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Effect of Simultaneous Administration of Drugs on Absorption and Excretion. XII.[1] Effect of Salicylic Acid on Hypoglycemic Activity and Blood Concentration of Carbutamide in Rabbits[2]

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The effect of salicylic acid on the hypoglycemic activity and blood carbutamide concentration after oral administration of carbutamide was investigated in rabbits. Salicylic acid was found to enhance the hypoglycemic activity, while on the other hand, it was found to decrease the blood carbutamide concentration. Salicylic acid markedly decreased the *in vivo* binding of carbutamide to rabbit plasma proteins, and caused a significant increase in the plasma concentration of unbound carbutamide, despite a significant decrease in the plasma concentration of total carbutamide. These results lead us to conclude that the displacement of carbutamide from its plasma protein binding sites by salicylic acid produces both the enhanced hypoglycemic activity and the decreased blood carbutamide concentration.

Keywords——carbutamide; salicylic acid; hypoglycemic activity; blood concentration; plasma protein bonding; rabbit

Many investigators have pointed out that salicylic acid markedly enhances the hypoglycemic activity of sulfonylureas.[3] In addition, Wishinsky *et al.*[4] and Brown *et al.*[5] have revealed that salicylic acid displaces sulfonylureas from their plasma protein binding sites. Thus, it is suggested that the displacement of sulfonylureas from their plasma protein binding sites by salicylic acid may result in enhanced hypoglycemic activity of sulfonylureas. However, since salicylic acid has recently been reported to decrease the blood (plasma) concentration of many acidic drugs[6] such as sulfadimethoxine and fenoprofen, the interactions between sulfonylureas and salicylic acid may be more complex.

The present study was undertaken to elucidate the effect of salicylic acid on the hypoglycemic activity and the blood concentration of carbutamide orally administered to rabbits.

Experimental

Materials——Carbutamide was kindly supplied by Ono Pharmaceutical Industry Co., Ltd. N^4-Acetylcarbutamide was synthesized from sulfanilamide as described previously.[7] Tolbutamide and chlorpropamide were kindly supplied by Hoechst Japan Co., Ltd. and Ono Pharmaceutical Industry Co., Ltd., respectively. Salicylic acid and other chemicals were obtained commercially.

***In Vivo* Experimental Method**——Male albino rabbits weighing 2.5—3.5 kg (Kuroda Junkei-Doubutsu, Kumamoto) were fasted for 38—42 h prior to the experiments but allowed free access to water.

a) Oral Administration: Carbutamide (200 mg/kg, 100 mesh powder) alone or in combination with salicylic acid (100 mg/kg, 100 mesh powder) was suspended in 40 ml of distilled water. The suspension was injected into the stomach through a rubber tube. Moreover, the glass syringe used was washed with 30 ml of distilled water and the washing was injected into the stomach through the rubber tube.

b) Intravenous Administration: N^4-Acetylcarbutamide (50 mg/kg as carbutamide) was dissolved in 1—2 ml of saline solution by adding the same molar amount of NaOH and administered intravenously.

c) Blood Sampling: Blood samples (0.5 ml) were collected periodically from the ear vein.

Ultrafiltration Method——Ultrafiltration was carried out as described previously.[7] The percentage binding of carbutamide to rabbit plasma proteins was calculated from the difference between the carbutamide concentrations in rabbit plasma and in its ultrafiltrate.

Analytical Method——The carbutamide concentrations in blood, plasma and ultrafiltrate were measured

by the Bratton–Marshall method.[8] Blood, plasma and ultrafiltrate samples were deproteinized with 10% trichloroacetic acid. The samples were vortex-mixed and then centrifuged at 3000 rpm for 10 min. The supernatants were used for measuring carbutamide concentration. The glucose levels in blood were measured by the Somogyi–Nelson method.[9]

Statistical Analysis——Statistical analyses were performed by the paired Student *t*-test. A *p*-value of 0.05 or less was considered significant.

Results and Discussion

Figure 1 shows the time course of hypoglycemic activity after oral administration of carbutamide alone or in combination with salicylic acid. The hypoglycemic activity was expressed as the % change in blood glucose levels before and after drug administration. Salicylic acid significantly enhanced the hypoglycemic activity after oral administration of carbutamide. Since salicylic acid itself did not possess hypoglycemic activity under the present experimental conditions, as shown in Fig. 2, salicylic acid was expected to increase the blood carbutamide concentration. However, salicylic acid caused an unexpected decrease in the blood carbutamide concentration after oral administration of carbutamide (Fig. 3). These findings imply that when carbutamide and salicylic acid are coadministered, the utilization of blood concentration data alone are inadequate for evaluating the potency of hypoglycemic activity.

