 0.012 min^{-1}, $t_{1/2}$ = 58.4 min). The concentration of benzylpenicillin was determined by microbioassay at the end of

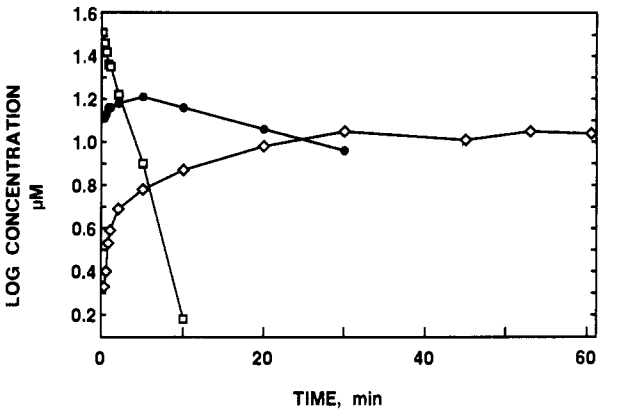


Figure 1. Kinetic investigations of CDS 9 in 10% rat brain homogenate at 37 °C: CDS 9 (□); quaternary salt 8 (●); β-(hydroxyethyl)benzylpenicillin (6) (◇).

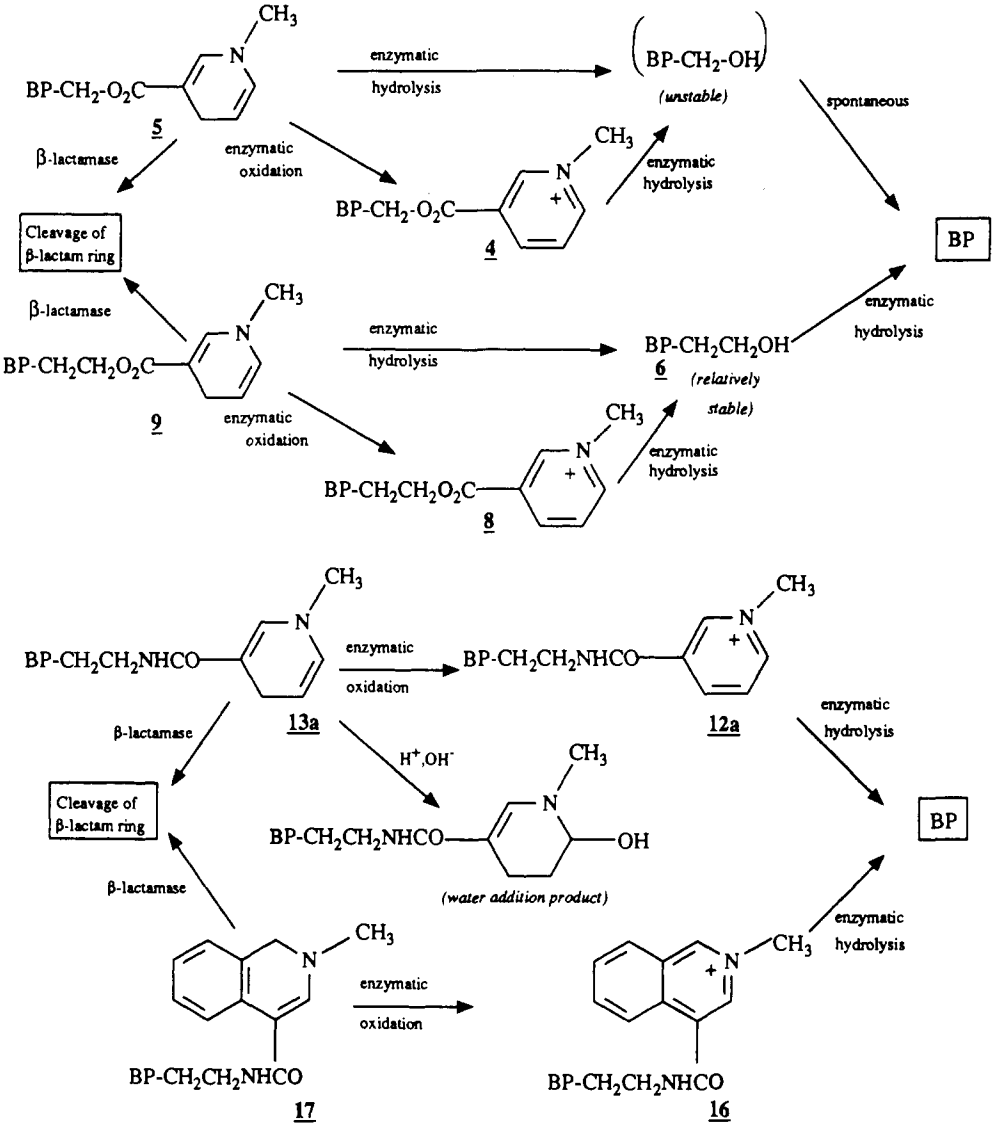
this study. All of the benzylpenicillin was accounted for, indicating quantitative conversion.

