who found that the degradation of a proteinaceous matrix was much slower than the drug release. The remaining drug amount (20 to 40 %) (Fig. 7) diffuses out of the nanocapsules very slowly because of the diminishing concentration gradient over the capsule wall. After 2 hours of drug release no excess crystalline triamcinolone acetonide remains in the capsules and therefore the release rate declines (15).

Figure 8 and Table I show the effects of the production variations with regards to drug content, size distribution, mean size and encapsulation efficiency. For the nanocapsules hardened at pH 10 the size distribution was shifted to greater diameters (Fig. 8). This effect is probably due to thicker capsule walls because of an enhanced coacervation of gelatin at higher pH values leading to a higher amount of deposited wall material (16).

With regard to the encapsulation efficiency no influence can be observed by changing the pH or encapsulating cholesterol (Table I). The lower drug content for the pH 10 and cholesterol nanocapsule batches in comparison with the pH 5.8 nanocapsules reflects the fact that a constant amount of encapsulated drug became surrounded by a higher amount of wall material. Moreover, Table I shows that the nanocapsules having a similar mean diameter as the nanoparticles possess a much higher drug content than the nanoparticles.

In conclusion the present investigation demonstrates that the described production process yielded nanocapsules with a high drug content in comparison with nanoparticles. Furthermore, it is possible to encapsulate lipophilic drugs, whereas the methods described by Widder (6), Oppenheim (7) and Yoshioka (5) are only suitable for hydrophilic substances. Moreover, these nanocapsules can be used as a drug delivery system to direct lipophilic drugs ot organs of the RES, because of their accumulation in liver, spleen and kidney after intravenous application (2).

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Effect of Salicylate on the Uptake of Cefmetazole into Brain of Mice

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Abstract: Cefmetazole distribution into mice cerebral cortex was minimal when the drug was administered alone. However, the co-administration of salicylate or diethyl maleate enhanced cefmetazole uptake into the cerebral cortex, while it decreased the level of reduced nonprotein sulfhydryls in cerebral cortex. The enhanced cerebral uptake of cefmetazole was suppressed by the simultaneous administration of cysteamine with a concomitant recovery of the reduced nonprotein sulfhydryl concentration in cerebral cortex.

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Rat colonic membrane permeability against polar compounds was previously shown to correlate with reduced nonprotein sulfhydryls levels in intestinal tissue (1); moreover, it has been suggested that the integrity of brain tissue may be controlled by glutathione (2). Therefore, agents such as diethyl maleate (1) and salicylate (3) that affect sulfhydryl levels and increase the colonic membrane permeability, may also change the uptake of polar compounds into brain tissue through the blood brain barrier.

In the present study, the effect of either diethyl maleate or salicylate on the distribution of cefmetazole into brain tissue of mice was examined.

Materials and Methods

Materials. Sodium cefmetazole was supplied from Sankyo Co. Ltd. (Tokyo, Japan). Sodium salicylate was obtained from Nakarai Chemicals Co. Ltd. (Kyoto, Japan). Diethyl maleate and cysteamine were obtained from Sigma Co. Ltd. (Mo. USA). Other reagents used were of analytical grade.

Animals. Male, d,d-strain mice, 45 to 50 g, were used; experiments were carried out at 10 a.m.

Uptake study of drugs into mice cerebral cortex. At 30 min after *i.p.* injection of 1 ml/kg saline solution containing cefmetazole with or without diethyl maleate or sodium salicylate, blood samples from the aorta and cerebral cortex tissues were collected. Blood samples were centrifuged to obtain plasma for the drug assay. Cerebral cortex was gently rinsed with saline immediately, after excision and weighed. Homogenized cerebral cortex was employed for the assay of drugs and reduced nonprotein sulfhydryls. Pretreatment with diethyl

maleate of mice cerebral cortex was carried out 15 min before the *i.p.* injection of cefmetazole. Diethyl maleate was injected freehand into the lateral ventricle of unanesthetized mice, using a modification of the procedure reported by Clark et al. (4). The injection needle was 26 gauge 5/8" and polyethylene-sleeved to expose only 3 mm of the needle chip. The mouse was held firmly behind the head and the injection was made at a point 2 mm lateral from an imaginary midsagittal line and at the intersection of an imaginary line drawn through the anterior base of the ears, orienting the needle perpendicular to the skull. The needle was pushed through the bone to the full 3 mm depth with the outer edges of the polyethylene sleeve just touching the scalp.

