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Improvement of (+)-catechin inhibitory activity on human PMN respiratory burst by (+)-3-O-propionyl and (−)-3-O-valeryl substitution

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Abstract

Catechins and their derivatives are abundant flavanols in the plant kingdom. Usually, catechin activity correlates with chemical structure. We hypothesized that by adding hydrophobic groups to the native catechin, we could ameliorate penetration of the cell and make the derivatives more active than native molecule in inhibiting polymorphonuclear leucocyte (PMN) oxidative burst. This study was designed to compare the antioxidant activity of native catechin with that of (+)-3-propionylcatechin and (−)-3-O-valerylcatechin esters by two cell-free colorimetric methods and by their effects on whole blood leucocytes as well as on isolated PMN chemiluminescence activity. The results showed that the colorimetric methods did not detect differences between catechins. On the contrary, cellular chemiluminescence studies showed that light emission by resting, as well as by phorbol myristate acetate (PMA)-stimulated PMNs and whole blood leucocytes was inhibited by catechin esters more intensively than native catechin. The compartmental chemiluminescence evaluation showed that the extracellular activity was similar with all catechins, while the intracellular activity was higher with esters. PMN pre-incubation, with catechins at various times before stimulation with PMA, enhanced the inhibitory activity of all compounds. Since the esterification with propionic or valeric acid increased the lipophilicity of (+)-catechin, we hypothesized that native and esterified catechins have different intracellular availability and therefore differ in effectiveness. An ancillary result obtained is that a single approach, chemical or cellular, is not sufficient to evaluate overall antioxidant activity in biological sytems. The results indicate that modified catechins may be very intriguing as possible future leucocyte modulating drugs, with possible applications in vascular and inflammatory diseases.

Introduction

Flavonoids are a large group of phenylchromones widely distributed in plant structures (Harborne 1986). They are important in human diet and are present in plant extracts that have been used for centuries in oriental medicine (Di Carlo et al 1999). The pharmacological and biochemical properties of flavonoids have now been extensively reviewed (Middleton et al 2000; Rice-Evans 2001). Antioxidant properties, reactive oxygen species (ROS) scavenging, and cell function modulation of flavonoids could account for the large part of their pharmacological activity (Limasset et al 1993). Catechins (flavanols) are water-soluble flavonoids with two phenol nuclei (A and B) and a heterocyclic ring (C), with −OH groups in the 3, 5, 7, 3′ and 4′ position. The orthodihydroxylic structure in the B ring confers greater stability to the radical form produced by scavenging and contributes to electron uncoupling (Rice-Evans et al 1996). An added −OH group in the 5′ position on the B ring slightly increases the molecule's antioxidant activity, as in epigallocatechin. Besides the hydroxylic group addition, the manipulation of the -OH groups (i.e., alkylation, glycosylation, esterification) somehow modulates antioxidant and other biological activities. Thus, some catechins with a 3-OH esterification by gallic acid (gallate catechins) enhance scavenging (Rice-Evans et al 1996).

The antioxidant activity of catechins can be studied using cell-free colorimetric or in-vitro cellular methods, such as chemiluminescence. Systems which selectively inhibit the generation of extra- or intracellular chemiluminescence shed light on the relationship

between the two sources of chemiluminescence (Briheim 1984). Further information about the interaction between catechins and leucocytes can be obtained by pre-incubation of cells with molecules before chemiluminescence assays.

Since function is determined by structure, we hypothesized that adding hydrophobic groups to the 3-OH position of the native catechin would better enable the molecule to enter the cell. Although this molecule would have similar scavenging properties to the native molecule, it should be more active in inhibiting the polymorphonuclear leucocyte (PMN) oxidative burst.

The aim of this study was to compare the antioxidant effects of two synthetic catechin esters, (+)-3-propionylcatechin ((+)-3-O-PC) and (−)-3-O-valerylcatechin ((−)-3-O-VC), with those of native catechin ((+)-catechin).

