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RESEARCH COMMUNICATION

Observations on the inhibition of HIV-1 reverse transcriptase by catechins

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The sensitivity and specificity of the inhibition of HIV-1 reverse transcriptase by various catechins have been examined. As previously reported, (−)epicatechin 3-gallate inhibits the viral polymerase. However, it is noted here that this inhibition is not observed in the presence of either serum albumin or Triton X-100. Other catechins behave similarly to (−)epicatechin 3-gallate in that they inhibit polymerase activity only in the absence of these reagents. Additionally, other DNA polymerases are inhibited to a similar degree by (−)epicatechin 3-gallate. Taken cumulatively, these results suggest that these catechins, and in particular (−)epicatechin 3-gallate, bind with no apparent selectivity and that the observed inhibition of HIV-1 reverse transcriptase is non-specific in nature.

INTRODUCTION

In the last few years, a number of natural compounds have been reported to inhibit HIV-1 reverse transcriptase (RT), the most notable being flavonoid derivatives [1–3] and galloylquinic acids [4]. In particular, (−)epicatechin 3-gallate has been reported to be an extremely effective and specific inhibitor of the viral polymerase activity [3]. Although the galloylquinic acids have been shown to inhibit HIV replication at non-cytotoxic concentrations [4], it was observed that (−)epicatechin 3-gallate will not inhibit HIV-1 replication at non-cytotoxic concentrations [3]. Nevertheless, while the inhibitory nature of the flavonoids and catechins against HIV-1 RT has been well studied, their selectivity and mechanism of action have not been clarified. It is reported here that (−)epicatechin 3-gallate inhibits other DNA polymerases to a similar extent, with no particular preference for HIV-1 RT. Moreover, this inhibition is absolutely dependent on the absence of detergent or competing non-related protein such as serum albumin. This is highly suggestive that these compounds have a similar binding capacity for any protein, and that the inhibition of RT activity is a non-specific event. The potential for development of catechins as specific inhibitors of HIV-1 RT for therapeutic benefit would therefore appear to be minimal.

MATERIALS AND METHODS

Materials

The known catechins **1**–**3** (see Fig. 1) were isolated from the methanol extract of *Detarium microcarpum* (Leguminosae), and catechins **4** and **5** were isolated from the latex of *Croton draconoides* (Euphorbiaceae), by sequential Sephadex LH-20 column chromatography and reverse-phase h.p.l.c. Quercetin, myricetin, baicalein and BSA (fraction V) were purchased from Sigma. [^{3}H]dTTP was from Amersham and poly(A)/poly(dT)$_{12-18}$ was from Pharmacia. Recombinant HIV-1 RT was a gift from Dr. Nick Cammack, Glaxo Group Research. Other DNA polymerases were purchased from either New England Biolabs or Pharmacia.

Polymerase assays

HIV-1 RT reactions were carried out in a final volume of 25 μl containing 50 mM-Tris, pH 8.0, 100 mM-KCl, 6 mM-$MgCl_2$, 5 mM-dithiothreitol, 10 μM-dTTP (^{3}H-labelled; 4 Ci/mmol) and 1.6 μg of template/primer per ml. The final enzyme concentration was 1.4 μg/ml (0.05 unit/ml). When appropriate, Triton X-100 was added to a final concentration of 0.05 % and BSA was added as indicated. Reactions were incubated for 20 min at 37 °C. Enzyme activity was linear with time for 30 min. For assay of avian myeloblastosis virus (AMV) RT, Moloney murine leukaemia virus (MMLV) RT and *Escherichia coli* DNA polymerase I, conditions were those recommended by the supplier; enzymes were present at a concentration of 0.05 unit/ml, as for HIV-1 RT. At the end of the incubation time, aliquots of the reaction were spotted on to DEAE-cellulose paper and dropped immediately into ice-cold trichloroacetic acid/50 mM-sodium pyrophosphate, followed by washes with water and finally with ethanol. Filters were then air-dried and radioactivity was measured by liquid scintillation counting.

RESULTS

Inhibition of HIV-1 RT by catechins: effects of Triton X-100 and BSA

Fig. 1 shows the structures of the various catechins used in this study. Compounds **1**–**3** all inhibit HIV-1 RT activity, with (−)epicatechin 3-gallate being the most potent (Table 1), having an IC_{50} value of about 0.5 μg/ml. The presence of an hydroxyl group at the R^2 position apparently abolishes any inhibitory capacity under the conditions tested, as judged from the results with compounds **4** and **5**. Additionally, the (−) isomer would appear to be more active than the corresponding (+) isomer with respect to compounds **1** and **2**. Thus one cannot exclude entirely the likelihood that some structure/activity relationships may

Abbreviations used: HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; AMV, avian myeloblastosis virus; MMLV, Moloney murine leukaemia virus.