In blood or brain homogenate CDS 13a was more stable than 5 and 9 (Table II). However, at biological pH of 7.4, 13a was found to disappear via hydrolysis, oxidation, and acid-catalyzed water addition reactions. Therefore, in test media, the parent compound, benzylpenicillin, the quaternary pyridinium salt 12a, and water addition compound were detected. The water addition compound is formed by the hydration of the 5,6 double bond of the dihydropyridine ring, as a result of a protonation of C-5 followed by a nucleophilic attack (by OH^-) at C-6. Since 13a was oxidized in rat 10% brain homogenate to the corresponding quaternary ion, 12a, in a fairly high proportion (data not shown), and 12a gradually released the parent compound, benzylpenicillin (12a in 10% rat brain homogenate K = 0.008 min^{-1}, $t_{1/2}$ = 92.1 min), it seemed that a good candidate for a brain chemical delivery system was found. However, the hydration reaction in blood may restrict the penetration of 13a in the brain, as resulted during the in vivo study.

Compound 17 was the most stable CDS of all the CDS's investigated in this study. The half-lives of 17a were twice and 5 times longer compared to those of 13a in rat or dog blood and in 10% rat brain homogenate, respectively. The hydration cannot occur in the case of the dihydroisoquinoline carrier; therefore, in the biological media, mainly the oxidation and hydrolysis products (16 and benzylpenicillin) were obtained. The oxidation product 16 was subsequently hydrolyzed to the parent compound.

As a result of the in vitro studies a proposed summary for the biotransformation pathways of benzylpenicillin CDS's is given in Scheme I. In biological media, all CDS's can be enzymatically oxidized by oxidase, yielding the corresponding quaternary pyridinium salts to match the brain "lock-in" concept requirement. The quaternary pyridinium salts are then subsequently hydrolyzed to the parent compound, benzylpenicillin. CDS's 5 and 9 as well as their corresponding quaternary salts 4 and 8, being diol esters, can be hydrolyzed in biological media by a two-step process to yield benzylpenicillin. The first hydrolysis product of 5 and 4 was the hydroxymethyl ester of benzylpenicillin, which was very unstable and decomposed spontaneously into benzylpenicillin. However, in the case of 8 and 9, the first two-step hydrolysis yielded the hydroxyethyl ester of benzylpenicillin, which was relatively stable and was hydrolyzed in a slower fashion to yield benzylpenicillin. The hydrolyses of 13a and 17 are essentially one-step reactions, the cleavage of the ester linkage resulting in the release of benzylpenicillin. The

Scheme I



acid-catalyzed hydration of the dihydropyridine CDS's was not observed for **5** and **9** in biological media. However, in the case of **13a**, this hydration reaction was irreversible and significant. Such hydration prevents further oxidation and decreases the amount of the quaternary salt in the brain. Besides, having a lower lipophilicity than CDS, the water addition compounds can restrict the permeability of CDS through the blood–brain barrier and so reduce the efficiency of the brain-specific delivery. The dihydroisoquinoline type CDS **17** has the advantage of not being susceptible to acid-catalyzed hydration. Although the effect of β-lactamase on the benzylpenicillin CDS's was not performed in this study, in biological media, there is a possibility of a β-lactamase attack of the β-lactam ring as in the case of benzylpenicillin itself.