Materials and Methods

Drugs

Native (+)-catechin and its derivatives (+)-3-O-PC and (−)-3-O-VC were prepared as reported by Lambusta patent (Lambusta et al 2001). The compounds were diluted to give 0.01–100 μM concentrations in modified Krebs-Ringer phosphate medium (KRP*; De Sole et al 1993).

Colorimetric antioxidant studies

DMPD method

N,N-dimethyl-*p*-phenylenediamine dihydrochloride (DMPD), ferric chloride and L-ascorbic acid were from Fluka (Switzerland). All solvents (HPLC grade) were from Carlo Erba (Italy). The spectrophotometric measurements were recorded using a UV-Vis Shimadzu 2100 (Japan) apparatus. The antioxidant activity colorimetric evaluation was performed according to Fogliano protocol (Fogliano et al 1999). Briefly, the coloured radical cation ($DMPD^{\bullet+}$) was obtained by adding 0.2 mL of 0.05 M ferric chloride solution to 100 mL of 100 mM DMPD (pH 5.25). One millilitre of this solution was evaluated at 505 nm. An optical density of 0.900 ± 0.100 units of absorbance was obtained and it represented the uninhibited signal. A standard solution of ascorbic acid was prepared at 1 mg mL^{-1} and then diluted to build the calibration curve. The concentrations tested ranged between 4.5×10^{-4} and 5.7×10^{-5} M. For ascorbic acid, a dose–response curve was derived by plotting the absorbance at 505 nm as percentage of the absorbance of the uninhibited radical cation solution (blank). DMPD values were expressed as ascorbic acid equivalent ($\mu g\,mL^{-1}$), calculated by calibration curves built with titred ascorbic acid solutions. Each assay was performed in triplicate.

ABTS method

Ferric chloride, L-ascorbic acid and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were from Fluka (Switzerland). All solvents (HPLC grade) were from Carlo Erba (Italy). Spectrophotometric measures were recorded using a UV-Vis Shimadzu 2100 (Japan) instrument. The evaluation of the sample antioxidant ability was carried out as described by Miller et al (1996) and by Rice-Evans & Miller (1994). Antioxidant activity measured by the ABTS method was reported as ascorbic acid equivalent ($mg\,mL^{-1}$), calculated by the calibration curve built with ascorbic acid titred solutions (range). Each assay was performed in triplicate.

Chemiluminescence studies

Blood collection and PMN isolation

Peripheral blood was collected from three healthy fasting donors between 0800 and 0900 h, to minimize daytime variability of phagocyte respiratory burst (Heberer et al 1982). Samples were withdrawn by K_3EDTA vacutainers (Becton Dickinson, Plymouth, UK). PMNs were isolated using a discontinuous gradient, consisting of 100% (density 1.1294 $g\,mL^{-1}$) and 70% (density 1.090 $g\,mL^{-1}$) isotonic Percoll (Pharmacia, Uppsala, Sweden) in calcium- and magnesium-free phosphate-buffered saline pH 7.4 (PBS; Sigma Chemical Co., St Louis, MO) (Harbeck et al 1982). The blood was centrifuged for 20 min at 250 *g* at room temperature. The PMN layer, banded at the interface of the two employed Percoll densities, was collected and washed twice in PBS. The PMN suspension was adjusted to 0.5×10^6 cells mL^{-1} with KRP* for chemiluminescence assay. The purity of isolated PMNs, evaluated on May Grunwald Giemsa-stained cytocentrifuged smears, and cell viability, checked with the trypan blue dye exclusion test both ranged between 90 and 95%.

