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Dietary polyphenols protect dopamine neurons from oxidative insults and apoptosis: investigations in primary rat mesencephalic cultures

Linda D. Mercer[a,c,*], Belinda L. Kelly[a,c], Malcolm K. Horne[b,c], Philip M. Beart[a,c]

[a]*Department of Pharmacology, Monash University, Clayton, Vic. 3800, Australia*
[b]*Department of Medicine, Monash University, Clayton, Vic. 3800, Australia*
[c]*Brain Injury and Repair Program, Howard Florey Institute, University of Melbourne, Parkville, Vic. 3010, Australia*

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Abstract

Naturally occurring polyphenols have the potential to prevent oxidative damage in various pathophysiological conditions. Various members of the flavonoid family were investigated to determine if they could protect mesencephalic dopamine (DA) neurones from injury and reduce apoptosis produced by oxidative stressors. Primary mesencephalic cultures were sensitive to oxidative insults (hydrogen peroxide, 4-hydroxynonenal, rotenone, 6-hydroxydopamine and *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium hydrochloride (MPP^+)) which produced concentration-dependent decreases in cellular viability across an apoptotic-necrotic continuum of injury. Flavonoids (catechin, quercetin, chrysin, puerarin, naringenin, genestein) protected mesencephalic cultures from injury by MPP^+, which was shown by DNA fragmentation studies and tyrosine hydroxylase (TH) immunocytochemistry of DA neurones to occur by apoptosis. Catechin also reduced injury produced by hydrogen peroxide, 4-hydroxynonenal, rotenone and 6-hydroxydopamine as shown by increases in cellular viability and [^{3}H]DA uptake. When the neuroprotection of catechin against MPP^+-induced injury was compared to that produced by the caspase-3 inhibitor, Z-DVED-FMK, both reduced DNA fragmentation and the injury patterns of TH-positive neurones. These data demonstrate the neuroprotective abilities of flavonoids which are able to attenuate the apoptotic injury of mesencephalic DA neurones. Since these DA neurones are under oxidative stress in Parkinsonism, our findings suggest that flavonoids could provide benefits along with other anti-oxidant therapies in Parkinson's disease.

Keywords: Oxidative stress; Flavonoids; Injury; Mesencephalic dopamine neurones; Neuroprotection; Cultures

1. Introduction

Oxidative stress (OS) as a consequence of mitochondrial dysfunction is believed to contribute to the pathological changes seen in Parkinson's disease (PD) and especially to the death of mesencephalic dopamine (DA) neurones [1,2]. Whilst there is general agreement as to the sensitivity of mesencephalic DA neurones to OS [3,4], exactly how this potentially cytotoxic mechanism eventuates and how its chronology determines the various pathological events remain a topic of some speculation [5]. Free radical generation (hydroxyl, superoxide) and impaired energy production are likely to occur as a consequence of impaired electron transport in the mitochondrial respiratory chain, with complex I particularly implicated as its dysfunction has been described in post-mortem Parkinsonian tissue [6]. The abilities of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone, two toxins targeting mitochondrial complex I, to produce experimental models of Parkinsonism, provide further evidence for the involvement of mitochondrial dysfunction and free radical generation in the pathology of this crippling neurological condition [7,8]. Moreover, the deposition of α-synuclein, considered one of the pathological hallmarks of PD, appears to be linked to oxidative damage [9,10]. Recently, genetic mapping studies have indicated that oxidative stress, possibly arising as a consequence of a hydroperoxide-responsive protein, may be causative in a familial form of early-onset PD [11]. Other toxic free radicals,

* Corresponding author. Tel.: +61 3 8344 7486; fax: +61 3 9348 1707.
E-mail address: l.mercer@hfi.unimel.edu.au (L.D. Mercer).

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including those arising from dopamine-, lipid- and nitrergic-related mechanisms [2,7,12], also may interact in diverse ways with cellular energetics, life/death signaling cascades, protein aggregation and DNA [13].

