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Natural Polyphenols–Iron Interaction

Its Biological Importance

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ABSTRACT

The iron-binding capacity of different fractions of natural polyphenols extracts was determined by chromatographic and electrophoretic methods. Their effects on iron-induced calcium homeostasis changes in liver tissue suspension showed that mate tea and green tea extracts provoke a very significant inhibition of the iron effects, whereas it is much less significant with red wine extract. The biological importance of this phenomenon is discussed.

Index Entries: Natural polyphenols; iron-induced cell calcium changes.

INTRODUCTION

Iron binds to polyphenols and catechins, forming colored anionic complexes *(1)*. Plant polyphenols have been suggested as cancer-preventive agents and an abundant bibliography supports this assumption *(2)*. The role of intracellular calcium ion concentration variations in cellular reproduction and proliferation seems to be an important factor in neoplastic transformation and spreading of the transformed cell *(3)*. The aim of this experimental work has been to study the iron interaction with several natural extracts rich in polyphenols and to assay their effects on liver tissular calcium homeostasis.

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MATERIALS AND METHODS

Polyphenol Extraction

Polyphenols were extracted from leaves of green tea (GT) (*Thea sinensis*) and mate tea (MT) (*Ilex paraguariensis*) by aqueous decoction at 80°C, followed by evaporation under vacuum at 60°C to reduce the original volume to 1/30, and from red wine (WI) (Bordeaux type) by reducing its original volume to 1/15 under the same conditions of evaporation. The polyphenols content of the final solution was determined using the Folin–Ciocalteaus reagent *(4)* and is referred to as catechin molarity by comparison with a catechin (Sigma, St. Louis, MO) solution.

The ferric ATP complex was prepared as described elsewhere *(5)*; ferric citrate and ferric chloride were from Merck (Darmstadt, Germany). When ^{59}Fe-labeled iron compounds were used, they were obtained with the general preparation technique already described *(6)*.

Chromatographic and Electrophoretic Studies of the Extracted Polyphenols

A separation of the different polyphenols in each extract was performed by ascending chromatography on 3MM Whatman (Clifton, NJ) paper using 0.02*M* sodium bicarbonate as solvent. With this solvent, ferric citrate and ferric ATP complex migrate with the solvent front, whereas ferric chloride remains at the origin. This analytical determination was done with aliquots of the original extracts and with aliquots of the same extracts incubated with ferric citrate, the ferric ATP complex, or ferric chloride (Fig. 1). This technique produces different migration zones of a characteristic color, without any reagent spray, that were cut off, eluted with water, and the elution liquid used for polyphenols quantitation with Folin–Ciocalteous reagent, and for iron estimation with *o*-phenanthroline *(7)*.

Electrophoresis of aliquots were performed using 0.02*M* sodium bicarbonate as buffer, and a 1-h run with a 20-V/cm gradient.

In Vitro Study of Polyphenol Effects on Liver Calcium Homeostasis

Liver tissue suspension of Swiss mouse liver was prepared according the technique published elsewhere *(8)*. To assess the changes in calcium uptake by liver tissue suspensions induced by the polyphenols, aliquots of the extracts were incubated with ferric ATP complex and ^{45}Ca-chloride. The radiocalcium uptake and the lipid peroxidation (absorption at 532 nm) were determined as described in a recent publication *(9)*.

The significance of change from control liver suspension was determined by statistic analyses of data using Student's *t*-test; $p < 0.05$ was considered significant.