Luminol-dependent chemiluminescence assay

Chemiluminescence assays were performed following the protocol described by De Sole et al (1993) and using an automatic luminometer (Autolumat LB 953, Berthold, Wildbad, Germany). Whole blood and isolated PMN chemiluminescence emission was evaluated within three hours after venipuncture. The reaction mixtures were prepared in 4-mL polypropylene vials. Each vial contained 100 μL of 10^{-4} M luminol (Sigma Chemical Co.), 100 μL of the various catechin concentrations, 100 μL of 1.5 μM phorbol myristate acetate (PMA; Sigma Chemical Co.), 100 μL of diluted whole blood (1:100) or isolated PMN suspension at 0.5×10^6 mL^{-1} and sufficient KRP* to yield a final volume of 1.0 mL. Samples without stimuli were also included as controls. To exclude the possibility that the catechins quenched the activated luminol, luminol and catechins, in the presence or absence of PMA, were included as controls. The reaction temperature was 37 °C and the resulting light emission was recorded for 0.5 s on each vial over a 90-min period. All measurements were performed in triplicate. The effects of catechins on leucocyte viability were determined by the trypan blue dye exclusion test with microscopic discrimination between stained (dead) and unstained (live) cells (Merchant et al 1969).

Intra- and extracellular chemiluminescence response. The quantification of extra- and intracellular chemiluminescence

was performed according to the Mundi protocol (Mundi et al 1991). Briefly, the extracellular chemiluminescence emission was measured by adding 1 mM azide (Sigma) and 4 U horseradish peroxidase (Sigma) to the luminol-dependent chemiluminescence assay reagents, whereas the evaluation of intracellular chemiluminescence was performed by adding superoxide dismutase (200 U; Sigma) and catalase (2000 U; Sigma) to the same reagents. Luminol-amplifed chemiluminescence is related to the myeloperoxidase–H_2O_2 system. The detection protocol for extracellular chemiluminescence response used azide, a low-molecular-weight myeloperoxidase inhibitor, which accesses intracellular compartments, and an azide-insensitive peroxidase (horseradish peroxidase), which cannot gain access to intracellular compartments. Azide inhibits myeloperoxidase and total chemiluminescence, but horseradish peroxidase regenerates extracellular response. The method for intracellular chemiluminescence response quantification used superoxide dismutase and catalase as high-molecular-weight scavengers of O_2^- and H_2O_2, respectively. In the presence of these molecules, the extracellular O_2^- and H_2O_2 are removed, thus allowing measurement of intracellular response alone.

Since the extracellular chemiluminescence evaluation is obtained by adding horseradish peroxidase in excess, this test must be considered only an assessment of the actual extracellular chemiluminescence production and the values cannot be added to the intracellular amount to calculate the total chemiluminescence activity.

The aim of this test was to obtain information on cell penetration by catechins.

Chemiluminescence studies on pre-incubated PMNs. Two types of experiments have been performed to study the effects of cell pre-incubation on chemiluminescence activity. Firstly, PMNs pre-incubated at 37 °C for 15, 30 and 45 min with each catechin (50 μM), washed and activated with PMA to evaluate their chemiluminescence activity. Control PMNs were incubated for the same times without catechins, washed and activated with PMA for chemiluminescence evaluation. Secondly, PMNs pre-incubated with each catechin in the same experimental conditions of the previous step, not washed and stimulated with PMA at the moment of chemiluminescence evaluation. Two controls were employed for this experiment: PMNs incubated without catechins, not washed and added to catechins and PMA at the moment of chemiluminescence evaluation; PMNs incubated without catechins, not washed and activated with PMA before chemiluminescence recording.

Statistical analysis

The work has been carried out in three steps: step 1, antioxidant activity evaluation of the three catechins, determined by colorimetric methods; step 2, construction of dose–response curves, drawn for each catechin in various experimental conditions; step 3, incubation time–response curves, made for each catechin at 50 μM concentration.

Test A was used to analyse step 1 — analysis of variance by orthogonal comparisons of the three molecules among them ((+)-catechin vs the two catechin esters and the two esters among them). To analyse the results of steps 2 and 3, test B was used — regression analysis with evaluation of the linearity of dose–response curves (dose logarithm as independent variable and chemiluminescence percent inhibition as dependent) or incubation time–response curves (pre-incubation time as independent variable and chemiluminescence percent inhibition as dependent). If response regression was linear, the angular coefficient significance was calculated, to verify the null hypothesis of an angular coefficient equal to zero.