The in vitro studies indicate that there are good prospects for the clinical use of some of the benzylpenicillin CDS's. Therefore, further in vivo studies were performed in rats.

In Vivo Studies. The distribution of benzylpenicillin and CDS's was investigated in rats. A dose of 60 μmol/kg of potassium benzylpenicillin or CDS's (equimolar dose of 20 mg/kg benzylpenicillin) was administered in the tail vein of conscious animals. CDS's, quaternary pyridinium salts, and related compounds [(hydroxyethyl)penicillin and water addition compound] in various tissues were determined by a high-performance liquid chromatography method. Benzylpenicillin was determined by microbioassay. Since CDS's and quaternary pyridinium salts were unstable, and the concentrations in almost all samples were below the detection limit (0.1 μg/mL), benzylpenicillin concentrations in blood or tissue determined by the microbioassay were used for the comparison of the activity of CDS's. The results obtained from these studies are shown in Table III and Figures 2 and 3. After iv administration of benzylpenicillin in a vehicle consisting

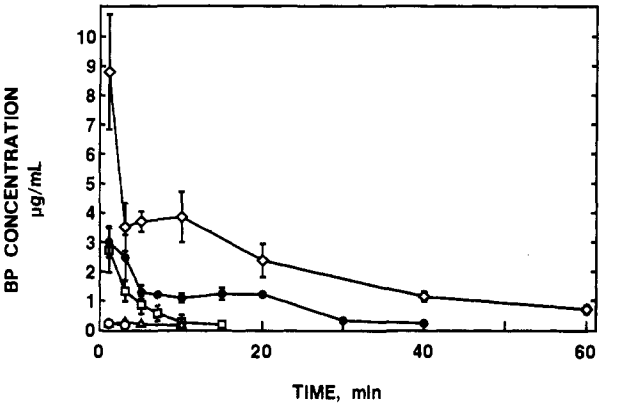


Figure 2. Blood concentrations after intravenous administration of 20 mg/kg BP or equimolar dose of CDS's: BP (□); **5** (●); **9** (◇); **13a** (△); **17** (▽).

Table III. Concentration of Benzylpenicillin (BP) (μg/mL or μg/g) in Blood and Various Tissues after Intravenous Injection of an Equimolar Dose of BP, 5, or 9

