Safety Assessment of Combinative Use of Food Additives by Platelet Function
(Received July 1, 1999)
(Accepted October 2, 1999)

Hiroyasu Yamazaki, Takatoshi Yoneda, and Takako Yamaguchi
Faculty of Pharmaceutical Sciences, Kobe-Gakuin University

Keywords: Food additives, Combinative use of preservative and artificial red colors, Daily intake level, Platelet function, Blood biochemical assay

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 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 simultaneous use on ADI and daily intake level.

Two commonly used preservatives (sodium benzoate and potassium sorbate) and four artificial red colors (Nos.3, 40, 102 and 106) 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 1/10 ADI and 1/40 ADI, and each red colors was used at concentrations of 0.01% and 0.001%.

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

Commercially used amount of each preservative and color was 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 B₂ (TXB₂) synthesis and the extent of platelet aggregation.

Among several combinations of preservative and artificial red color, mixtures of sodium benzoate and red No.3 had inhibitory effect on both agonists' induced TXB₂ synthesis (Fig.1, 2) in vitro. On the other hand, mixtures of potassium sorbate and red colors revealed no effect on both agonists induced TXB₂ synthesis (Fig.3, 4).

In an ex vivo experiment, only a weak inhibitory effect was observed at thrombin induced TXB₂ synthesis in combination of potassium sorbate and red No.106 (Fig.6B). There was not any significant effect on platelet aggregation (Fig.7-10) or blood biochemical assay data (Table 1, 2).

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