Cellular death in PD is likely to occur by apoptosis, not only because of its slow time-course, but because post-mortem Parkinsonian tissue presents evidence of DNA fragmentation and apoptotic neurones [14]. Moreover, the death pathway requiring the cooperation of mitochondria and the downstream recruitment of caspases operates in mesencephalic DA neurones [15]. Based on these data, anti-oxidants may provide protection against the oxidative damage occurring in PD. The use of anti-oxidant therapies in the management of PD has been limited, but the existing evidence is that this strategy may be beneficial as coenzyme Q_{10} provides beneficial outcomes in early PD [16]. One family of naturally occurring compounds possessing free radical scavenging properties is the flavonoids. These polyphenols are found in fruit, vegetables and plant-derived beverages and may have important roles as dietary components via cytoprotective actions in many organs [17]. In addition to being potent antioxidants, flavonoids have capacities to prevent lipid peroxidation and modulate nitrergic-related mechanisms [18]. Indeed, there has been much recent interest in dietary strategies to combat OS-related damage in various pathophysiological conditions [17,19]. Given recent evidence that the green tea polyphenol, (−)-epigallocatechin-3-gallate, was neuroprotective in the MPTP mouse model of PD [20], we decided to investigate whether various flavonoids could protect mesencephalic DA neurons from injury produced by oxidative stressors and reduce their death by apoptosis by undertaking investigations in primary mesecephalic cultures.

2. Materials and methods

2.1. Reagents

The 96- and 48-well culture plates were obtained from Nunc (Medos; Mount Waverley, Australia). Neurobasal™ medium (NBM), dialysed foetal calf serum, L-glutamine, B27 supplement, Hank's balanced salt solution (HBSS) and penicillin/streptomycin were purchased from Gibco Life Technologies (Melbourne, Australia). Paraformaldehyde was purchased from Merck (Darmstadt, Germany). Mouse anti-tyrosine hydroxylase (TH), mouse anti-microtubule-associated protein 2 (MAP2), rabbit anti-glial fibrillary acid protein (GFAP), 4-nitroblue tetrazolium chloride, 5-bromo-4-chloro-3-indolyl-phosphate-4-toluidine salt, digoxigenin-11-dUTP, and anti-digoxigenin alkaline phosphatase were purchased from Boehringer Mannheim (Sydney, Australia) and INCSTAR Corporation (Stillwater, MN, USA), respectively. Horse radish peroxidase (HRP) conjugated IgG anti-mouse and anti-rabbit antiserum were purchased from Silenus Laboratories (Melbourne, Australia). Stable peroxidase suppressor solution and 3,3′-diaminobenzidine (DAB) were purchased from Pierce (Rockford, Illinois, USA). 4-Hydroxynonenal (4-HNE) was purchased from Sapphire Bioscience (Crows Nest, Australia). Z-DVED-FMK was obtained from Calbiochem Merck (Kilsyth, Australia). [^{3}H]DA (specific activity 58.4 Ci/mmol) obtained from NEN (Perkin Elmer; Melbourne, Australia). All other reagents, including flavonoids and proteinase inhibitors, were purchased from Sigma (Sydney, Australia) and were of cell culture grade or the highest commercially available grade. Flavonoids were dissolved in dimethyl sulfoxide or ethanol, diluted in H_2O and then culture medium (final concentrations: DMSO 0.03–0.2%, ethanol 0.04%).