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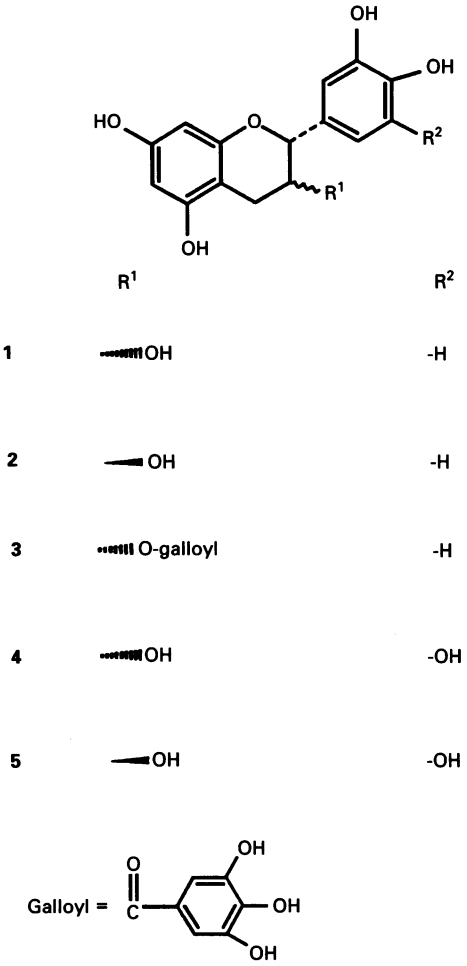

Fig. 1. Structures of the various catechins used in this study

1, (−)Epicatechin; **2**, (+)catechin; **3**, (−)epicatechin 3-gallate; **4**, (−)epigallocatechin; **5**, (+)gallocatechin.

Table 1. Effects of Triton X-100 and BSA on the inhibition of HIV-1 RT by catechins

Compounds **1–5** were present at 5 μg/ml, except for **3**, which was present at 2 μg/ml. Data are present as pmol of nucleotide incorporated/min per ml. A duplicate experiment showed essentially the same results.

		RT activity (pmol/min per ml)		
Compound	Addition...	None	BSA (100 μg/ml)	Triton X-100 (0.05%)
None		28.1	38.2	37.0
(−)Epicatechin (**1**)		1.5	35.0	34.8
(+)Catechin (**2**)		10.9	36.5	36.7
(−)Epicatechin 3-gallate (**3**)		1.4	37.8	36.4
(−)Epigallocatechin (**4**)		28.6	39.0	37.5
(−)Gallocatechin (**5**)		28.0	40.4	35.3
ddTTP (50 μM)		0.6	0.7	0.7
$H_4SiW_{12}O_{40}$ (10 μM)		1.4	2.0	1.9
Phosphonoformate (0.1 mM)		0.5	0.6	0.5

exist in this class of compounds. In any case, the above results are more or less in accordance with previously published data [3].

As previously observed, the presence of BSA or Triton X-100

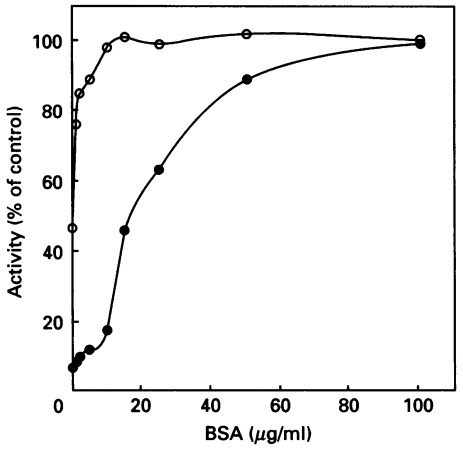

Fig. 2. Effect of BSA on the inhibition of HIV-1 RT by (−)epicatechin 3-gallate

Reactions containing inhibitor were normalized to the appropriate control reaction containing the same amount of BSA minus inhibitor. (−)Epicatechin 3-gallate concentrations: ○, 0.5 μg/ml; ●, 2 μg/ml.