compd	time, min	concentration ± SE,[a] μg/mL or g				
		brain	blood	kidney	liver	lung
BP	1	2.7 ± 0.8	64.6 ± 10.6	33.6 ± 9.5	15.1 ± 3.2	52.5 ± 8.0
	3	1.3 ± 0.4	25.2 ± 4.9	120.5 ± 25.5	38.9 ± 6.3	30.3 ± 3.7
	5	0.9 ± 0.3	22.8 ± 4.8	88.5 ± 1.3	30.7 ± 4.5	22.4 ± 3.7
	10	0.3 ± 0.3	8.5 ± 2.2	27.6 ± 0.6	17.7 ± 2.7	9.5 ± 2.0
	20	[b]	2.8 ± 0.8	20.8 ± 6.0	7.5 ± 2.3	2.3 ± 0.7
	40	[b]	1.3 ± 0.6	6.2 ± 0.8	2.9 ± 0.5	1.4 ± 0.5
	60	[b]	0.4 ± 0.1	1.1 ± 0.1	0.7 ± 0.3	0.7 ± 0.3
6	1	3.0 ± 0.5	41.8 ± 8.7	59.0 ± 33.5	18.0 ± 8.4	82.5 ± 17.7
	3	2.5 ± 0.8	23.9 ± 6.1	65.9 ± 22.4	28.1 ± 3.8	75.3 ± 21.3
	5	1.3 ± 0.2	12.1 ± 2.0	74.6 ± 7.5	27.8 ± 3.5	36.8 ± 5.1
	10	1.1 ± 0.2[d]	6.4 ± 1.5	50.2 ± 14.7	24.3 ± 5.7	28.9 ± 6.2
	20	1.2 ± 0.1	3.4 ± 0.5	68.3 ± 31.4	12.0 ± 2.2	22.1 ± 1.3
	40	0.2 ± 0.0	0.9 ± 0.1	5.0 ± 0.5	3.2 ± 0.4	10.3 ± 1.6
	60	[b]	0.4 ± 0.2	3.2 ± 0.4	1.9 ± 0.3	4.7 ± 1.6
9	1	8.8 ± 2.0[d]	22.7 ± 4.6	37.9 ± 12.9	11.1 ± 1.3	37.7 ± 4.7
	3	3.5 ± 0.8[d]	9.0 ± 1.7	56.4 ± 10.9	10.9 ± 0.9	32.4 ± 3.3
	5	3.7 ± 0.4[d]	10.7 ± 1.2	54.6 ± 4.2	12.6 ± 0.5	31.2 ± 7.0
	10	3.9 ± 0.9[d]	5.6 ± 0.6	30.3 ± 2.4	11.4 ± 0.6	24.5 ± 2.3
	20	2.4 ± 0.6[d]	1.9 ± 0.2	10.3 ± 0.9	4.7 ± 0.8	7.2 ± 1.2
	40	1.1 ± 0.2[d]	0.7 ± 0.1	3.4 ± 0.6	1.5 ± 0.3	5.0 ± 1.3
	60	0.7 ± 0.2[d]	0.3 ± 0.0	2.1 ± 0.2	0.6 ± 0.1	2.8 ± 0.6
13a	1	0.2 ± 0.0	5.0 ± 1.2	[c]	[c]	[c]
	3	0.3 ± 0.1	7.3 ± 1.9	[c]	[c]	[c]
	5	0.2 ± 0.1	3.8 ± 1.2	[c]	[c]	[c]
	10	0.1 ± 0.1	4.9 ± 2.2	[c]	[c]	[c]
	20	[b]	0.8 ± 0.3	[c]	[c]	[c]
	40	[b]	0.3 ± 0.1	[c]	[c]	[c]
	60	[b]	0.2 ± 0.0	[c]	[c]	[c]
17	1	0.2 ± 0.0	6.6 ± 1.2	[c]	[c]	[c]
	3	0.2 ± 0.0	3.0 ± 0.3	[c]	[c]	[c]
	5	[b]	2.5 ± 0.9	[c]	[c]	[c]
	10	[b]	1.2 ± 0.3	[c]	[c]	[c]
	20	[b]	0.2 ± 0.0	[c]	[c]	[c]
	40[b]	[b]	[c]	[c]	[c]	

[a] Four animals for each datum. [b] Below detection limit. [c] No data were available. [d] Significantly different from benzylpenicillin ($P < 0.05$).

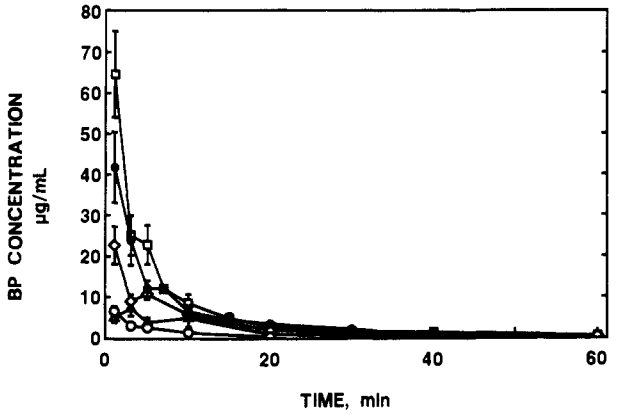


Figure 3. Brain concentrations after intravenous administration of 20 mg/kg BP or equimolar dose of CDS's: BP (□); 5 (●); 9 (◇); 13a (△); 17 (▽).

of H_2O, ethanol, and propylene glycol, relatively high levels of benzylpenicillin were found in blood and other organs at the beginning, except for the brain where permeation of the organic acid was restricted by the blood–brain barrier. At the 1-min sampling point, a small amout of benzylpenicillin passed through the blood–brain barrier; however, its levels in blood and all organs then dropped rapidly. At the 20-min sampling time, the benzylpenicillin level in brain was below the detection limit. In blood and other tissues, at the 60-min sampling time, all benzylpenicillin concentrations were about 1 μg/mL of blood or g of tissues or lower.