2.2. Primary mesencephalic cultures

All procedures involving animal experimentation received ethical approval from Monash University and were undertaken according to the guidelines of the National Health and Medical Research Council (Australia). Cultures were established by a modification of a procedure previously used in our laboratories [21]. Briefly, freshly dissected ventral mesencephalon (VM) was removed from the brains of rat embryos (gestational day E15-16) and placed in ice-cold HBSS. Typically cultures were established from 40–60 foetuses. Tissue was digested in 20 ml HBSS containing trypsin (0.2 mg/ml) and deoxyribonuclease (60 U/ml) at 37 °C for 20 min with gentle shaking. Digestion was terminated by the addition of HBSS containing soyabean trypsin inhibitor (83.2 μg/ml), followed by centrifugation (2000 × *g*, 5 min). The supernatant was aspirated and the tissue pellet was resuspended in 20 ml HBSS containing BSA (3 mg/ml), 1.5 mM $MgSO_4$, soyabean inhibitor (0.52 mg/ml) and deoxyribonuclease (60 U/ml) before trituration through a 24-gauge needle. This suspension was centrifuged (3500 × *g*, 5 min), the supernatant aspirated and the pellet resuspended in NBM containing 2% B27 supplement, penicillin (100 U/ml), 100 μg/ml streptomycin, 0.5 mM L-glutamine, 25.4 mM KCl and 10% dialysed foetal calf serum. Cells were plated in 96 (biochemical procedures) and 48 (morphological analyses) microwell plates coated with 0.05% poly-D-lysine – densities 0.15 and 0.3 × 10^6 cells/well, respectively. After 24 h cultures were changed to serum-free medium as above and maintained at 37 °C in a humidified incubator (5% CO_2, 8.5% O_2) for a further 5–7 days in vitro (div). Medium changes (50%) were performed at 3–4 div.

2.3. Immunocytochemistry

Procedures employed here were essentially those used previously in our laboratories [21,22]. After the culture medium had been aspirated, the cells were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline, pH

7.4, washed extensively and stored at 4 °C until required. Following permeabilization (0.2% Triton X-100), washing, and quenching of endogenous peroxidases, non-specific binding was blocked with 10% normal goat serum (NGS) and 0.1% Triton X-100 for 1 h at room temperature. After further washing, cells were incubated with primary antibodies, TH (1:2000), MAP2 (1:400) or GFAP (1:25), in 2% NGS and 0.1% Triton X-100 overnight at 4 °C. After washing, incubation (3 h) with appropriate secondary antibodies was followed by washing and incubation with 3,3′-diaminobenzidine (0.5 mg/ml in stable peroxidase substrate buffer) until sufficient colour had developed. Photographs were taken of representative fields using bright field microscopy. These analyses revealed that the cultures contained 15–20% GFAP-positive and 5% TH-positive cells (four independent cultures). TH-positive cells were presumed to represent DA neurons because of their morphologies (cf. [21]) and sensitivity to DA neurotoxins.

2.4. Cell viability

Cultures were exposed to various insults and drug treatments at 6 div for 48 h in anti-oxidant free medium (NBM, 100 U/ml penicillin and 100 μg/ml streptomycin) which replaced culture medium. At 8 div media were aspirated and 3(4,5-dimethylthiazole-2yl)-2,5-diphenyltetrazolium bromide (MTT; 0.5 mg/ml) was added to cells in 96-well plates for the estimation of mitochondrial activity as described previously [22]. Raw MTT data were standardized to values from control or vehicle-treated cultures as appropriate (100% cell viability) and cultures treated with 0.1 M Triton X-100 (100% cell death).

2.5. [^{3}H]DA uptake

The procedure employed cells plated at day zero in 96-well view plates (Packard) coated with 0.05% poly-D-lysine and maintained as described above. Cultures were exposed to treatments (see Section 2.4) and at 8 div the medium replaced with gassed, pre-warmed Kreb's bicarbonate containing 10 μM pargyline, 10 μM desmethylimipramine, 100 nM fluoxetine and 1 mM ascorbic acid for 15 min at 37 °C in a humidified atmosphere. [^{3}H]DA (100 nM) was added and the incubation continued for 20 min at 37 °C. The reaction was stopped by rapid washing 3 times with ice-cold Kreb's bicarbonate, and the wells dried at 37 °C for 30 min before the addition of Microscint™ PS (Packard). After solubilization overnight, the accumulated [^{3}H]DA was determined by scintillation spectrometry (Topcount™). Wells treated with 0.1 M Triton X-100 (100% cell death) served as blank estimates of [^{3}H]DA uptake.

2.6. TUNEL

The procedure was a minor modification of that previously employed in our laboratories [22]. VM cultures were grown in 48-well plates and after exposure to MPP^+ on 6 div in the presence and absence of flavonoids or vehicles for 48 h were fixed overnight in 4% (v/v) paraformaldehyde in 0.1 M phosphate-buffered saline pH 7.4. Next day, after extensive washing, the TUNEL procedure was employed with labeled cells being detected by the digoxigenin/alkaline phosphatase method [22]. Colour was developed for 3 h at 4 °C, cells were visualized and random fields photographed.