Drug Assays. Assays of cefmetazole and salicylate were carried out by a high pressure liquid chromatographic method described in a previous paper (5). The assay of reduced nonprotein sulfhydryls in cerebral cortex was carried out according to the method described by Owen and Belcher (6).

Results

Uptake of salicylate into cerebral cortex in mice after *i.p.* injection is shown in Fig. 1B. The fraction of the salicylate dose reaching the cerebral cortex was slightly increased with increasing salicylate dose and plasma concentration (Fig. 1A). Uptake of salicylate into cerebral cortex was not affected by the coadministration of cefmetazole (Fig. 1B). Further the increase of salicylate dose resulted in a significant decrease of reduced nonprotein sulfhydryl levels in mice cerebral cortex (Fig. 1C).

Distribution of cefmetazole into cerebral cortex after *i.p.* injection of cefmetazole alone at a dose of 22.5 mg/kg was 0.043 ± 0.021 % against the dose (n = 8), as shown in Fig. 2. The coadministration of salicylate at various doses of 3.75 to 50 mg/kg increased the fraction of the cefmetazole dose in the cerebral cortex (Fig. 2). The cerebral uptake of cefmetazole was inversely related to the amount of reduced nonprotein sulfhydryls levels in cerebral cortex, which decreased with increasing salicylate levels in cerebral cortex

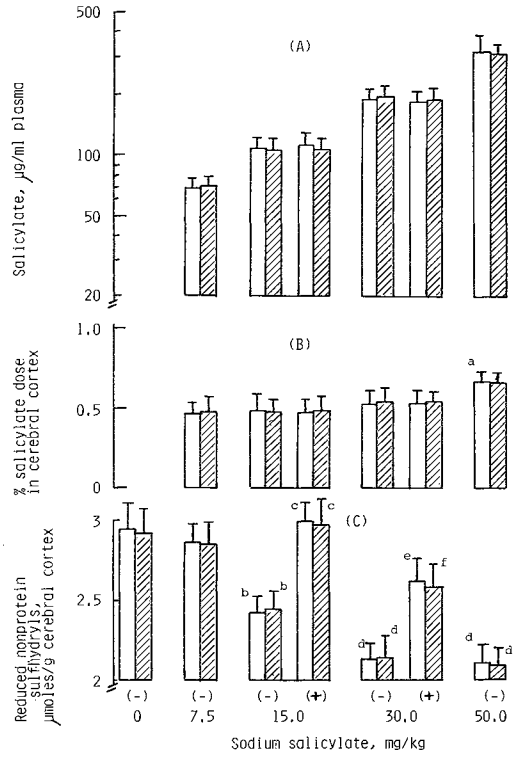

Fig. 1 Effect of salicylate dose on the plasma salicylate concentration (A), uptake of salicylate into cerebral cortex (B), and reduced nonprotein sulfhydryl levels in cerebral cortex (C) 30 min after *i.p.* injection of sodium salicylate without (–) or with (+) cysteamine (50 mg/kg). Columns, □ and ■, represent the results without and with sodium cefmetazole (22.5 mg/kg), respectively. Each value represents the mean ± S.D. (n = 8). [a]$p < 0.1$ versus the results obtained when sodium salicylate was administered at 7.5 mg/kg. (Student's t-test). [b]$p < 0.05$ versus no salicylate. [c]$p < 0.05$ versus no cysteamine, [d]$p < 0.01$ versus no salicylate. [e]$p < 0.05$ versus no cysteamine, [f]$p < 0.1$ versus no cysteamine.