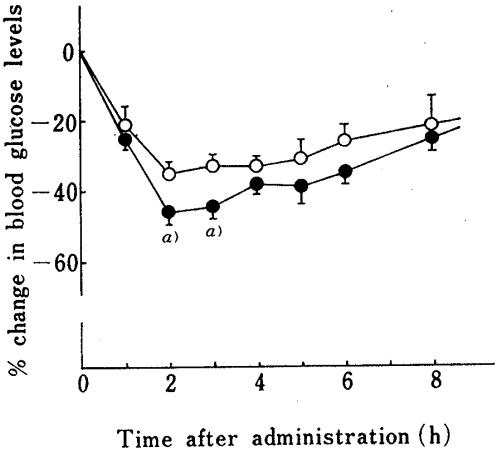

Fig. 1. Time Course of Hypoglycemic Activity after Oral Administration of Carbutamide alone or in Combination with Salicylic Acid

Each point represents the mean ± S.E. of 4 rabbits.
—○—, carbutamide alone; —●—, with salicylic acid. *a*) $p<0.05$.

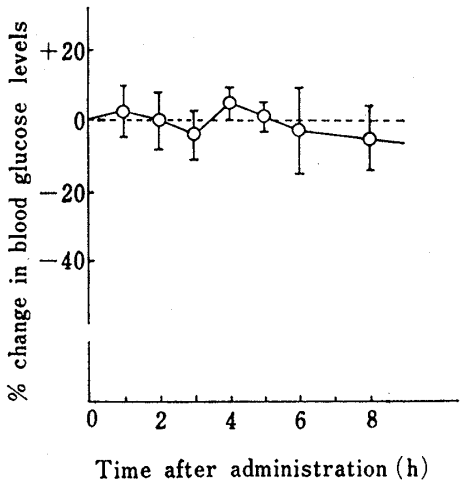

Fig. 2. Time Course of Hypoglycemic Activity after Oral Administration of Salicylic Acid

Each point represents the mean ± S.E. of 5 rabbits.

The binding of carbutamide to rabbit plasma proteins 1.0 h after oral administration of carbutamide alone or in combination with salicylic acid was determined according to the ultrafiltration method described previously.[7] As shown in Fig. 4, salicylic acid markedly decreased the *in vivo* binding of carbutamide to rabbit plasma proteins. In addition, salicylic acid was found to markedly decrease the *in vitro* binding of carbutamide to rabbit plasma proteins (Fig. 5). These findings suggest that the displacenent of carbutamide from its plasma protein binding sites by salicylic acid may change the usual relationship between the hypoglycemic activity and the blood carbutamide concentration. Ariëns,[10] Levy[11] and Sheiner *et al.*[12] have already pointed out that the displacement of one drug from its plasma protein binding sites by another drug raises some questions regarding the significance of blood drug concentration as an indicator of pharmacological activity.

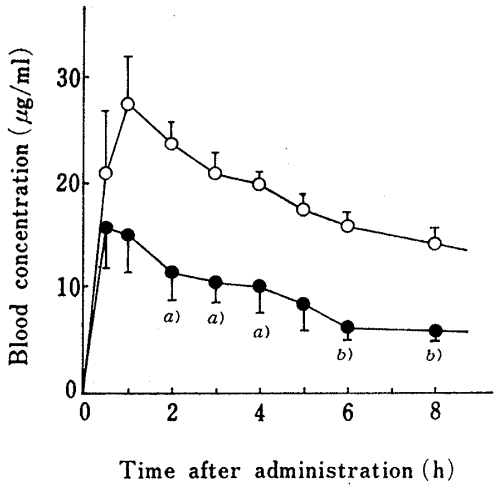

Fig. 3. Time Course of Blood Carbutamide Concentration after Oral Administration of Carbutamide alone or in Combination with Salicylic Acid

Each point represents the mean ±S.E. of 4 rabbits.
—○—, carbutamide alone; —●—, with salicylic acid.
a) $p<0.05$, *b*) $p<0.01$.