2.7. Statistical methods

Raw MTT data were standardized to values from control cultures (100% cell viability – media only) and cultures treated with 0.1 M Triton X-100 (100% cell death) for the same time course as drug treatment. Vehicle controls were included as appropriate. Data are expressed as mean ± standard error of mean (S.E.M.). Concentration–response curves were generated by non-linear regression (variable slope) using the GraphPad PRISM™ computer program. EC_{50} values are given as the mean and 95% confidence intervals and are from analyses of multiple independent (3–5) experiments. TH-positive cells in cultures treated with vehicle and MPP^+ with and without catechin or Z-DEVD-FMK, as appropriate, were scored on a scale of 0–3 according to their numbers of neurites to compare the morphologies of DA neurones. ANOVA with appropriate post-hoc tests were carried out using GraphPad PRISM™ ($P < 0.05$ considered significant).

3. Results

3.1. Injury profile of VM cultures

VM cultures were exposed to a range of insults on 6 div to establish the patterns of change in cell viability and their sensitivities to injury. Agents considered to produce oxidative stress, hydrogen peroxide, 4-hydroxynonenal, 6-hydroxydopamine and MPP^+ [7,23] displayed variable potencies, but all induced maximal cell death that was essentially identical to that of cultures treated with 0.1 M Triton X-100 (100% cell death) – EC_{50} values, 39 μM (31–49), 8.3 μM (5.9–12), 50 μM (33–75) and 910 μM (54–2100) ($n = 3-5$), respectively (Fig. 1A). Data from the MTT assay demonstrated that all four insults caused concentration-dependent cell death, which morphological observations demonstrated was widespread (Fig. 2 and data not shown). By contrast, rotenone was much more cytotoxic (EC_{50} 0.14 μM (0.06–0.31)) and produced injury in a concentration-dependent manner, but the maximal cell death was consistently only 55–60% of vehicle control. Staurosporine (EC_{50} 0.84 μM (0.22–3.2)) was employed to demonstrate the patterns of injury expected of an apoptotic insult (data not shown). Morphological examination of the cultures was undertaken to correlate cellular changes with