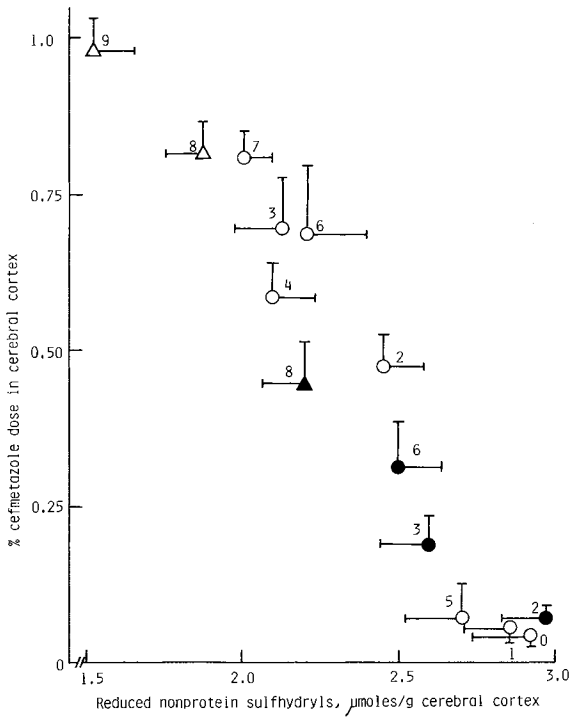

Fig. 2 Effect of salicylate or diethyl maleate (open symbols) on the uptake of cefmetazole into mice cerebral cortex and the concentration of reduced nonprotein sulfhydryls in cerebral cortex 30 min after *i.p.* injection of sodium cefmetazole (22.5 mg/kg) with salicylate or diethyl maleate, or 30 min after *i.p.* injection of sodium cefmetazole (22.5 mg/kg) at 15 min after pretreatment with diethyl maleate into the brain. Each number represents the dose of salicylate and diethyl maleate as follows; 0: no additive, 1: 7.5 mg sodium salicylate/kg, 2: 15 mg sodium salicylate/kg, 3: 30 mg sodium salicylate/kg, 4: 50 mg sodium salicylate/kg, 5: 2.5 mg diethyl maleate/kg, 6: 7.5 mg diethyl maleate/kg, 7: 15 mg diethyl maleate/kg, 8: 1.0 mg diethyl maleate/kg, and 9: 2.5 mg diethyl maleate/kg. For 0 to 7 salicylate or diethyl maleate were coadministered by *i.p.* injection. For 8 and 9, the dose of diethyl maleate was injected into brain 15 min before the *i.p.* injection of cefmetazole solution. Closed symbols represent the results obtained when cysteamine at a dose of 50 mg/kg was coadministered with cefmetazole by *i.p.* injection. Each value represents the mean ± S.D. (n = 8).

(Fig. 1 C and 2). Although the simultaneous administration of cysteamine at a dose of 50 mg/kg by *i.p.* injection did not affect the uptake of salicylate into cerebral cortex (Fig. 1 B), coadministration of cysteamine reversed the loss of reduced nonprotein sulfhydryls levels and concomitantly suppressed the enhanced uptake of cefmetazole into cerebral cortex by salicylate (Fig. 2).

Diethyl maleate which was coadministered with cefmetazole by *i.p.* injection or was injected into brain 15 min before the *i.p.* injection of cefmetazole solution also increased the uptake of cefmetazole into cerebral cortex in a dose-dependent fashion and decreased reduced nonprotein sulfhydryl levels in cerebral cortex (Fig. 2). Coadministration of cysteamine at a dose of 50 mg/kg again restored reduced nonprotein sulfhydryl levels in cerebral cortex and suppressed the increased uptake of cefmetazole into cerebral cortex by diethyl maleate.

Discussion

Diethyl maleate and salicylate enhance the cerebral uptake of cefmetazole, a polar compound, probably by modifying the blood-brain barrier via a decrease of reduced nonprotein sulfhydryl levels in cerebral cortex as was previously observed with intestinal membranes (1, 3, 7, 8). Since simultaneous administration of cysteamine suppressed at least partly the enhancing action of salicylate and diethyl maleate on the uptake of cefmetazole into cerebral cortex with the recovery of reduced nonprotein sulfhydryls in cerebral cortex, it can be suggested that the permeability of the blood brain barrier may be regulated to some degree by reduced nonprotein sulfhydryls. On the other hand, the slight increase of the fraction of salicylate doses reaching the cerebral cortex with increasing doses may be partly due to the increase of free salicylate fraction in plasma because of saturable salicylate binding to albumin.

The present study documents that salicylate increases the uptake of cefmetazole into cerebral cortex. From these results, it may be considered that bacterial infection in brain tissue can be treated more effectively by the concomitant administration of cefmetazole with salicylate. Since antiinflammatory salicylate doses result in salicylate plasma levels of 150 to 300 μg/ml in patients (10), one should also consider that combined treatment of drugs with salicylate may result in unexpected side effects through the modification of drug distribution into brain tissue.

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