The administration of CDS produced a distribution consistent with the lipophilic nature of the molecule. Therefore, in the case of the iv administration of CDS, a rapid distribution was observed. Blood levels of benzylpenicillin were lower compared to those seen after benzylpenicillin administration. However, brain benzylpenicillin levels were increased after the first 10 min in a sustained manner. At the sampling time of 10 min, the concentration of benzylpenicillin in brain tissue was 1.1 μg/g which is about 4.3 times higher than in the case of benzylpenicillin iv administration, yielding a brain/blood ratio of 0.28, and the brain level was over 0.2 μg/g for 40 min. The data indicate that the CDS probably penetrated into brain tissue, and the oxidation product of CDS, the quaternary pyridinium salt, was certainly "locked in" the brain tissue in order to obtain an evident sustained effect. The higher levels of benzylpenicillin in lung after the administration of 5 can be explained by its higher lipophilicity or lower solubility, which may have caused some precipitation of 5 in lung after tail vein injection.

The benzylpenicillin levels obtained from the administration of CDS 9 look different compared to 5. As described previously, the hydrolysis of 9 or its quaternary pyridinium salt form 8 produces a relatively stable hydroxyethyl ester of benzylpenicillin which does not have bacterial growth inhibiting activity, as the free carboxylic group of benzylpenicillin is essential for this. In the in vivo study, the concentration of (hydroxyethyl)penicillin in blood or tissues could not be determined by either HPLC or microbioassy methods; therefore, the levels of benzylpenicillin in blood or tissues were relatively lower than in the case of CDS 5. However, after the administration of CDS 9, the brain levels of benzylpenicillin were interestingly increased and more sustained than in the cases of other CDS's or benzylpenicillin administration. At 10-min

sampling time, the brain level was more than 15 times higher than by administration of benzylpenicillin and 3 times higher than by administration of CDS **5**, yielding a brain/blood ratio of 0.69. By 60 min, the level of benzylpenicillin was higher in the brain than in the blood. As reported previously, the lipophilicities of **5** and **9** are very similar, their R_m values (lipophilicity index) being 2.89 and 2.83, respectively. However, the concentration of benzylpenicillin in brain after the administration of **9** was much higher and more sustained than in the case of **5**. This result indicates that lipophilicity should not be the only factor considered in describing the requirements for a chemical delivery system. Higher levels of BP after administration of **9** compared to **5** may be due to its higher stability in blood. The remarkable increase of BP levels as well as the prolonged effect after CDS **5** and **9** administration may be considered also due to other factors. This can include tissue affinity of CDS and its quaternary pyridinium salts, or the roles that oxidative or hydrolytic enzymes played in the chemical delivery systems in the brain, as well as the brain "lock-in" effect of the quaternary pyridinium salt.

The concentrations of benzylpenicillin in the brain and in blood after administration of the amide–ester type CDS **13a** and the isoquinoline type CDS **17** are shown in Table III. In brain, benzylpenicillin disappeared after 10 min in the case of **13a** and after 3 min in the case of **17**. In blood, both **13a** and **17** produced relatively low concentrations of benzylpenicillin compared to the diester type of CDS's. The low brain concentrations indicate that **13a** and **17** are not proper candidates for the brain-specific chemical delivery systems. The amide–ester type CDS **13a** did not correspond to the requirements of a proper chemical delivery system to the brain, mainly because of the water addition reaction which irreversibly converted the CDS to the less lipophilic 6-hydroxytetrahydropyridine derivative at physiological pH and thereby lowered the amount of CDS penetration into the brain. The isoquinoline type CDS **17**, in spite of the promising in vitro behavior, was proved not brain specific in the in vivo study.

Figure 2 represents the plot of blood benzylpenicillin concentration versus time obtained after administration of a 20 mg/kg dose of benzylpenicillin or an equimolar dose of chemical delivery systems **5**, **9**, **13a**, and **17** to rats. At the 10-min time point after administration of benzylpenicillin, higher blood levels of benzylpenicillin were observed than after CDS administration. However, after 20 min, the blood benzylpenicillin levels were essentially identical with those obtained after benzylpenicillin treatment. The administration of **13a** and **17** resulted in very low blood levels compared to the other compounds tested.