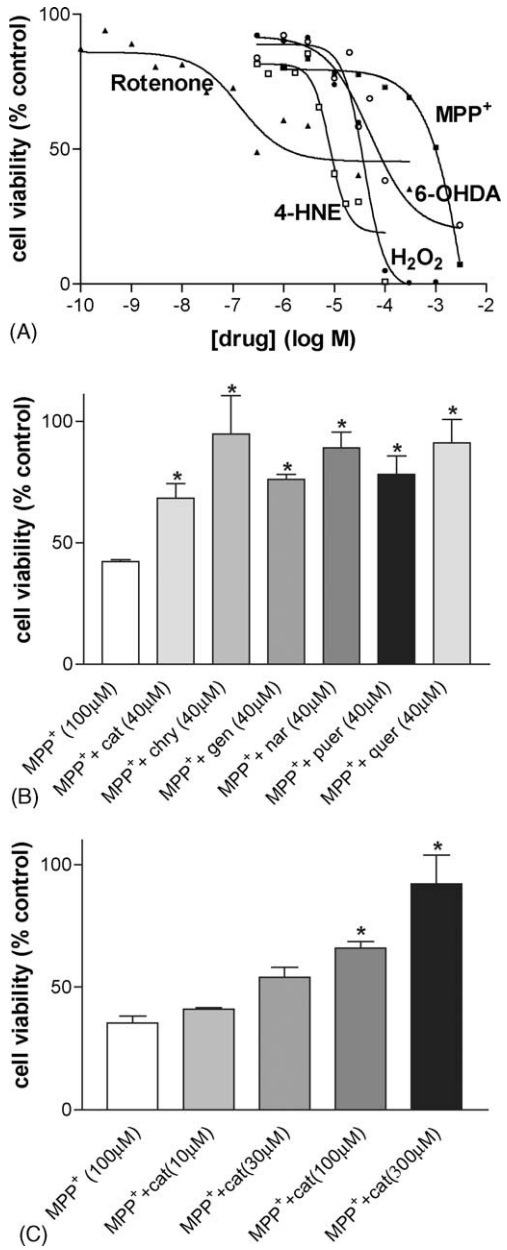

Fig. 1. (A) Injury of primary mesencephalic cultures by various drugs producing oxidative insult. Concentration-dependence of cytotoxicity in cultures of ventral mesencephalon was determined 48 h after drug addition at div 6 using the MTT cell viability assay. Concentration–response curves were generated for all oxidative stressors by computer-assisted, non-linear regression. Each point represents the mean of three to five independent experiments involving triplicate determinations. (B) Cultures of ventral mesecephalon were exposed to MPP^+ (100 μM, 48 h) alone or in the presence of various flavonoids (all 40 μM) before the determination of cell viability: catechin (cat), chrysin (chry), genestein (gen), naringenin (nar), puerarin (puer) or quercetin (quer). Values are the mean ± S.E.M; two to three independent experiments involving triplicate determinations; $P < 0.05$, ANOVA. (C) Catechin (10–300 μM) provided concentration-dependent cytoprotection against injury produced by the exposure of ventral mesencephalic cultures to MPP^+ (100 μM, 48 h). Values are the mean ± S.E.M. of two to three independent experiments involving triplicate determinations. $^*P < 0.05$ relative to MPP^+, ANOVA. Further details are given in the text.

MTT viability data. All oxidative insults produced concentration-dependent neuronal damage, demonstrating morphological changes consistent with apoptosis and necrosis, dependent on the severity of the insult. High concentrations of drugs produced initial rapid swelling followed by appreciable levels of debris and diminished cell density. After exposure (24 and 48 h), low concentrations of these drugs produced neurite blebbing and shrinkage, consistent with apoptosis (Fig. 2 and data not shown) and the pattern of injury found with staurosporine. Overall, our data are consistent with existent findings with these insults in VM cultures [15,24,25].

3.2. Effect dietary polyphenols on MPP^+-mediated injury

Preliminary investigations of the neuroprotection provided by flavonoids were performed in a standardized procedure where MPP^+ induced apoptotic injury. MPP^+ (100 μM, 48 h) produced 30–40% cell death, which was slow in time-course and involved TH-positive neurons and apoptosis, as confirmed by immunocytochemistry and TUNEL, respectively (Fig. 2). Under these conditions a number of flavonoids significantly attenuated the cellular injury produced by MPP^+ as indicated by changes in cellular viability (Fig. 1B).

3.3. Effects of catechin versus diverse oxidative insults

The actions of catechin, one of the more water-soluble flavonoids, were evaluated in more detail to fully examine its neuroprotective profile against MPP^+ and a range of oxidative insults. Catechin (10–300 μM) produced concentration-dependent cytoprotection standardized MPP^+ procedure ($P < 0.05$), with the highest concentration evaluated returning cell viability almost to control values (Fig. 1C). In further studies, catechin (40 μM) protected VM cultures from injury by a range of oxidative insults, including the inhibition of mitochondrial complex I (rotenone and MPP^+), dopamine- (6-hydroxydopamine) and lipid-related (4-hydroxynonenal) mechanisms, or generation of hydroxyl radicals (H_2O_2; Fig. 3). Protection with catechin was found with all insults (Fig. 3 and data not shown), which at the concentrations used produced injury by apoptosis, and even with H_2O_2 under conditions where injury was by necrosis. While these experiments examined overall effects on cell viability, subsequent studies employed [^{3}H]DA uptake as a sensitive, quantitative index of injury to the population of DA neurons of VM cultures [26]. Catechin (40 μM) produced significant increases ($P < 0.05$) in [^{3}H]DA uptake when VM cultures were exposed to all oxidative insults (Fig. 3). The variable degrees of injury and of neuroprotection found with catechin appeared to result at least partially from the difficulty of producing approximately 40–50% injury given the steep concentration–response relationships. However, in some cases the effects of catechin when compared to the level of