Test C was used to compare dose–response curves. Test C1 was used when the curves of the three catechins were linear — the analysis of variance applied to regression (analysis of variance-R) was performed to compare the angular coefficients by orthogonal comparisons ((+)-catechin vs the two catechin esters and the two esters among them); test C2 was used if one or more curves were non-linear — analysis of variance was carried out comparing the three molecules at each dose by orthogonal comparisons ((+)-catechin vs the two catechin esters and the two esters among them).

The results of all experiments were expressed as mean ± s.d. In all tests, values of $P < 0.05$ were regarded as significant.

Results

Antioxidant activity in cell-free systems

Statistical analysis (Test A) demonstrated that the ABTS and DMPD methods did not allow us to distinguish between the three catechins on the basis of their antioxidant activity. In fact, the ascorbic acid equivalents of (+)-catechin, (+)-3-O-PC and (−)-3-O-VC, determined by the DMPD method, were 5.62 ± 0.49, 5.00 ± 0.14 and 5.09 ± 0.18 μg mL^{-1}, respectively, and those determined by the ABTS method were 1.51 ± 0.25, 0.98 ± 0.16, 1.04 ± 0.18 μg mL^{-1}.

Whole blood leucocyte chemiluminescence studies

Total leucocyte, PMN number and haemoglobin concentration were very similar in the three donors. Catechins did not show any effect on background luminescence. The trypan blue exclusion test indicated that leucocytes were healthy after exposure to all compounds studied, at each concentration used; catechins did not quench luminol chemiluminescence background in either the presence or absence of PMA (data not shown).

In the absence of catechins, total chemiluminescence counts were $4.73 \times 10^6 \pm 4.14 \times 10^5$ and $4.40 \times 10^7 \pm 3.60 \times 10^6$ for resting and PMA-stimulated cells, respectively. Resting whole blood leucocyte chemiluminescence results showed that all catechins significantly inhibited chemiluminescence emission in a linear fashion (Test B). Three curves were confronted by analysis of variance-R (Test C1): esterified catechins were more active than

native catechin ($P < 0.05$) and (−)-O-VC was more active than (+)-O-PC ($P < 0.05$; data not shown).

PMA-stimulated whole blood leucocytes were inhibited by all catechins (0.01–100 μM). In these experimental conditions, native (+)-catechin inhibited chemiluminescence emission in a non-linear fashion (Test B). On the contrary, esterified catechins showed a linear pattern of inhibition in the range of concentrations tested (Test B) ($P < 0.05$; data not shown). To compare the inhibitory effects of the three molecules, statistical test C2 was applied. At concentrations of 10 and 100 μM, esterified catechins inhibited chemiluminescence emission more than (+)-catechin ($P < 0.05$; data not shown); no differences were found between esterified catechins.

PMN chemiluminescence studies

Isolated PMNs remained healthy after exposure to all compounds and concentrations employed (data not shown).

In the absence of catechins, chemiluminescence total counts for resting and PMA-stimulated cells were $2.69 \times 10^7 \pm 3.79 \times 10^6$ and $9.35 \times 10^7 \pm 3.88 \times 10^6$, respectively. Native and modified catechins (0.01–100 μM) inhibited chemiluminescence emission either by unstimulated or PMA-stimulated PMNs (Figure 1).

The inhibitory effects of native as well as esterified catechins on resting PMN light emission showed a linear trend (Test B; Figure 1A). The three molecules were compared by analysis of variance-R (Test C1). Esterified catechins were more active than native catechin ($P < 0.05$) and (−)-O-VC was more active than (+)-O-PC ($P < 0.05$).

When PMNs were stimulated with PMA, native (+)-catechin poorly inhibited chemiluminescence emission in a non-linear fashion (Test B; Figure 1B). On the contrary, esterified catechins had a linear pattern of inhibition in the range of concentrations tested (Test B); comparing the effects of the three molecules at each dose (Test C2), in all range of doses tested, esterified catechins were more active than (+)-catechin and, of the esterified catechins, (−)-O-VC was the most active (Figure 1B; $P < 0.05$).

