

BACKGROUND

Polyphenolic compounds present in many foods are known to have a preventive but also curative effect on carcinogenic progression by multiple effects on the cell physiology. A direct effect of polyphenols on the enzyme activity should therefore be considered.

GOAL

Herein we studied *in vitro* the inhibitory effects of two polyphenolic compounds (+)-Catechine & Epigallocatechin Gallate (EGCG) on the performance of the polymerase and reverse transcriptase, as a model for eukaryotic and viral enzyme activity.

MATERIAL & METHODS

Since in real-time RT-PCR the reaction kinetics trajectory can be recorded, we compared several amplification histories obtained with or without polyphenols.

Two different approaches of RT-PCR were adopted:

1. **A one-step RT-PCR approach** (RT and PCR together in one run), where y_0 is showing the efficiency of the prior RT reaction;
2. **A two-step RT-PCR approach**, where the mRNA was separately reverse transcribed, and polyphenols were added only into PCR;

In each approach, reaction setups without any additional agent as a background control ($n = 8$), and three serial dilutions of both polyphenols were performed: $1 \cdot 10^{-5}$, $1 \cdot 10^{-6}$, $1 \cdot 10^{-7}$ and $1 \cdot 10^{-8}$ M ($n = 3$). We determined various parameters describing the enzyme properties derived from the sigmoidal shaped reaction trajectory, using an established four parametric sigmoid model (Tichopad et al., *Biotech. Lett.* 2002; *Mol. & Cel. Probes* – 2003, in press).

$$f(x) = y_0 + \frac{a}{1 + e^{-\frac{(x-x_0)}{b}}}$$

four parametric sigmoidal model

Raw fluorescence data were fitted, where $f(x)$ is the function computed fluorescence at cycle x , y_0 is the background fluorescence, a the plateau height ($a = y_{max} - y_0$), e is the natural logarithm base, x is the cycle number, x_0 is the first derivative maximum (FDM), the second derivative maximum (SDM), and b describes the slope at x_0 , representing an „inverse estimator“ of the polymerase efficiency. Further the area under the melting curve peak (AUC) of a final PCR product was determined, representing the amount of amplified product. All statistics were done in SAS 8.02 using GLM, checking for differences between the groups.

RESULTS

In one-step RT-PCR, only the effect of EGCG addition was significantly present as a decrease of final cDNA product after RT reaction (Table 1). This is in accordance with known antiviral properties of EGCG. Decrease in PCR product was a consequence of decreased prior template cDNA.

Employing two-step RT-PCR approach one can see in table 1 an effect of both compounds on PCR performance. Parameters were altered in a sense of PCR inhibition and lower PCR efficiency. The range of added polyphenols was biologically relevant (10 nM to 10 μ M) and able to inhibit the enzyme activities.

CONCLUSION

Our results suggest that polyphenols are suppressing the polymerase as well as reverse transcriptase activity *in vitro*. This may lead to the hypothesis, that organs exposed to polyphenols exhibit lower DNA replication and proliferation rate, as well as lower viral activity caused by retroviruses.

Table 1: The significance of the alteration of parameters by EGCG and (+)-Catechin.

a , b , x_0 (= FDM), y_0 , CP and SDM are the parameters of the four-parametric sigmoid model. Significantly altered parameters are indicated. The lower field contains comment on the impact of the finding on the reaction. AUC represents the amount of amplified real-time PCR product.

| One-step real-time RT-PCR approach | | | | | | |
|------------------------------------|--------|-------------------------------------|-------------------|------------------|-------------------|-------------------|
| | a | b | x_0 = FDM | y_0 | SDM | AUC |
| p value | 0.8052 | 0.1858 | 0.9416 | 0.4086 | 0.5315 | 0.9273 |
| addition of Catechin caused | - | - | - | - | - | - |
| p value | 0.1051 | 0.5921 | 0.6113 | 0.0294 | 0.5171 | 0.0462 |
| addition of EGCG caused | - | - | - | lower RT product | - | lower PCR product |
| Two-step real-time RT-PCR approach | | | | | | |
| p value | 0.0815 | 0.0015 | <0.0001 | - | 0.8843 | 0.4303 |
| addition of Catechin caused | - | decrease of delay efficiency of PCR | - | - | - | - |
| p value | 0.0919 | 0.777 | <0.0001 | - | <0.0001 | 0.4090 |
| addition of EGCG caused | - | - | delay of PCR | - | delay of PCR | - |

Figure 1: Effect of EGCG on RT reaction efficiency (parameter y_0).

Figure 2: Effect of (+)-Catechin on real-time PCR reaction efficiency (parameter b).

Box Plot: The first box represents 8 reactions with no EGCG added. Following boxes represent 3 reactions with various concentration of polyphenols added. The length of the box represents the inter-quartile range (the distance between the 25th and the 75th percentiles), the dot in the box interior represents the mean, the horizontal line in the box interior represents the median, the vertical lines issuing from the box extend to the minimum and maximum values of the analysis variable.

