Fate of Oral $^{35}$S-Cloxacillin in Man

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Summary. $^{35}$S-Cloxacillin was administered orally, intravenously or by intrajejunal instillation to healthy subjects. An oral dose of the $^{35}$S-penicilloic acid of cloxacillin was given to two subjects. The absorption of radioactive material from the upper part of the digestive tract was calculated by reference to an unabsorbed marker in the test solution. After an oral dose of $^{35}$S-cloxacillin, the average cumulative absorption of radioactivity from the upper jejunum was 60%, and although some $^{35}$S-cloxacillin was degraded in the stomach, the uptake of radioactivity appeared mainly to represent absorption of intact drug. The uptake of radioactivity after administration of $^{35}$S-penicilloic acid was about 30% of that of labelled cloxacillin. After $^{35}$S-cloxacillin, up to 25% of radioactivity in urine and about 80% of that in bile were attached to microbiologically inactive cloxacillin metabolite(s). The almost identical pattern of degradation after oral and intravenous administration of $^{35}$S-cloxacillin suggested that the metabolite(s) were formed outside the gastrointestinal tract. A comparison of the concentration of total cloxacillin equivalents (measured as total radioactivity) and intact cloxacillin (determined by microbiological assay) showed that a substantial part of the plasma radioactivity represented microbiologically inactive metabolites of cloxacillin. The half-life of the plasma label was much longer than that reported for the microbiologically active compound.

Key words: Cloxacillin, gastrointestinal absorption, metabolism, penicillins, penicilloic acid.

Most of our knowledge of the fate of penicillins in man has depended on estimates of antibacterial activity in body fluids and excreta. Sidell et al. (1963), and Bunn and Milicich (1963) used this type of technique and reported large inter-individual variations in the plasma levels produced by oral cloxacillin. Under similar experimental conditions, Knudsen et al. (1962), Gravenkamper et al. (1963), Bunn and Milicich (1963) and Sutherland et al. (1970) found that about 40% of an administered dose could be recovered from urine. The aim of the present study was to discover whether the latter figure reflected incomplete absorption, substantial biliary excretion or extensive breakdown of the drug when taken by mouth, and for this purpose $^{35}$S-cloxacillin was given to healthy volunteers.

Materials and Methods

a) Experimental Procedures

The volunteers investigated were healthy male transport workers, 41—62 years of age, all of whom had been treated previously with penicillin without any apparent adverse effects. $^{35}$S-Cloxacillin (15 µCi, 250 mg) was administered orally to 10 of them, by intrajejunal instillation to one and intravenously to two others (Table 1). Two additional subjects received an oral dose of the disodium salt of $^{35}$S-penicilloic acid prepared from labelled cloxacillin (15 µCi, 20 mg). The oral doses were dissolved in 50 ml water containing 5 g polyethylene glycol (PEG) as an unabsorbable marker (Fordtrac, 1966; Foch, 1969). 100 ml water was drunk immediately after the test solution. For intrajejunal instillation, the drug was injected as a bolus in 5 ml water, followed by 8 ml water to rinse the tube. $^{35}$S-cloxacillin given intravenously was dissolved in 5 ml saline, ultrafiltered and infused during a 5 min period.

All experiments were begun in the morning after an overnight fast. When required, an intestinal double lumen tube was inserted through the nose and allowed to pass to the desired level in the intestine. Its position was checked by X-ray just before the experiment began. Intrajejunal doses were instilled 200 cm from the nose. Gastric juice was sampled through a separate tube passed on the day of the experiment. Gastrointestinal aspirates (2—5 ml) were drawn intermittently until the concentration of radioactivity was less than 3% of the peak level. Cholecystokinin (37.5 Ivy units) was injected intravenously to obtain concentrated duodenal bile (Table 1). The aspirates were analyzed for radioactivity, PEG, pH and, in some instances, bilirubin. Blood samples were collected at intervals for at least 24 h. Urine and faeces were collected continuously for 7 days. Radioactivity was measured in all specimens. The radioactivity in some of the intestinal aspirates and urine samples was fraction-
ated on a Sephadex column or by thin layer chromatography (TLC). The antibacterial activity of some specimens of gastrointestinal aspirates, plasma and urine was also measured. The methods employed are described below.

