Effect of tea catechins on erythrocyte Ca++-pump in type 2 diabetes mellitus

Syed Ibrahim Rizvi, and Mohd Abu Zaid

Department of Biochemistry, University of Allahabad, Allahabad, India

Abstract
An altered intracellular calcium metabolism is known to play an essential role in the pathophysiology of diabetes and its complications. Tea [Camellia sinensis L. (Theaceae)] contains polyphenolic compounds collectively known as catechins belonging to the flavonoids family. Catechins have been reported to possess several biological properties including an antidiabetic effect. The present paper reports the effect of tea catechins on erythrocyte membrane Ca++-pump in normal and type 2 diabetic patients. The activity of erythrocyte Ca++-ATPase was significantly decreased in type 2 diabetes. In vitro incubation with tea catechins (10⁻⁴ M) caused an increase in the activity of Ca++-ATPase in both normal and type 2 diabetic subjects; the effect was more pronounced in type 2 diabetes and was observed at much lower concentrations (10⁻⁵ M) compared to normal (10⁻³ M). The order of effectiveness of individual catechins was in the order: EGCG > EGC > EC > EGC. The increase in Ca++-ATPase activity after incubation with tea catechins may be explained on the basis of alteration in fluidity due to a direct effect of catechins on the membrane.

Keywords: Diabetes mellitus; tea catechins; Ca-pump; erythrocyte

Introduction
Diabetes mellitus makes an individual prone to various complications such as macro and microvascular disease, hypertension, neuropathy, cataracts, cardiomyopathy, and premature aging, thereby indicating that these complications develop through a similar pathway common to diabetic conditions (Brownlee, 2005). It is known that intracellular calcium concentration is increased in most tissues in the diabetic condition, and this altered intracellular calcium metabolism seems to result from a common, underlying abnormality linking the metabolic, cardiovascular, ocular, and neural manifestations of the diabetic disease process (Levy, 1994).

The red blood cell along with its membrane has always been an important medium for study due to the important role it plays in various physiological and metabolic aspects. Several alterations in erythrocytes have been reported in type 1 and type 2 diabetes mellitus (Watala, 1993).

Membrane-bound calcium-transporting proteins are important in regulating various signal functions of calcium (Carafoli, 1987). The regulation of this calcium is performed by Ca++-ATPase or calcium pump. An altered activity of erythrocyte membrane Ca++-ATPase has been reported in the diabetic condition (Gonzalez-Flecha et al., 1990). Reduced membrane Ca++-ATPase activity, as seen in type 2 diabetics, may be responsible for an increase in intracellular calcium and, consequently, for elevated vascular resistance which is frequently associated with hypertension (Zemel et al., 1990).

Tea – Camellia sinensis L. (Theaceae) – is the most popular beverage worldwide. Epidemiological studies indicate that tea consumption may reduce the risk of cardiovascular disease (Tijburg et al., 1997). Tea contains polyphenolic compounds (collectively known as catechins) belonging to the flavonoid family. Quantitatively, the most important catechins in green tea are: epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) (Figure 1). Catechins are known to possess antioxidant, anticancer, cardioprotective, vasorelaxant, and hypoglycemic activities (Cabrera et al., 2006). The exact mechanisms underlying these
Effect of tea catechins on Ca++-pump remain speculative. In earlier reports we have demonstrated the effect of tea catechins on biomarkers of oxidative stress on diabetic erythrocytes (Rizvi et al. 2005) and on erythrocyte Na/H antiport (Rizvi & Zaid, 2005). The present study was undertaken to evaluate the effect of tea catechins (EGCG, EGC, ECG, and EC) on erythrocyte Ca++-ATPase in normal (control) and type 2 diabetic patients.

Materials and methods

Selection of subjects

The criteria for selection of type 2 diabetic patients were the same as reported earlier (Rizvi et al., 2005; Rizvi & Zaid, 2005). Blood from 31 diabetic patients (18 men, 13 women) was taken after informed consent had been obtained from all patients, with the following characteristics: mean age 58 ± 7 years, fasting plasma glucose level 183.5 ± 42.4 mg/dL, BMI 27 ± 4 kg/m², total plasma cholesterol 5.4 ± 1.3 mM/L and duration of diabetes was 12 ± 5 years. None of the patients had high blood pressure or microalbuminuria. Care was also taken to exclude patients who had a family history of hypertension.

The control group consisted of 31 healthy volunteers age and sex matched with diabetic subjects, mean age 56 ± 8 years, fasting plasma glucose level 85.2 ± 14.4 mg/dL, BMI 24.8 ± 3.8 kg/m², and total plasma cholesterol 5.3 ± 1.3 mM. None of the controls was affected by hypertension. Care was taken to select control subjects with no family history of diabetes mellitus or hypertension (two generations). None of the women studied was receiving any hormonal treatment. All volunteers (diabetic patients and normal subjects) were informed about the nature of the study.

