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Fate of Sulpyrin in Rat Muscle after Intramuscular Injection

A rapid separative determination of sulpyrin, 4-methylaminoantipyrine and the metabolites in muscle was performed by the use of high-speed liquid chromatography. After intramuscular injection of sulpyrin in rats, a rapid removal of the drug from the muscle was observed.

Keywords——sulpyrin; separative determination; high-speed liq. chromatog.; rat muscle; fate in muscle

Recently, considerable attentions have been focused on the contractures of muscles, mainly quadriceps femoris, deltoid and gluteus, caused by repeated intramuscular injections of drugs at the same site of infants.[1] Among the drugs which were recognized as the agents for the tissue damage, sulpyrin has been widely used as an antipyretic drug.[2]

In our preliminary examination with rats, 4-methylaminoantipyrine (MAA), pharmacologically active hydrolyzate of sulpyrin, caused the similar tissue damage to that by the drug.[3] As a possible factor to cause such damage, the retention of sulpyrin, MAA and further metabolites in the tissue was investigated.

Several different procedures for the determination of sulpyrin and MAA have been reported,[4] however, these methods had limited sensitivity and specificity for the direct determination of a small quantity of sulpyrin and others in the muscle. One of the reasons was that sulpyrin in aqueous solution was easily split into MAA and hydroxymethanesulfonate to reach equilibrium. The difficulties were overcome by the use of methanol for solvent of homogenization of the muscle in order to suppress the dissociation of sulpyrin, and by the use of a high-speed liquid chromatography (HSLC) for determination of sulpyrin and others.

Wistar male rats (280—330 g) were placed in supine position without anesthesia. The quadriceps femoris of the right side was carefully injected with the solution of 12.5 mg of sulpyrin in 25 μl of water, its osmotic pressure was about 12 times as large as physiological one, through a 100 μl syringe fitted with 26-gage needle. The muscle of the left side was used as the control to check the inflow of sulpyrin and others into the muscle from the systemic circulation. At various times after the injection, the animals were sacrificed by decapitation. The whole quadriceps femoris (about 2 g) was dissected from other tissues, immediately placed in a disperser (Hansen Co., Ultra Turrax TP-10, West Germany), added with methanol and homogenized in ice bath. The homogenate was added with the methanolic solution of antipyrine as an internal standard, and with methanol to make up 7 ml. The mixture was centrifuged for 10 min at 3500 rpm in a centrifugal separator (Kubota KC-70, Tokyo, Japan) and the supernatant was filtrated with a membrane filter of pore size 10 μm (Nihon Millipore Ltd., Tokyo, Japan). Twenty microliters of the filtrate was applied to a Shimazu model 830 HSLC equipped with a column of 500×4 mm i.d. glass tube packed with Bondapak (Nihon Waters Ltd., Tokyo, Japan) and a fixed-wavelength (254 nm) UV detector. The mobile phase was 0.05 M ammonium acetate (adjusted pH 6.0 with 0.01 N HCl)–acetonitrile (80: 20,

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2) Y. Miyata, *Acta Paed. Jap.*, **80**, 851 (1976); Y. Uchida, *Oyoyakuri*, **12**, 95 (1976); M. Umeda, K. Takatori and M. Saitoh, *Igaku no Ayumi*, **100**, 447 (1977).
3) H. Nakamura, Y. Okuno, S. Terai, S. Isozaki and Z. Tamura, unpublished data.
4) K. Kato, M. Umeda and S. Tsubota, *Yakuzaigaku*, **24**, 116 (1964); S. Ono, R. Onishi and K. Kawamura, *Yakugaku Zasshi*, **86**, 11 (1966); H. Nogami, M. Hanano, S. Awazu and K. Imaoka, *Yakugaku Zasshi*, **90**, 378 (1970).

v/v) and its flow rate was 2.0 ml/min. The UV absorbance detector was set at 0.02 absorbance unit scale. All analyses were performed at ambient temperature.

The good separation of biological constituents, sulpyrin and others was obtained as shown in Fig. 1, in which, only sulpyrin and MAA were actually detected in the muscle. Linearity of the peak height ratio with their amount in muscle was attained between 0.18 and 1.4 mg.

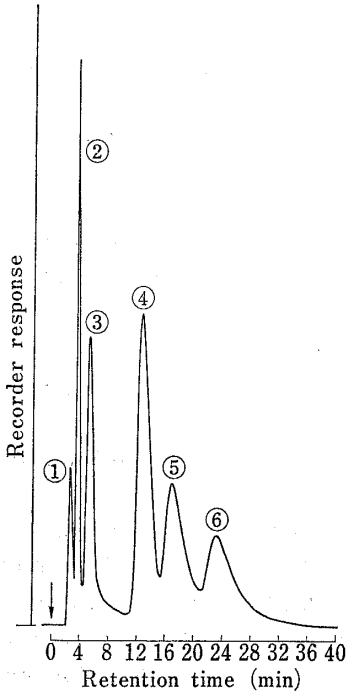

Fig. 1. Chromatogram of Sulpyrin and Others

Peak ①, biological constituents; ②, sulpyrin; ③, 4-acetylaminoantipyrine; ④, antipyrine (internal standard); ⑤, 4-aminoantipyrine; ⑥, 4-methylaminoantipyrine.

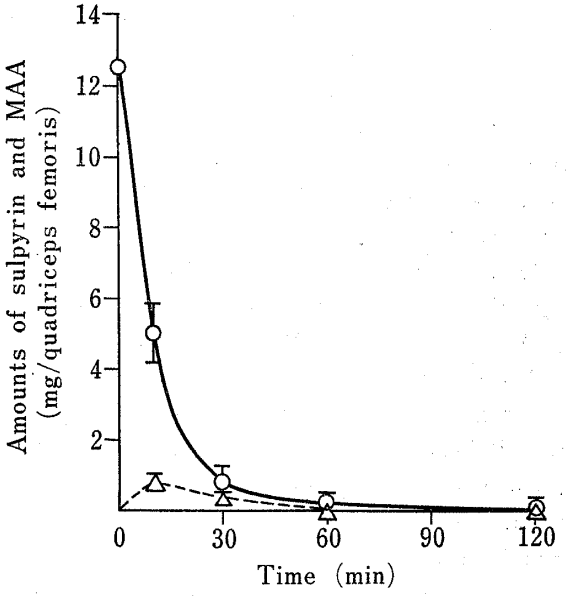

Fig. 2. Fate of Sulpyrin in Rat Muscle after Intramuscular Injection in a Dose of 12.5 mg ($n=3$, mean $\pm$ S.E.)

—○—, sulpyrin,
---△---, 4-methylaminoantipyrine (MAA).

The amount of sulpyrin decayed exponentially with time as shown in Fig. 2. The elimination rate constant (k) and the half life were estimated by the use of the first order kinetic equation ($C=C_0e^{-kt}$, C_0: initial amount of sulpyrin) as 8.8×10^{-2} min^{-1} and 7.9 min, respectively. Negligible amount of the drug in the control muscle was detected at any time after injection.

These results suggest that the removal of sulpyrin from the muscle is fairly rapid similar to those of other water soluble compounds such as benzylpenicillin and urea,[5] and that the conversion of sulpyrin into MAA in muscle is comparably slow.

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