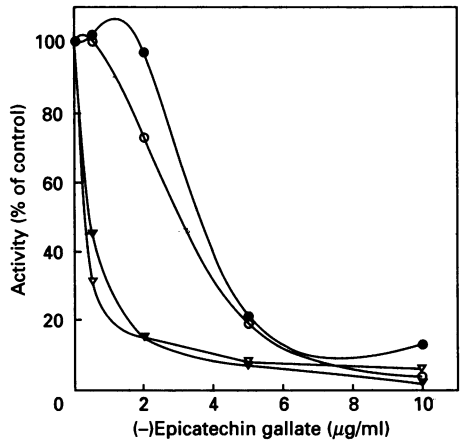

Fig. 3. Effect of (−)epicatechin 3-gallate on the activity of various DNA polymerases

Values were normalized to reactions containing no inhibitor. None of the reactions contained either Triton X-100 or BSA. ▼, HIV-1 RT; ▽, MMLV RT; ○, AMV RT; ●, *E. coli* DNA polymerase I. 100% activity values ranged from 10 to 30 pmol/min per ml.

increases the activity of HIV-1 RT, presumably by acting as a stabilizing agent or by preventing aggregation [5]. However, when either of these reagents was present in the polymerase reaction, no inhibition was observed by any of the aforementioned compounds, including (−)epicatechin 3-gallate (Table 1). The detergent Nonidet P-40 was equally effective in negating any inhibitory effect (results not shown). The inhibition of polymerase activity by other structurally diverse compounds such as ddTTP, polyoxytungstates and phosphonoformate was not affected by the addition of either BSA or Triton X-100 (Table 1).

To further characterize this effect, a dose–response experiment was performed. Fig. 2 shows the effect of adding increasing amounts of BSA to polymerase reactions containing either 0.5 or 2 μg of (−)epicatechin 3-gallate/ml. At the higher concentration, BSA at 50 μg/ml was sufficient to restore more than 90% of control activity. At the lower concentration, 99% of control activity was observed in the presence of as little as 10 μg of BSA/ml. This result is highly suggestive that (−)epicatechin

3-gallate has a binding capacity for BSA similar to that for HIV-1 RT (reactions contained 1.4 μg of HIV-1 RT/ml).

Effect of (−)epicatechin 3-gallate on the activity of other DNA polymerases

Fig. 3 shows the inhibition of other DNA polymerase activities by compound **3** (in the absence of added protein or detergent). In the range 0–5 μg/ml, AMV RT and *E. coli* DNA polymerase I were less sensitive to inhibition than HIV-1 RT; MMLV RT was slightly more sensitive, having an IC_{50} of about 0.25 μg/ml. However, at 5 μg/ml, more than 80% inhibition was observed with all polymerases tested. While some preference was shown for MMLV and HIV-1 RTs, this difference in IC_{50} values is only about 5-fold. It is, however, expected that that are some differences in these polymerases, since they vary in properties such as specific activity, subunit composition and rates of processivity. In spite of this, no striking specificity was seen for HIV-1 RT.

DISCUSSION

It would not be expected that a specific inhibitor would have a similar binding capacity for BSA as for the target protein. Similar findings were seen for other reported flavonoid inhibitors such as quercetin and myricetin [2], i.e. no inhibition in the presence of BSA or Triton X-100 (results not shown). Contrary to previous reports [1,2], however, no inhibition was observed with baicalein under any conditions (results not shown). The reason for this discrepancy is unclear.

Flavonoids have been demonstrated to inhibit a wide variety of functionally diverse enzymes (see [6] for a review). Similarly, the catechins have been shown to inhibit, among other enzymes, endo-polygalacturonase and succinoxidase [7]. Suramin is another natural compound which was postulated to inhibit the RT of HIV-1 [8], but it will likewise inhibit a large number of other enzymic activities [9], as well as several DNA polymerases [10]. In the case of suramin, it was found that the drug binds with no domain-specific interactions and with no selectivity, and thus the protein–suramin interaction is non-specific [11]. It has been known for some time that suramin will bind with high affinity to serum albumin [12].

Preincubation of HIV-1 RT with template/primer, dNTP or a combination of the two will not protect the enzyme from inactivation by (−)epicatechin 3-gallate (results not shown), further suggesting that the interaction is non-specific. While the (−)epicatechin 3-gallate-mediated inhibition of HIV-1 RT is partially reversible by the addition of Triton X-100, no such reversibility is seen after the addition of BSA (results not shown). It is still unclear what the protein-binding site for (−)epicatechin 3-gallate may be.

Nonetheless, the facts that (−)epicatechin 3-gallate will inhibit a number of functionally unrelated enzymes, that the compound shows no marked preference for HIV-1 RT over other DNA polymerases, and that this inhibition is observed only in the absence of serum albumin, strongly suggest that this catechin has little selectivity and that the protein–(−)epicatechin 3-gallate interactions are non-specific in nature. Whatever the mechanism for protein binding might be, it would seem that (−)epicatechin 3-gallate and other flavonoids have little potential for development as specific inhibitors of HIV-1 RT.

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