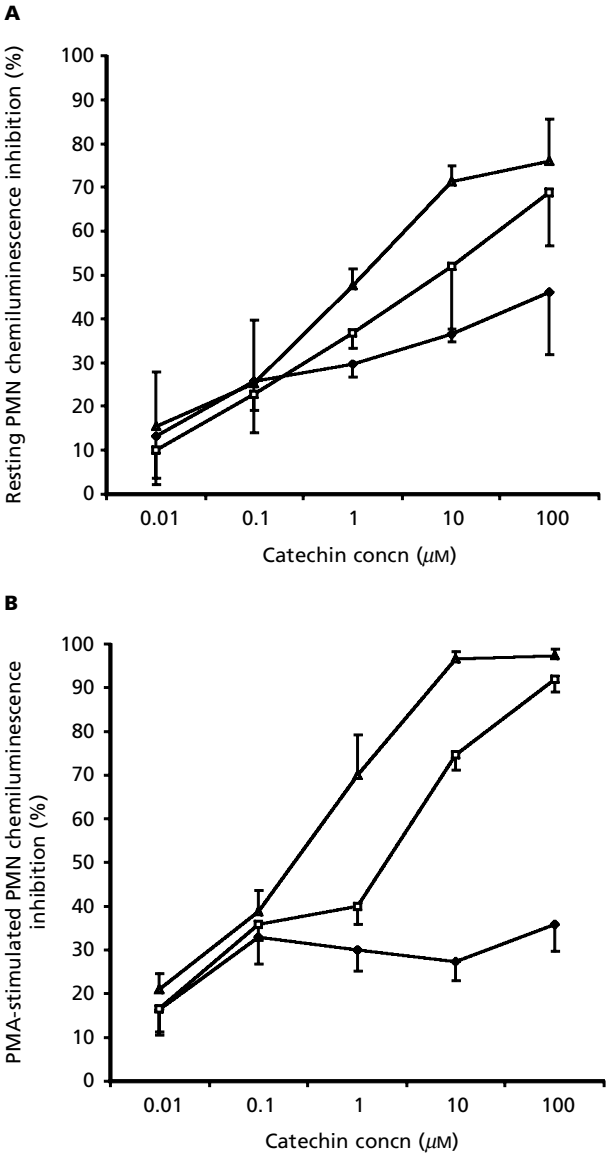

Figure 1 Inhibitory effect of catechins on chemiluminescence emission from resting (A) and PMA-stimulated (B) human isolated PMNs. Values are expressed as percent inhibition (mean ± s.d.). In the absence of catechins, extracellular chemiluminescence counts were $2.69 \times 10^7 \pm 3.79 \times 10^6$ and $9.35 \times 10^7 \pm 3.88 \times 10^6$ for resting and PMA-stimulated cells, respectively. ◆, (+)-catechin; □, (+)-O-PC; △, (−)-O-VC.

Intra- and extracellular chemiluminescence response

Since luminol is a small uncharged molecule and can freely pass through the plasma membrane, the luminol chemiluminescence assay allows us to quantify extra- and intracellular free radical production using myeloperoxidase inhibitors and extracellular ROS scavengers, respectively.

Whole blood leucocyte extracellular chemiluminescence emission in resting and PMA-stimulated cells was $2.19 \times 10^6 \pm 2.16 \times 10^5$ and $2.71 \times 10^7 \pm 2.71 \times 10^6$, respectively, whereas the intracellular ones were $1.53 \times 10^6 \pm 7.32 \times 10^4$ and $5.93 \times 10^6 \pm 1.56 \times 10^6$, respectively.

PMN extracellular chemiluminescence emission for resting and PMA-stimulated cells was $1.12 \times 10^8 \pm 1.06 \times 10^7$ and $9.82 \times 10^8 \pm 1.33 \times 10^8$, respectively, whereas the intracellular one was $2.36 \times 10^7 \pm 7.83 \times 10^5$ and $2.99 \times 10^8 \pm 9.44 \times 10^7$, respectively.

The catechins inhibited both intracellular and extracellular chemiluminescence activity of whole blood leucocytes (data not shown) or isolated PMNs (Figure 2), either resting or stimulated.