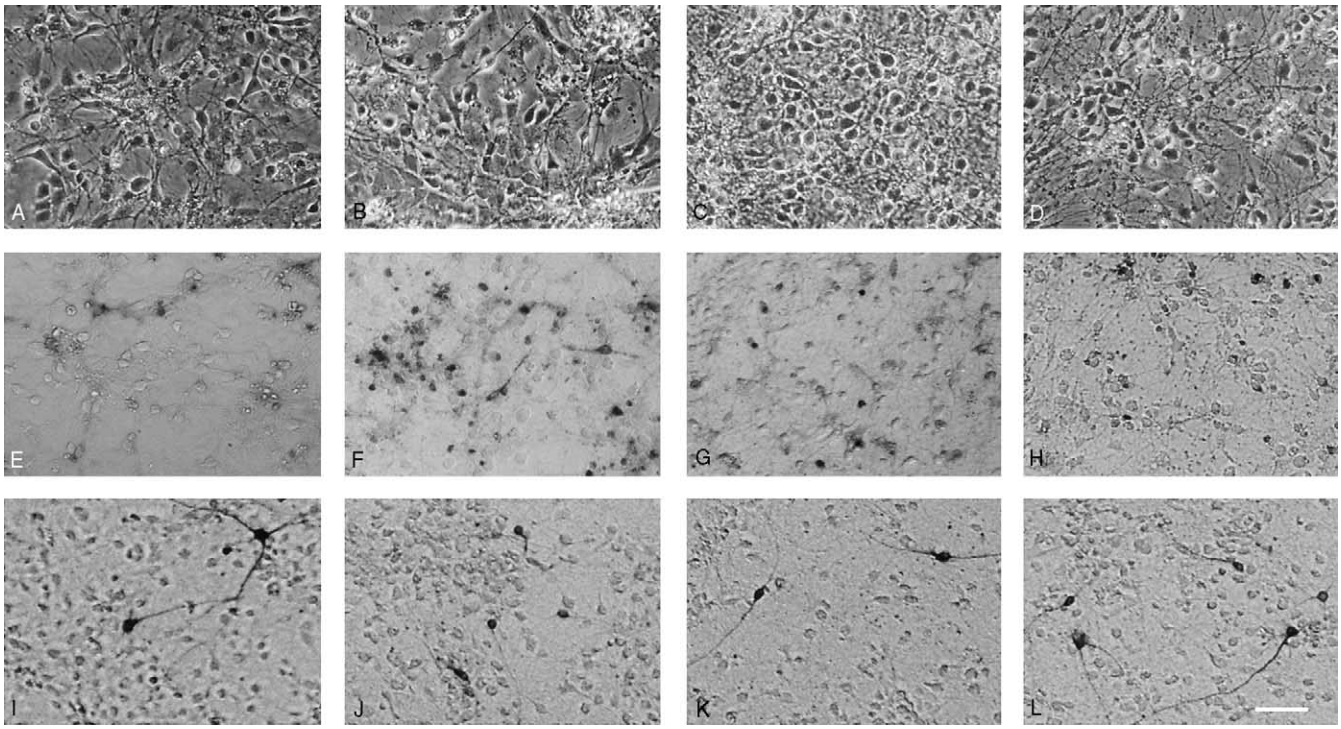

Fig. 2. Analyses of the morphology of ventral mesencephalic cultures by various methods 48 h after drug treatments. On div 6, cultures were exposed to vehicle (A, E, I), MPP^+ 300 μM (B, F, J), MPP^+ 300 μM + catechin 40 μM (C, G, K), or MPP^+ 300 μM + Z-DVED-FMK 40 μM (D, H, L). Phase contrast microscopy (A–D) showed that catechin and Z-DVED-FMK reduced the cellular damage produced by MPP^+. Bright field photomicrographs of DNA fragmentation by the TUNEL technique (E–H) revealed decreases in the extent of labeling after treatment with catechin and Z-DVED-FMK relative to that produced by MPP^+ alone. Immunohistochemistry for tyrosine hydroxylase (I–L) revealed that relative to vehicle-treated cultures, MPP^+ produced extensive neurite blebbing indicative of apoptosis, whereas inclusion of catechin and Z-DVED-FMK reduced the injury to dopamine neurones as shown by the preservation of neurites. ANOVA indicated significantly different numbers of neurites across the four groups ($F_{3,56} = 22.07$, $P < 0.05$) and a significant interaction between treatments and neurites ($F_{9,56} = 7.159$, $P < 0.05$). Further details are given in the text. Scale bar represents 150 μm.

uptake after injury was indicative of extensive neuroprotection of DA neurones (Fig. 3).