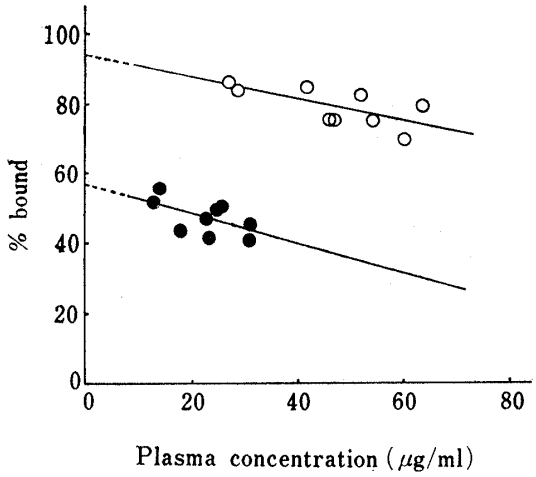

Fig. 4. Effect of Salicylic Acid on the *in Vivo* Binding of Carbutamide to Rabbit Plasma Proteins

○, carbutamide alone ($Y=94.2-0.32X$); ●, with salicylic acid ($Y=56.5-0.41X$).

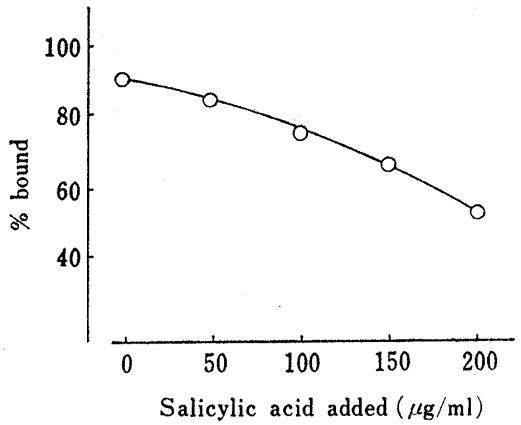

Fig. 5. Effect of Salicylic Acid on the *in Vitro* Binding of Carbutamide to Rabbit Plasma Proteins

Carbutamide added: 50 μg/ml.

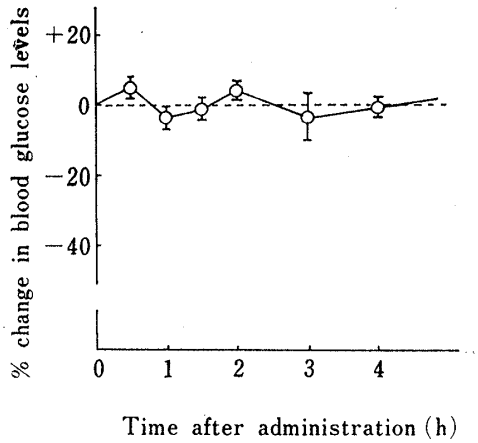

Fig. 6. Time Course of Hypoglycemic Activity after Intravenous Administration of N^4-Acetylcarbutamide

Each point represents the mean ± S.E. of 4 rabbits.

The major metabolite of carbutamide in rabbits is well known to be N^4-acetylcarbutamide. For example, Root[13] has reported that from 20 to 60% of carbutamide in rabbit's blood after oral administration of carbutamide is in the N^4-acetylated form. We also obtained a similar result in rabbits.[7] If N^4-acetylcarbutamide possesses hypoglycemic activity, it will change the usual relationship between the hypoglycemic activity and the blood carbutamide concentration. However, it is evident from the result shown in Fig. 6 that N^4-acetylcarbutamide does not possess hypoglycemic activity. Recently, we have demonstrated that N^4-acetylcarbutamide decreases the *in vitro* binding of carbutamide to rabbit plasma proteins.[7] This suggests that the displacement of carbutamide from its plasma protein binding sites by N^4-acetylcarbutamide may be partly responsible for the enhanced hypoglycemic activity after oral administration of carbutamide in combination with salicylic acid. Detailed studies are in

progress to elucidate whether N^4-acetylcarbutamide has any effect on the displacement of carbutamide from its plasma protein binding sites by salicylic acid.

