Cellular Effect of Combined Food Additives on Platelet Function

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Abstract

Food additives have contributed to safe and stable supply of food. Although acceptable daily intake (ADI) has been used for the safety assessment of each food additive, their synergistic and/or combined effects on our health are unclear.

Actually, several food additives are usually used together in a food, therefore, they may have some adverse effect on cellular levels and/or whole animals even if their individual used levels are below ADI, or commercially used levels. In this paper, we carried out to investigate their cellular effects as well as whole body effects in case of combined use on ADI and daily intake level. Two commonly used preservatives (potassium sorbate and sodium benzoate) and four artificial colors (Yellow No.4 and 5, Red No.102 and 106), which have been certified by the Japanese Standard of Food Additives, were chosen and used.

Each preservative was dissolved in Ca$^{2+}$, Mg$^{2+}$ free Tyrode buffer pH 7.4 at final concentrations corresponded to 10mg/mL (potassium sorbate) and 1.77mg/mL (sodium benzoate), and each color was used at concentrations of 0.01%.

Washed rabbit platelets were prepared by the method described. An aliquot (2 x $10^7$ platelets/mL) was incubated with calcium ionophore A-23187 (10$^{-6}$M) or thrombin (1U), or without either agonist in the presence or absence of preservative and coloring mixture.

In addition, commercially used amount of each preservative and color were added together to drinking water and administered to a rabbit for 5 days, then washed platelets were prepared and subjected to incubation.

Incubation lasted for 5 min at 37°C with vigorous shaking and was terminated by placing the reaction mixture on ice followed by centrifugation. The magnitude of platelet activation was determined by the amount of thromboxane B$_2$ (TXB$_2$) synthesis and the extent of platelet aggregation.

In spite of an inhibitory effect of these additives when each of them was used, in combinations of preservative and 2 or 3 artificial colors used in this experiment, no inhibitory effect was observed on both agonists induced TXB$_2$ synthesis (Table 1, 2) or platelet aggregation in vitro. On the other hand, in an ex vivo experiment, an inhibitory effect was observed at A-23187 or thrombin induced TXB$_2$ synthesis in combination of potassium sorbate and 2 (Yellow No.4 and 5) or 3 (Yellow No.4, 5 and Red 102, Yellow No. 4, 5 and Red 106) colors (Fig. 1, 2).

Platelet aggregation was also inhibited or delayed in combinations of these additives. In contrast, no significant change was observed in blood biochemical assay data (Table 5).

These results indicate that it is important to consider both an interactive effect of food additives used together and an effect on cellular level when we make safety assessment of food additives.