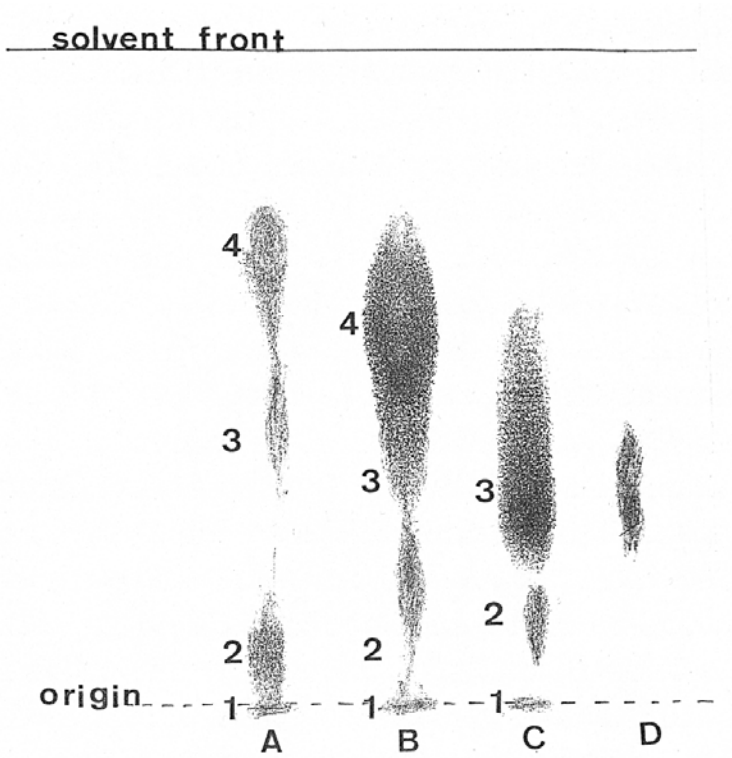

Fig. 1. Polyphenol chromatographic separation of different fractions corresponding to A = red wine, B = mate tea, C = green tea, and D = catechin. Sample volume = 10 µL (1 µmol) and 0.02*M* sodium bicarbonate as solvent.

RESULTS

The chromatography of the polyphenol solutions has shown spots of characteristic colors (Fig. 1): For WI, spot 1 was black, spot 2 was blue, spot 3 was yellow, and spot 4 was pink. For MT, spot 3 was light yellow and spot 4 was dark green on a light pink-yellow background. For GT, the diffuse spot 3 showed a superposition of dark green and pink-yellow. Standard catechin (CA) migrates as a yellow spot coincident with fraction 3 of the polyphenol solutions. The iron load produced by incubation with ferric citrate, the ferric ATP complex, and ferric chloride in all of the cases strongly increases the color intensity of fraction 4.

The chromatographic analyses of the original polyphenols extracts show that there is a direct relationship between the polyphenols and iron contents of each migrating fraction (Figs. 1 and 2) and that most of the iron (over 60%) is present in the upper migrating fractions (fractions 3 and 4 in Fig. 2). On the other hand, after incubation with ferric citrate and the ferric ATP complex, the higher iron incorporation is in those fractions (Fig. 3). When the incubation was done with ferric chloride, this

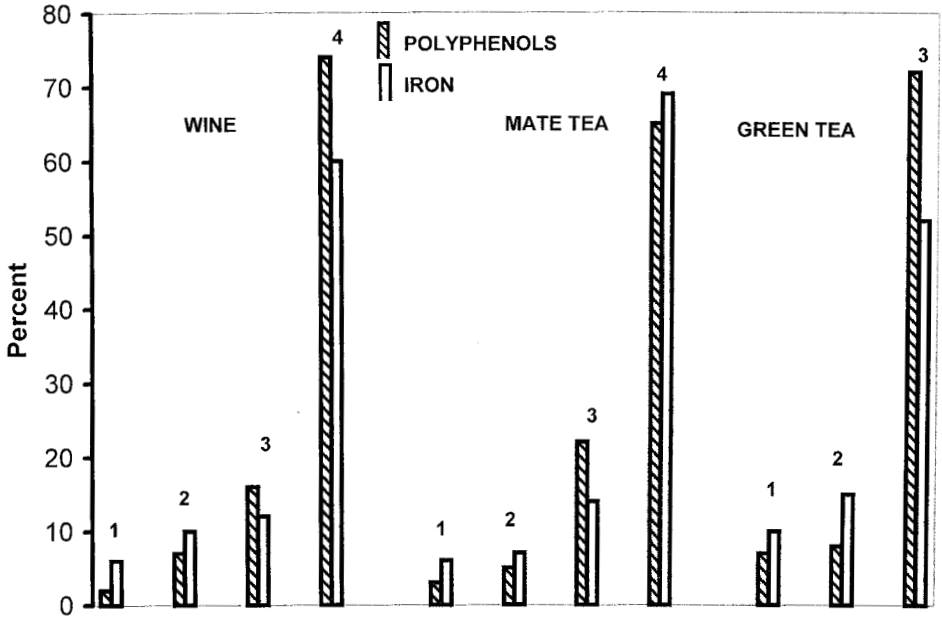

Fig. 2. Polyphenols and iron distribution (as percentage of the total) in the different chromatographic fractions of original extracts. Number 1 to 4 correspond to spots in Fig. 1.

form of inorganic iron remains on the origin and makes the evaluation of its combination with the fraction 1 uncertain; however, in all the cases, the iron present in the other migrating fractions reflects a very significant incorporation in these fractions, particularly in fraction 4 of MT (over 50% of the total iron). When incubated with ferric citrate, WI polyphenols show an iron distribution that is similar to one of the other two extracts, but with ferric ATP complex, a decrease in the upward migrating iron, that is concomitant with an increase in nonmigrating iron, is observed. GT shows a similar behavior, and the black deposit on the origin is similar to that observed with WI and GT extracts alone (without incubation with iron compounds). This black deposit is missing with the MT extract.