In extracellular compartments, all catechins inhibited chemiluminescence emission with a concentration-dependent linear pattern (Test B; $P < 0.05$). By analysis of variance-R (Test C1), no significant differences were found between the three molecules, either in resting or in PMA-stimulated whole blood leucocytes (data not shown). Similar results were obtained both in resting (Figure 2A) and in activated (Figure 2C) PMNs.

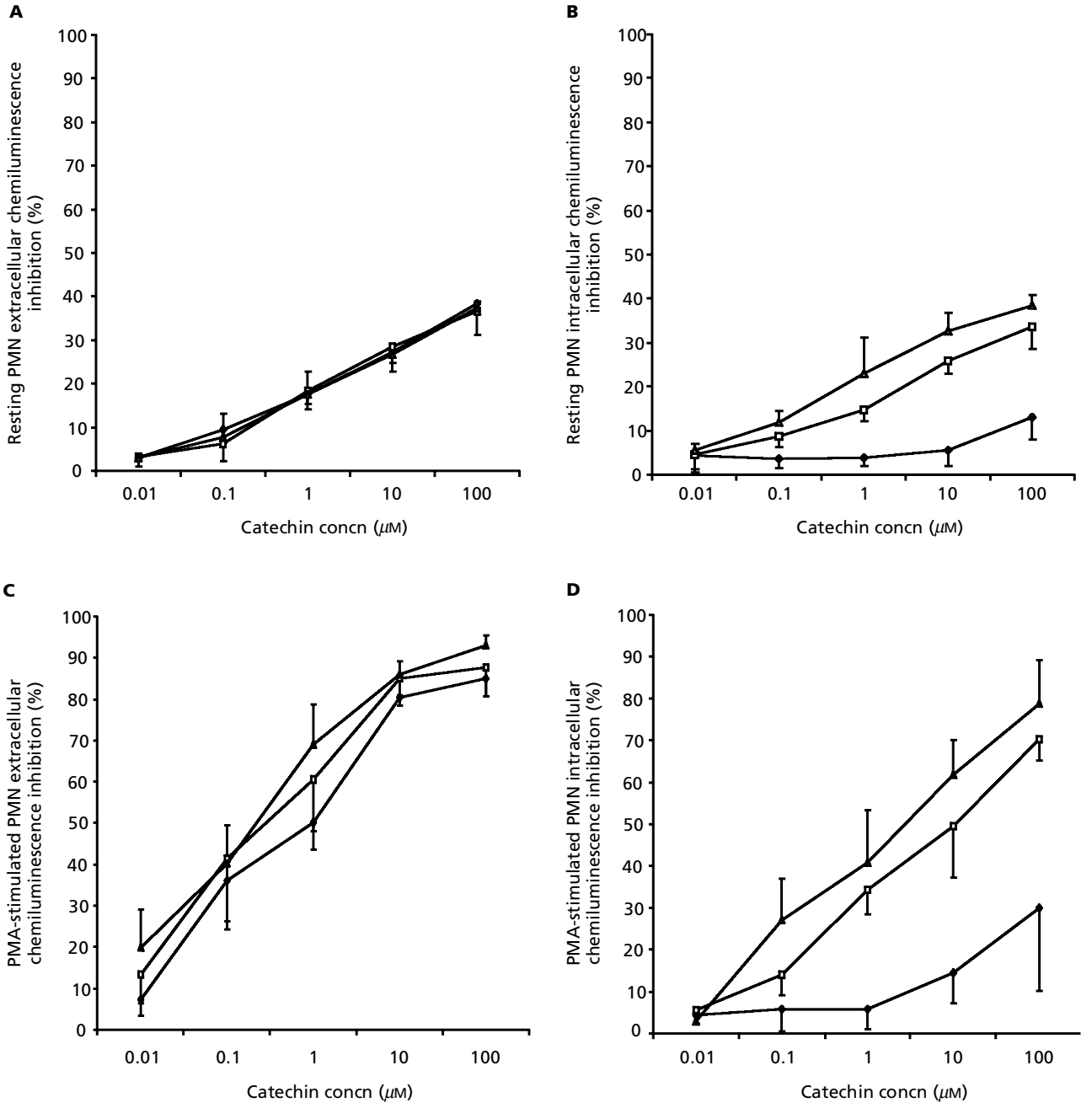

Figure 2 Inhibitory effects of catechins on extra- and intracellular chemiluminescence emission from resting (A, B) and PMA-stimulated (C, D) human isolated PMNs. Values are expressed as percent inhibition (mean ± s.d.). In the absence of catechins, extracellular chemiluminescence counts were $1.12 \times 10^8 \pm 1.06 \times 10^7$ and $9.82 \times 10^8 \pm 1.33 \times 10^8$ for resting and PMA-stimulated cells, respectively, whereas the intracellular counts were $2.36 \times 10^7 \pm 7.83 \times 10^5$ and $2.99 \times 10^8 \pm 9.44 \times 10^7$, respectively. ◆, (+)-catechin; □, (+)-O-PC; △, (−)-O-VC.

At the intracellular level, the chemiluminescence inhibition on resting and PMA-stimulated whole blood leucocytes by native (+)-catechin was low and nonlinear (Test B; data not shown). Esterified catechins exhibited a linear pattern of inhibition in the range of concentrations tested (Test B). The three molecules were compared at each dose (Test C2): esterified catechins were more active than the native one at each dose ($P < 0.05$), whereas no differences were found between them in the range of doses tested. The results obtained with resting (Figure 2B) and PMA-stimulated (Figure 2D) PMNs were similar to those obtained with whole blood leucocytes: esterified catechins were the most active and showed a linear inhibition, whereas native catechin showed a nonlinear slope. No differences were found between esterified catechins.

PMN pre-incubation studies