Figure 3 shows brain benzylpenicillin concentrations versus time, and Figure 4 shows the area under the curve (AUC 1–∞ min) values of benzylpenicillin in brain following intravenous administration of benzylpenicillin or CDS's. The estimated AUC values were obtained by calculating the total area of approximate triangles and trapezoids which were divided by each time point. The brain levels after benzylpenicillin adminstration were low and disappeared after 20 min, yielding an AUC (1–∞ min) value of 10.4 μg·min/mL. Administration of CDS **5** did not show much difference in brain level at the beginning. However, a significant difference was observed after 10 min, the sustained effect giving an AUC 1–60 min value of 39.6 μg·min/mL, which is about 4 times higher than in the case of benzylpenicillin administration. Compound **9** was the most potent CDS in delivering benzylpenicillin to the brain. After an equimolar dose of **9**, the benzylpenicillin level in brain was significantly higher, and a sustained effect was observed for more than 60 min. The AUC (1–∞ min) value 133.6 μg·min/mL was about 13 times higher than in the case of benzylpenicillin administration. CDS **13a** and **17** did not deliver benzylpenicillin effectively to the brain; therefore, the AUC 1–∞ min values obtained from the administration of CDS **13a** or **17** were as low as 1.9 and 0.6 μg·min/mL, respectively.

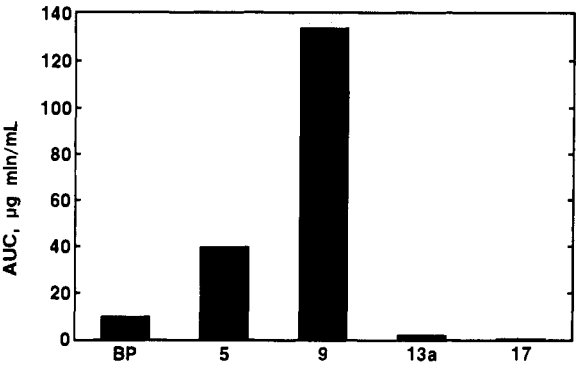


Figure 4. Area under the curve (AUC 1–∞ min) of time versus BP concentration in brain after intravenous administration of BP or CDS's.

Experimental Section

Materials. Potassium benzylpenicillin was obtained from Fluka A.G., and CDS's and their quaternary salts were synthesized as shown in a previous paper.[13] Chemicals were reagent grade.

Analytical Method. (a) High-performance liquid chromatography (HPLC) methods were used for quantitative analysis of CDS's, quaternary salts, and (hydroxyethyl)penicillin. The HPLC system consisted of a solvent delivery system (Kontron 410), a variable-wavelength UV detector (LDC spectromonitor D), an autosampler (Kontron MSI 66), and a recorder. An ASI reversed-phase C8 column (30 cm × 3.9 mm i.d.) was used to separate CDS's and related compounds. The mobile phase consisted of various combinations of acetonitrile (40–50%) and sodium phosphate solutions (5–10 mM). At a flow rate of 2 mL/min, the retention times were 3–10 min. The CDS's were detected at UV 360 nm, and the others at UV 230 nm. The detection limits of all compounds were less than 0.1 μg/mL. The preparation of biological samples (blood or tissues) is described in the following paragraphs. (b) The concentrations of benzylpenicillin in biological media were determined by microbioassay (disk method) with *Bacillus subtilis* ATCC 6633 as a test organism. Blood and brain samples were diluted or homogenized by more than 5 volumes of sterile water to minimize the protein binding effect which might affect the antibacterial activity of benzylpenicillin. The assay limit was 0.05 μg/mL in biological media.

Stability Determinations in Biological Media. The stability of CDS's and quaternary salts was determined in rat (trunk), rabbit, and dog (venous) blood, as well as in rat and rabbit brain homogenates. Freshly collected animal whole blood was used. Brain homogenates were prepared by homogenizing freshly collected brain tissues with pH 7.4 phosphate buffer (1/20 m, μ = 0.15) to obtain 10% w/v tissue suspension. Aliquots of 8 mM test compound in dimethyl sulfoxide solution were added to the prewarmed (37 °C) biological medium to yield a final concentration of 40 μM. At appropriate time intervals, samples (0.3 mL) were taken and mixed with 0.7 mL of 8% dimethyl sulfoxide in acetonitrile solution. After centrifuging, supernatants were removed, filtered, and analyzed by HPLC. By preparing calibration standards in the same way, it was proved that CDS and quaternary compounds could be completely extracted in supernatants by this method. Pseudo-first-order rate constants of the disappearance of compound in biological media were determined by linear regression analysis from plots of log peak height versus time.