The electrophoretic study shows that the migrating fractions are similar to that obtained by chromatography, but the use of ^{59}Fe-labeled iron compounds that permits the use of much lower amounts of solution shows a slightly higher iron in the forward migrating fraction. The migration in the electric field is an indication of the anionic character of the fraction, where a high polyphenol content is reflected by a high migration.

The electrophoresis of the CA–^{59}Fe–iron chloride interaction product has shown that with a stoichiometric ratio (µmol CA : µmol $FeCl_3$) of 1 : 0.5, only 22% of the iron migrates with CA; for a 2 : 0.5 ratio, the amount increases to 79%, and for a 4 : 0.5 ratio, it is 91%. On the other hand,

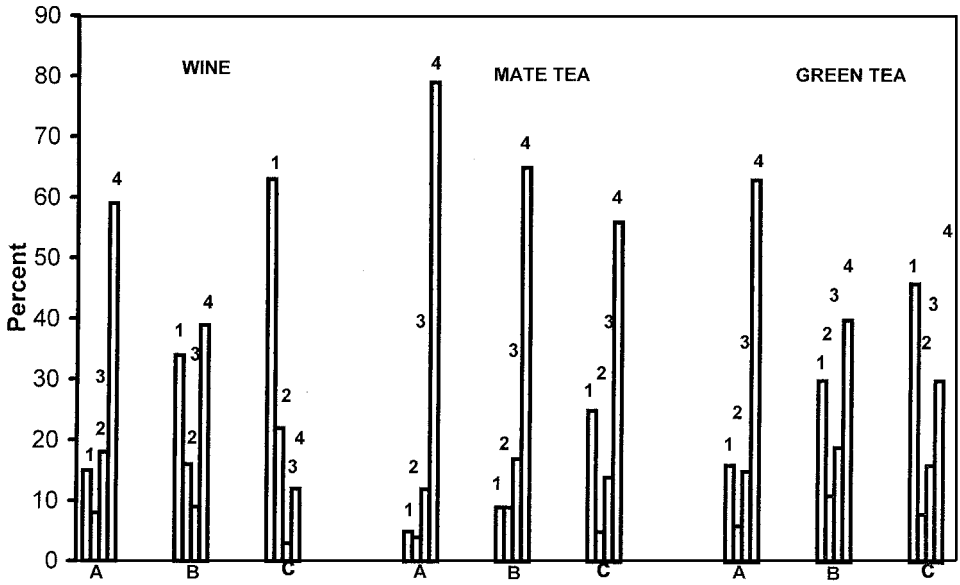

Fig. 3. Iron distribution (as percentage of the total iron) in the chromatographic fractions obtained from original extracts (2 mol) incubated with A = 1 µmol ferric citrate, B = 1 µmol ferric ATP complex, and C = 1 µmol ferric chloride. Incubation for 30 min at 37°C. Numbers 1 to 4 correspond to spots in Fig. 1.

increased combined iron deceases the migration distance from 5 to 2 cm. Similar electrophoretic analyses of the interaction product of each natural polyphenols extract with the ^{59}Fe–ferric ATP complex (ratio = 1 : 0.5) have shown that for WI about 73% of the iron remains on the origin line, whereas in the case of MT, GT, and CA, more than 80% migrates with the polyphenols as an anionic moiety.

Table 1 shows that compared with liver suspension alone, the presence of 1 µmol catechin equivalent of the natural polyphenol extracts produce a decrease in calcium uptake: (A) for WI, 54%; for MT, 60%; for GT, 38%; for CA, 57%. When 0.5 µmol of ferric ATP complex was present in the incubation medium, the decrease in calcium uptake compared to the uptake of the ferric ATP complex control (without polyphenols) was as follows: (B) for WI, 29%; for MT, 84%; for GT, 68%; for CA, 42%. On the other hand, the ferric ATP complex control increased 3.3-fold the calcium uptake of liver tissue suspension alone.

The observed lipid peroxidation values, as a percentage of the liver control value were as follows: (A) for WI, 115; for MT, 106; GT, 87; for CA, 71. In case B, as a percentage of the ferric ATP complex control value, they were as follows: for WI, 102; for MT, 74; for GT, 53; for CA, 49. The presence of only the ferric ATP complex (ferric ATP control) increases 1.6-fold the value of liver tissue suspension alone (liver control).