Chemiluminescence activity after pre-incubation and cell washing. When PMNs were incubated for 15, 30 or 45 min with each catechin (50 μM) and stimulated with PMA after cell washing, a significant inhibitory activity of all catechins on chemiluminescence emission versus control was observed ($2.59 \times 10^8 \pm 2.37 \times 10^7$, $2.76 \times 10^8 \pm 1.92 \times 10^7$, $2.71 \times 10^8 \pm 2.11 \times 10^7$ for 15, 30 and 45 min, respectively; data not shown).

Based on statistical data analysis (test B), the native (+)-catechin inhibition was unrelated to incubation time, whereas the (+)-O-PC and (−)-O-VC inhibitions were related ($P < 0.05$). Comparing results at each incubation time (Test C2), derivatives were more active than native (+)-catechin ($P < 0.05$) and (−)-O-VC was more active than (+)-O-PC.

Chemiluminescence activity after pre-incubation without cell washing. Isolated PMNs were also pre-incubated for 15, 30 or 45 min with each catechin (50 μM) and PMA-activated, without cell washing, at the moment of the chemiluminescence study.

In the first control, PMNs were preincubated without catechins, which were added at the moment of the chemiluminescence study. The chemiluminescence values in the presence of (+)-catechin were $4.96 \times 10^7 \pm 8.59 \times 10^6$, $5.03 \times 10^7 \pm 8.82 \times 10^6$ and $4.98 \times 10^7 \pm 2.61 \times 10^6$ at 15, 30 and 45 min pre-incubation time. The chemiluminescence values in the presence of (−)-PC were $8.46 \times 10^6 \pm 8.08 \times 10^5$, $8.76 \times 10^6 \pm 8.44 \times 10^5$ and $8.45 \times 10^6 \pm 1.02 \times 10^6$ at 15, 30 and 45 min, respectively. The chemiluminescence values in the presence of (+)-VC were $6.98 \times 10^6 \pm 4.57 \times 10^5$, $6.90 \times 10^6 \pm 2.53 \times 10^5$ and $7.19 \times 10^6 \pm 3.56 \times 10^5$ at 15, 30 and 45 min, respectively.

In the second control, PMNs were preincubated without catechins, unwashed and PMA-activated, in the absence of catechins, at the moment of the chemiluminescence study. The chemiluminescence values were $2.20 \times 10^8 \pm 3.66 \times 10^7$, $2.11 \times 10^8 \pm 2.98 \times 10^7$ and $2.25 \times 10^8 \pm 1.97 \times 10^7$ at 15, 30 and 45 min, respectively.