In morphological studies, we sought to determine that the biochemical effects seen with catechin translated into increases in neuronal integrity. Catechin (40 μM) greatly improved the integrity of cultures, which contained more healthy neurones and extensive networks of neurites relative to VM cultures treated with 300 μM MPP^+ alone. Numbers of TUNEL-positive neurons were reduced and TH-positive neurons possessed more extensive neuritic morphologies (Fig. 2). Similar results were obtained in terms of protection against other oxidative insults, except hydrogen peroxide (data not shown). Given that neuroprotection against apoptosis was a key area of our investigation, we compared these actions of catechin to those of the caspase-3 inhibitor, Z-DEVD-FMK, against MPP^+-induced injury. Here the findings obtained by light microscopy, TUNEL labeling and TH immunocytochemistry were very similar to those noted with catechin, with Z-DEVD-FMK proving protective by all three methods of morphological assessment (cf. [27]). Specifically, both catechin and Z-DEVD-FMK when included with MPP^+ increased the number of neurites on TH-positive DA neurones relative to MPP^+ alone – there were different numbers of neurites across the four groups ($P < 0.05$) and a significant interaction between treatments and neurites ($P < 0.05$). These data confirmed that MPP^+-induced injury was predominantly apoptotic under our defined experimental conditions, and demonstrated the ability of catechin to protect DA neurons from apoptosis.

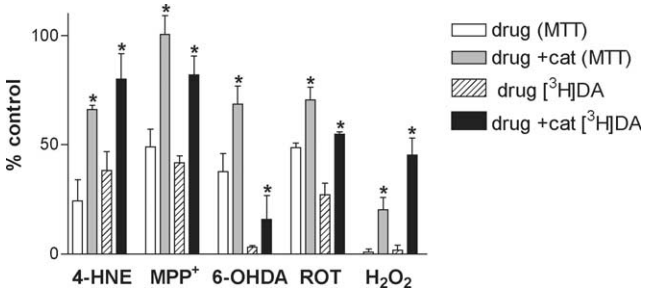

Fig. 3. Cytoprotection by catechin (40 μM) against cellular injury produced by 4-hydroxynonenal (4-HNE, 10 μM), MPP^+ (100 μM), 6-hydroxydopamine (6-OHDA, 50 μM), rotenone (ROT, 1 μM) and H_2O_2 (1 mM). Oxidative insults and catechin were added to ventral mesencephalic cultures at 6 div and estimates of cellular viability and [^{3}H]DA uptake were made 48 h later. Data for cellular viability shown in first two columns (white = insult alone, grey = insult + catechin), while the last two columns are data from [^{3}H]DA uptake (striped = insult alone, black = insult + catechin). Control [^{3}H]DA uptake was 20–45 fmol/well. Values are the mean ± S.E.M. of three to four determinations from a single independent experiment that was replicated at least once. $^*P < 0.05$ relative to insult alone, ANOVA. Further details are given in the text.