Table 1
Calcium Uptake and Lipid Peroxidation Induced by 0.5 µmol of Ferric ATP Complex in the Presence of 1 µmol (as Catechin Equivalent) of Natural Polyphenols Extracts

	Alone		+ 0.5 µmol ferric ATP	
	nmol Ca	A532	nmol Ca	A532
Red wine	22+/- 3*	113+/- 4*	111+/- 9*	159+/- 11*
Mate tea	19 +/- 1*	104+/- 7	25+/- 2*	115+/- 2*
Green tea	30+/- 2*	85+/- 2*	49 +/- 7*	82+/- 2*
Catechin	21 +/- 8*	70+/- 4*	91+/- 19*	76+/- 10*
Ferric ATP (control)	-	-	57+/- 8	156+/- 3
Liver (control)	48 +/-5	98+/- 2	-	-

Note: All data after 1 h incubation at 37°C of liver tissue suspension (4 mg protein). The values are the mean ± SD of four incubations for calcium uptake and three incubations for lipid peroxidation. * = $p < 0.05$.

DISCUSSION

The experimental data indicate that there is a clear correlation between the polyphenols and iron content in the original extracts and that most of both are present in the fast ascending fraction (Fig. 2). On the other hand, the incubation with iron compounds shows a chromatographic iron distribution that is characteristic of each polyphenols extract and of the used iron compound (Fig. 3). The differences between the different iron compounds are related to the different stability constant and degree of dissociation.

The choice of liver as an assay tissue for the polyphenols–iron interaction was done considering the reported in vitro and in vivo susceptibility of liver to the action of low-molecular-weight iron complexes *(8)* and the fact that liver is the body's largest pool of enzymatic activity where metabolic elimination of a part of polyphenols and catechins may

take place. It is interesting to mention that an iron–catechin complex formation is known to be implicated in the action of phenolic oxigenases that catalyze the cleavage of the aromatic ring of polyphenolic compounds in the presence of molecular oxygen *(10)*.

An explanation of the different effects of WI, MT, GT, and CA on the ferric ATP complex-induced calcium uptake by liver can be inferred from their chromatographic and electrophoretic behavior. In a previous work *(11)*, it has been shown that the iron responsible for the modificationn of the cell calcium homeostasis is bound to molecular structures behind the cell plasma membrane barrier and that there is an iron threshold concentration that must be exceeded to trigger calcium-ion influx to the cell. On the other hand, the iron effects depend on the availability of ionic iron at physiological pH that is determined by the stability constant of the iron complex *(12)*. When ionic iron $[Fe(H_2O)_6]^{3+}$ reacts with the polyphenols, it can be linked by coordination to give nondissociable complexes, in which it is incorporated into the molecular structure, as in the case of $Na_3[Fe(catechin)_3]$ *(1)*, or less stable complexes with a residual coordination capacity that can lead, at physiological pH, to hydrolysis and insolubilization. This last possibility seems to be the WI and GT cases, which show a decreased iron in fraction 4 of Fig. 3 at the same time that there is an increase in the nonmigrant fraction 1. This formation of an hydrolyzed insoluble fraction has been corroborated by the electrophoretic analyses: In the case of WI, 73% of iron does not migrate, whereas more than 80% does, as an anion in the cases of MT, GT, and CA. The fact that ferric ATP complex-induced calcium uptake by liver is decreased by only 29% in the presence of WI, whereas the values of 60% and 38% for MT and GT, respectively (Table 1), can be related to a highly dissociated iron in the presence of WI and a much lower dissociation with MT and GT, that at physiological pH determines a lower availability of ionic iron with WI.

Iron is required for the growth of all living cells. It is quite obvious that the chelating capacity of polyphenols is of importance in the biological systems. Enzyme iron is the smallest iron compartment, but it is very important to living organisms. There are enzymes that contain iron in their molecules such as the cytochromes, lipoxidase, catalase, peroxidases, iron–flavoproteins–cytochrome C reductase, succinate oxidase, acyl-CoA dehydrogenase, NADH dehydrogenase, and xanthine oxidase, and enzymes requiring iron as cofactor such as aconitase and succinate dehydrogenase.

Considering the importance of iron in the biochemical functions of the cell, these experimental results emphasize the possibilities of polyphenols to control certain biological processes, as they do in the polyphenol concentration and ionic iron-dependent inhibition of iron-induced liver calcium homeostasis modification. Work is in progress to evaluate the effects of MT and GT polyphenols extracts on the experimental carcinogenesis induced by ferric ATP complex.

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