Pre-incubation with catechins markedly enhanced the inhibitory activity of all compounds versus controls ($P < 0.05$). The inhibitory effects were linearly dependent on pre-incubation time only in the presence of esterified catechins that were again the most active ($P < 0.05$, Test C2). Also in this case, (−)-O-VC was more active than (+)-O-PC.

Discussion

In this study we attempted to evaluate the effects of chemically changed (+)-catechin on in-vitro human PMN activity. Whole blood leucocyte chemiluminescence studies were also performed. In particular, we compared the action of native (+)-catechin with that of its synthetic derivatives obtained by 3-O-esterification with a hydrophobic propionyl or valeryl group.

The colorimetric approach revealed no differences between the antioxidant activity of native (+)-catechin and its new derivatives, but the same molecules acted differently in the in-vitro cellular assays. In whole blood leucocytes and isolated PMNs, esterified catechins were more active than the native one, both in resting and in PMA-stimulated cells (Figure 1). The degree of inhibition was (−)-O-VC > (+)-O-PC > (+)-catechin. No substantial differences have been found on the effects of any of the molecules on whole blood leucocytes and isolated PMNs, except for a higher chemiluminescence emission of PMNs for lack of haemoglobin quenching (De Sole et al 1983). This result strengthens the reliability of previous results and confirms that the effects of catechins on whole blood leucocyte chemiluminescence emission is principally due to their activity on PMNs. For this reason, we decided not to show whole blood leucocyte data. The effect of (+)-catechin may have been limited because of its limited penetration of the cell. Since esterification with propionic or valeric acid increases the lipophilicity of catechin, we argue that native (+)-catechin and its derivatives have different intracellular availability and, therefore, different effectiveness. This hypothesis is further supported by morphology of dose–response curves: native (+)-catechin showed a trend toward nonlinear inhibition on PMA-stimulated whole blood leucocytes or isolated PMNs and a linear trend when acting on resting cells. On the contrary, the esterified catechins always showed a linear inhibition when acting either on whole blood leucocytes or PMNs, both at rest and after stimulation.

The compartmental PMN and chemiluminescence pre-incubation study confirmed our hypothesis. Firstly, while extracellular inhibition was similar for the three molecules, intracellular inhibition was greater and linearly dose-dependent only with esterified catechins. Moreover, in the same compartmental study, the percent of intra- and extracellular inhibitory activity for each catechin, both in whole blood leucocytes (data not shown) and isolated PMNs (Figure 2A, B), was lower than that obtained from the same cells when PMA-activated (data not shown and Figure 2C, D, respectively). This could depend on the fact that both native and esterified catechins, besides acting like ROS scavengers, can also interfere with cellular activation mechanisms (membrane or cytoplasmic receptors or enzymes) as already demonstrated, and recently reviewed, for flavonoids (Middleton et al 2000). In such a case, the unactivated cells could be down-regulated to a lesser extent than the PMA-stimulated cells.

This study demonstrates that it is possible to modify catechin antioxidant properties on whole blood leucocytes and PMNs by changing functional groups that do not alter the scavenging activity.

Conclusion

The catechin esters (+)-3-propionylcatechin and (−)-3-O-valerylcatechin reduce the oxidative burst of human phagocytes more efficiently than the native (+)-catechin ((−)-O-VC > (+)-O-PC > (+)-catechin), without affecting antioxidant activity when evaluated by the cell-free colorimetric methods DMPD and ABTS.

An ancillary result obtained is that a single chemical or cellular approach is not enough for an accurate assessment of the overall amount of the antioxidant activity.

Finally, the results show that, with a suitable manipulation of the native (+)-catechin structure, it is possible to enhance its antioxidant properties. The new catechin derivatives act at pharmacological concentrations. These results pave the way to synthetically modifying other natural flavonoids, with applications in vascular and inflammatory diseases, a field where these substances could be of remarkable usefulness.

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