4. Discussion

Although there is not an abundant clinical literature, the therapeutic benefits found with coenzyme Q_{10} [16], when taken with evidence of oxidative stress in human and experimental Parkinsonism (see Section 1), together provide a strong case for the use of anti-oxidant therapies in the management of PD. The dietary polyphenols investigated here have the potential to prevent oxidative damage and directly scavenge free radicals [18,28,29]. When six such flavonoids were evaluated for their ability to prevent injury in mesencephalic cultures, all protected neurons from damage by the dopaminergic toxin MPP^+. One flavonoid, catechin, was studied in detail and could attenuate damage to DA neurons by MPP^+, which under the conditions employed was by apoptosis. Catechin also protected mesencephalic DA neurons from a wide range of oxidative insults including the generation of hydroxyl radicals, inhibition of complex I, and dopamine- and lipid-related mechanisms. Our study is the first to demonstrate directly that polyphenol molecules of the flavonoid and isoflavonoid classes provide neuroprotection for DA neurons against oxidative stressors. Although flavonoids theoretically possess both anti- and pro-oxidant properties [28] we focused our attention on neuroprotective actions by excluding in preliminary experiments a number of flavonoids that were cytotoxic in VM cultures. Our new evidence strongly supports existing findings that extracts of green tea containing polyphenols protect DA neurons [24]. Evidence in vivo that both green tea extract and its main polyphenol, (−)-epigallocatechin-3-gallate, when administered peripherally are neuroprotective in the MPTP mouse model of PD, provides other key parts of this exciting story. Taken together one can speculate, as have many others, that naturally occurring polyphenols and other anti-oxidants deserve further attention for the clinical management of PD [17,20,30].

The focus of our study in VM cultures was on apoptotic injury since this mechanism appears relevant to the slow death of DA neurons in PD [31]. Firstly, by examining the concentration–response of cellular viability to the various oxidative stressors, we were able to employ MPP^+ and other insults at concentrations $<EC_{50}$ values that produced injury that was slow in time-course resulting in numerous TUNEL-positive neurones. Other evidence that these insults produced apoptosis were the shrinkage of neurons and the loss of neurites [22], whilst with MPP^+ and rotenone we found mobilization of the mitochondrial pro-apoptotic proteins cytochrome *c* and apoptosis-inducing factor in DA neurons [15], which displayed DNA fragmentation as shown by labelling with propidium iodide [32]. Under these defined conditions, all injuries were attenuated also by the caspase-3 inhibitor, Z-DEVD-FMK (Fig. 2 and data not shown). H_2O_2 proved difficult to work with but interestingly catechin provided some protection when injury was by necrosis (Fig. 3). Secondly, by studying [^{3}H]DA uptake and TH immunocytochemistry, we specifically analysed DA neurons present in the VM cultures demonstrating that catechin reduced the apoptotic injury caused by various oxidative insults. However, what is clear is that while flavonoids could reduce injury to DA neurons, they were generally protective in VM cultures. Most of our attention was directed at the neuroprotective actions of catechin which is a component of tea, fruit and wine [28]. However, we also found that two other related molecules, one a flavone (chrysin, a component of vegetables and citrus fruits) and the other a flavonol (quercetin, found in wine grapes, onions, tea and fruit) were cytoprotective. Another active molecule was puerarin, a key component of kudzu root, widely used in Chinese herbal medicine and which, amongst diverse actions, is antidipsotropic [33]. Two isoflavonoids, naringenin (found in grapefruit and other citrus fruits) and genestein (a component of soy extracts and legumes) were also protective in our system.

The polyphenolic flavonoids investigated here have been well documented to possess activity as anti-oxidants and to be cytoprotective against insults such as hydrogen peroxide, nitric oxide and lipids [28,29]. Moreover, (−)-epigallocatechin-3-gallate when administered in vivo has the capacity to interact with anti-oxidant enzymes [34,35]. However, it is very clear that flavonoids have other, beneficial pharmacological actions unrelated to antioxidant activities, especially on signal transduction (particularly on various kinases; [18,20]), as typified by (−)-epigallocatechin-3-gallate, which has multiple effects on cellular life/death signaling cascades [20]. Other important studies reveal that flavonoids, presumably because of their lipophilicity, are able to cross the blood–brain barrier from the periphery including after oral administration [17,36]. Clearly dietary compounds such as flavonoids have the potential to protect at-risk mesencephalic DA neurons from OS. Overall, the evidence implicating mitochondrial dysfunction and free radical generation in the pathology of Parkinsonism supports the importance of a healthy diet (rich in fruit, vegetables and plant-derived beverages) providing flavonoids [19] and the use of anti-oxidant therapies in PD.

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