Figure 7 shows the plasma concentrations of unbound and total (unbound+bound) carbutamide 1.0, 2.0 and 3.0 h after oral administration of carbutamide alone or in combination with salicylic acid. Salicylic acid caused a significant increase in the plasma concentration of unbound carbutamide, but a significant decrease in the plasma concentration of total carbutamide. These findings indicate that since only the unbound fraction of the drug in plasma is available for direct exchange with the connected compartment, a part of the carbutamide displaced from its plasma protein binding sites by salicylic acid is redistributed into the tissue compartment until equilibrium is established between plasma and tissue compartments. Therefore, it is concluded that the displacement of carbutamide from its plasma protein binding sites by salicylic acid produces both the enhanced hypoglycemic activity and the decreased blood carbutamide concentration.

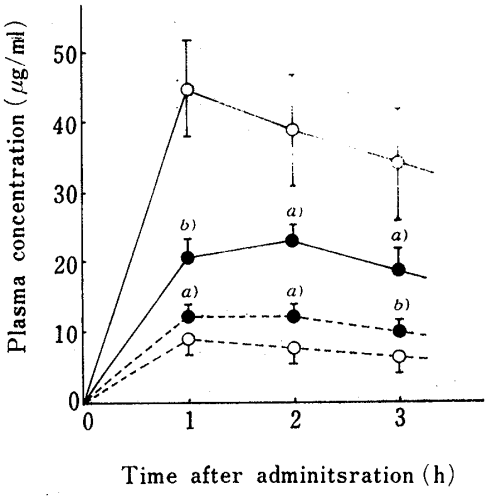

Fig. 7. Time Course of Plasma Concentration of Unbound and Total Carbutamide after Oral Administration of Carbutamide alone or in Combination with Salicylic Acid

Each point represents the mean±S.E. of 6 rabbits. --○--, carbutamide alone (unbound carbutamide); —●—, with salicylic acid (unbound carbutamide); —○—, carbutamide alone (total carbutamide); —●—, with salicylic acid (total carbutamide). *a*) $p<0.05$, *b*) $p<0.01$.

At 1.0 h after oral administration of carbutamide, salicylic acid caused a significant increase in the plasma concentration of unbound carbutamide, but did not cause a significant enhancement of the hypoglycemic activity (see Fig. 1 and Fig. 7). This discrepancy may arise from experimental errors, since two distinct rabbit groups were used in these experiments.

In this study, we have obtained evidence that salicylic acid significantly enhances the hypoglycemic activity after oral administration of carbutamide. A similar interaction was

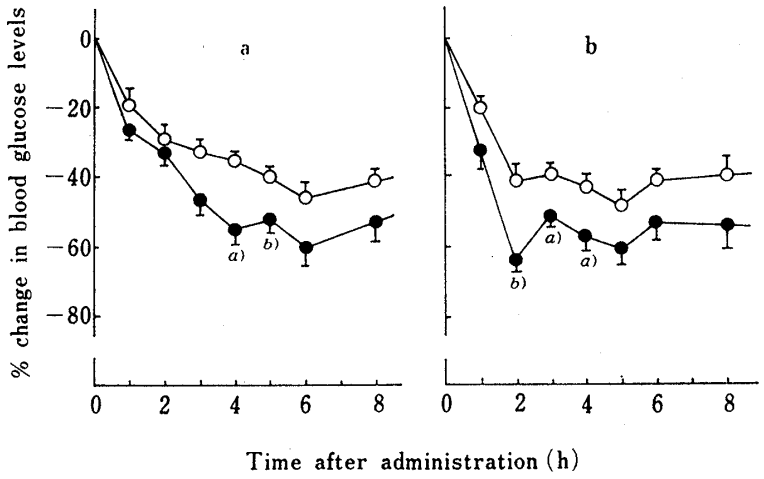

Fig. 8. Time Course of Hypoglycemic Activity after Oral Administration of Sulfonylureas alone or in Combination with Salicylic Acid

Each point represents the mean±S.E. of 5 or 6 rabbits. a, tolbutamide; b, chlorpropamide; —○—, sulfonylureas alone; —●—, with salicylic acid. *a*) $p<0.05$, *b*) $p<0.01$.

observed when other sulfonylureas, tolbutamide and chlorpropamide, were orally administered in combination with salicylic acid (Fig. 8). This finding suggests that the displacement of tolbutamide or chlorpropamide from its plasma protein binding sites by salicylic acid may be an important factor enhancing the hypoglycemic activity. However, further studies are necessary, because it has also been proposed that salicylic acid enhances the hypoglycemic activity of these two sulfonylureas by interfering with the renal tubular secretion.[3)]

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References and Notes

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