Stability of $^{35}$S-cloxacillin and penicilloic acid in vitro: $^{35}$S-cloxacillin (0.1 $\mu$Ci, 0.3 mg) was incubated in specimens (0.15 ml) of gastrointestinal aspirates, duodenal bile, urine and 0.1 M buffer solution (Clark-Lub, pH 1—9) for 0.5 and 2 h at 37°C. The stability of $^{35}$S-penicilloic acid of cloxacillin was evaluated by incubation in buffer solutions of pH 1, 2, 5 and 9 for one hour. Paper and/or thin layer chromatography were used in the subsequent fractionation of the incubated radioactivity.

and the penicilloate precipitated by addition of cold (−80°C) ethyl ether. The radio purity of the penicilloic acid was 98 and 96% as indicated by column and thin layer chromatography, respectively. $^{35}$S-labelled 6-APA (6-aminopenicillanic acid) was a gift from Astra AB. Labelled penicic acid was synthesized by incubation of $^{35}$S-6-APA with beta-lactamase (Neutrapen, Merck, Sharpe and Dohme), as described by Kulhánek and Tadra (1968). Unlabelled penicilloic acid from cloxacillin was obtained from Astra AB. Cholecystokinin was purchased from the Gastrointestinal Hormone Research Group, Chemistry Department, Karolinska Institutet, Stockholm, Sweden. Polyethylene glycol (PEG m.w. 4000) was manufactured by KEBO AB, Stockholm and

\begin{table}[h]
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\begin{tabular}{|l|l|l|l|}
\hline
Subject & Compound administered and route of administration & Intestinal aspiration: & Injection of cholecystokinin: \\
 & & distance from nose & time after administration of label \\
 & & (cm) & (h) \\
\hline
1. L.V. & $^{35}$S-cloxacillin, oral & 75, 105 & 4, 7, 10, 24 \\
2. L.-E. G. & $^{35}$S-cloxacillin, oral & 75, 105 & 4, 7 \\
3. H.D. & $^{35}$S-cloxacillin, oral & 80 & 4, 7, 10, 24 \\
4. K.J. & $^{35}$S-cloxacillin, oral & 85, 115 & 4 \\
5. J.L. & $^{35}$S-cloxacillin, oral & 85, 115 & 4, 7, 10, 24 \\
6. L.-E.E. & $^{35}$S-cloxacillin, oral & 90, 120 & 4, 7, 10, 24 \\
7. A.K. & $^{35}$S-cloxacillin, oral & 105, 135 & 4, 7 \\
8. R.K. & $^{35}$S-cloxacillin, oral & 155, 185 & 4, 7 \\
9. A.T. & $^{35}$S-cloxacillin, oral & 165, 195 & 4, 7 \\
10. G.J. & $^{35}$S-cloxacillin, oral & 170, 200 & 4, 7 \\
11. D.D. & $^{35}$S-cloxacillin, intrajejunal & 80 & 4, 7, 10, 22 \\
12. T.R. & $^{35}$S-cloxacillin, intravenous & 80 & 1, 2, 4, 7, 10, 22 \\
13. A.A. & $^{35}$S-cloxacillin, intravenous & 80 & 1, 3 \\
14. A.K. & $^{35}$S-penicilloic acid, oral & 100, 150 & 4, 7 \\
15. K.J. & $^{35}$S-penicilloic acid, oral & 105 & 4, 7 \\
\hline
\end{tabular}
\caption{Experimental procedures}
\end{table}

\paragraph*{b) Materials}

$^{35}$S-cloxacillin (initial specific activity $1.0\, \mu$Ci/mg), the disodium salt of $^{35}$S-penicilloic acid (initial specific activity $0.8\, \mu$Ci/mg) and unlabelled samples of both compounds were obtained from Astra AB, Sodertalje, Sweden. The radiopurity of $^{35}$S-cloxacillin was 99%, as shown by chromatography on a Sephadex column; the corresponding values after fractionation by paper and thin layer chromatography were 99 and 95—97%, respectively. As minor amounts of the radioactivity applied to TLC plates were found to lag behind the major spot, it appears that the radiopurity of the labelled compounds was better represented by the column or paper chromatography than by TLC. $^{35}$S-penicilloic acid was synthesized by alkaline hydrolysis of $^{35}$S-cloxacillin in 0.8 N sodium hydroxide. After evaporation of the solvent, the residue was dissolved in ethyl alcohol and Sephadex G 10 by Pharmacia AB, Uppsala, Sweden. The Clark-Lub buffer solutions were obtained from the Pharmaceutical Department, Sodersjukhuset, Stockholm, Sweden.

\paragraph*{c) Methods}

Radioactivity was measured in a liquid scintillation counter (Packard model 3003 or 2002). Plasma samples (1 ml) were dissolved in 10 ml of an emulsifier (Insta-Gel, Packard). Aliquots of gastrointestinal aspirates (0.1 ml) or urine (1 ml) were pipetted into 15 ml of Bray's (1960) scintillation fluid. Faeces were homogenized and lyophilized, and aliquots of the dry powder combusted and analyzed for radioactivity by a slight modification of the method of Jeffay et al. (1960).

Insta-Gel was used as scintillation liquid. The recovery of label from mixtures of $^{35}$S-cloxacillin