In Vivo Distribution Studies. Male Sprague-dawley rats weighing 200–250 g were used. Benzylpenicillin K or CDS's

(equimolar dose of benzylpenicillin 20 mg/kg) was administered in the tail vein of conscious animals. Benzylpenicillin K was dissolved in a vehicle consisting of water, ethanol, and propylene glycol, and CDS's were dissolved in a vehicle consisting of ethanol and propylene glycol (7:3) to obtain a concentration of 20 mg/mL. The animals were sacrificed by decapitation at appropriate time intervals after the intravenous injection. For each time point four to six animals were used. Trunk blood was collected into heparinized tubes. Brain, kidney, liver, and lung were removed and frozen immediately. Blood and tissue samples obtained from CDS-treated animals were each separated into two parts for HPLC and microbioassay analysis. In preparing the samples for HPLC analysis, the blood or brain tissues were mixed or homogenized by 3 volumes of 8% dimethyl sulfoxide in acetonitrile solution and centrifuged. The supernatant layer was separated, filtered through 0.45-μm nitrocellulose membranes, and analyzed by HPLC. The samples for microbioassay analysis were prepared as described in the analytical method section for determining the total benzylpenicillin concentrations in blood or tissues. The same microbiossay method was used to analyze the blood and brain samples obtained from benzylpenicillin-treated animals. In all cases (HPLC and microbioassay), standard calibration curves were prepared. Different concentrations of compounds were added to blood or tissue homogenates and then prepared as described previously for HPLC and microbioassay determinations.

Conclusion

By comparing the brain delivery of benzylpenicillin after either benzylpenicillin or CDS administration in rats, the diester types of CDS's **9** and **5** were found to be superior. Although the hydrolysis of **9** or its oxidation product **8** in vitro resulted in the enzymatically more stable compound, the (hydroxyethyl)penicillin ester, in rats, due to the high degree of hydrolytic activity of the esterases[14] this was hydrolyzed to benzylpenicillin in the brain. On the other hand, CDS **5** was hydrolyzed rapidly and completely to benzylpenicillin in the presence of esterases. The lower benzylpenicillin concentration in brain after intravenous administration of **5** compared to **9** in rats probably is due to the instability of **5** in blood where a great part of **5** was hydrolyzed or oxidized before penetrating into the brain. The stability studies performed in vitro demonstrated that **5** was much more stable in the media obtained from dog or human sources; therefore, **5** seems to be a good CDS model for dog or human use. Although in the rat, the best results were obtained with **9**, it is known that simple esters, such as the hydroxyethyl, are not able to be hydrolyzed in an efficient manner by the enzymes present in larger mammals, including humans. Because of this inconvenience, the drug of choice for further investigation was **5**.

In clinical use of benzylpenicillin for the treatment of central nervous system infections, such as meningitis or neurosyphilis, "seizures" are undesirable side effects[1] which sometimes are caused by the high dose of parenteral injection of benzylpenicillin. In this study, high brain benzylpenicillin levels were obtained following the administration of CDS in conscious animals; however, no seizure symptoms were observed in any animal after treatment. It is mentioned that benzylpenicillin penetrates into the CSF or brain more easily when the meninges are inflamed, but it is rapidly eliminated from CSF or brain into the blood stream via an active transport process.[3,15] The advantage of using the benzylpenicillin CDS, besides a much better CNS uptake, should be the control of the rapid increase and especially decrease of the benzylpenicillin in CNS because of the "lock-in" feature of its oxidation form. An appropriate sustained effect of benzylpenicillin in CNS therefore could be achieved.

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Registry No. 4, 121125-45-9; **5**, 121125-49-3; **8**, 121125-46-0; **9**, 121125-50-6; **12a**, 121125-47-1; **13a**, 121125-51-7; **13a** water addition product, 121125-53-9; **16**, 121125-48-2; **17**, 121125-52-8; benzylpenicillin, 61-33-6; esterase, 9013-79-0.

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