Collection of blood, isolation of packed RBC and preparation of ghosts

Venous blood was collected from control and type 2 diabetic patients after an overnight fast using ACD (citric acid/sodium citrate/dextrose) as anticoagulant. The blood sample was kept at 37°C for 3 h prior to experiments for degradation of endogenous insulin. The blood sample were centrifuged at 4°C for 10 min at 1000 g to remove plasma and buffy coat and the isolated erythrocytes were washed 4 to 5 times with 0.154 M NaCl, and finally packed erythrocytes were obtained. The erythrocyte membrane from leukocyte-free red cells were prepared following the method of Marchesi and Palade (1967), that involves the principle of osmotic shock treatment with hypotonic and hypertonic buffers (pH 7.4).

Determination of erythrocyte membrane Ca++-ATPase activity

Ca++-ATPase activity was assayed as described earlier (Rizvi & Luqman, 2003). The assay mixture (2.25 mL) contained 80 mM NaCl, 15 mM KCl, 3 mM MgCl₂, 18 mM Tris-HCl (pH 7.4), 0.1 mM ouabain, 0.1 mM
EGTA, 0.2 mL of the membrane solution containing 0.4 to 1.5 mg membrane protein per mL and ± 0.2 mM CaCl₂. The reaction was initiated by addition of 0.1 mL of 30 mM ATP. The incubation of the assay mixture was carried out at 37°C for 30 min. The reaction was stopped by adding 3.5 mL of a solution containing 0.5 M H₂SO₄, 0.5% ammonium molybdate and 2% SDS. Liberated inorganic phosphate was estimated by a modified method of Fiske and Subbarow (1925) and Ca⁺⁺-ATPase activity is expressed in terms of μmol of Pi released/h/mg membrane protein at 37°C. Values are mean ± SD.

Results and discussion

The activity of erythrocyte membrane Ca⁺⁺-ATPase was significantly decreased in type 2 diabetic subjects (control 0.343 ± 0.017; type 2 diabetes 0.199 ± 0.027). Our results are in agreement with other reports (Gonzalez-Flecha et al., 1990; Zemel et al., 1998). Reduced Ca⁺⁺-ATPase activity in type 2 diabetes may be responsible for increase in intracellular calcium and consequently for elevated vascular resistance which is frequently associated with hypertension (Zemel et al., 1990). An altered erythrocyte Ca⁺⁺-ATPase activity has been linked to the development of neuropathy in diabetes (Jain & Lim, 2000). The decrease in Ca⁺⁺-ATPase activity in type 2 diabetes may be due to altered membrane properties including functional and compositional changes (Shin et al., 2007; Bakan et al., 2006). A relationship has been reported between the blood glucose levels and erythrocyte membrane ATPases (Adamson et al., 1986). It is reported that glycosylation of erythrocyte membrane proteins significantly inhibit Ca⁺⁺-ATPase activity (Davis et al., 1985). The decreased Ca⁺⁺-ATPase observed in our studies in diabetic erythrocytes may be due to glycosylation of membrane protein.

The concentration-dependent effect of tea catechins (EGCG, EGC, ECG, and EC) on the activity of Ca⁺⁺-ATPase in normal (control) subjects is shown in Figure 2a and type 2 diabetic subjects in Figure 2b. In vitro incubation with tea catechins caused an increase in the activity of Ca⁺⁺-ATPase in both normal and type 2 diabetic subjects: the effect was more pronounced in type 2 diabetes. The effect was concentration-dependent, and a significant activation was visible at concentrations up to 10⁻³ M in normal (control) and 10⁻⁵ M in type 2 diabetes. The order of effectiveness of individual catechins was in the order: EGCG > ECG > EC > EGC. Catechins are bioavailable to humans after drinking tea. Catechin levels in human plasma reach their peak 2 to 4 h after ingestion (Yang et al., 1998). A study comparing the pharmacokinetics of equimolar doses of pure EGC, ECG, and EGCG in healthy humans reported that the peak plasma levels of each catechin reached micromolar concentrations, although there was a difference in the plasma level of individual catechins (Higdon & Frei, 2003). Chow et al. (2005) report a peak plasma level of 7.4 μmol/L of EGCG after a dose of 1,200 mg of EGCG in the fasting condition.
Catechins have been reported to modulate membrane fluidity and this property has been suggested to contribute to their medicinal utility (Tsuchiya, 2001). An altered membrane fluidity has been speculated to explain the effect of tea catechins (EGCG, EGC, ECG, EC) on erythrocyte membrane Na’K’ATPase and Na/H antiport activity (Rizvi & Zaid, 2005). Our observation of an increase in Ca++-ATPase activity after incubation with tea catechins may be explained on the basis of alteration in fluidity due to a direct effect of catechins on the membrane. It has been shown that EGCG is capable of protecting erythrocyte membrane-bound ATPases against oxidative stress (Saffari & Sadrzadeh, 2004). This effect may also contribute to the elevation in activity of Ca++-ATPase after treatment with catechins.

The importance of our finding on the effect of tea catechins on Ca++-ATPase lies in the fact that altered intracellular calcium metabolism plays an essential role in the pathophysiology of diabetic complications (Levy, 1994) and other diseases (Fujita, 2000). The effect on Ca-ATPase may help to explain, in part, the antidiabetic effects of tea catechins.

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References
