L-Selegiline Potentiates the Cellular Poly(ADP-Ribosyl)ation Response to Ionizing Radiation

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ABSTRACT

DNA strand breaks induced by alkylating agents, oxidants, or ionizing radiation trigger the covalent modification of nuclear proteins with poly(ADP-ribose), which is catalyzed for the most part by poly(ADP-ribose) polymerase-1 and plays a role in DNA base-excision repair. Poly(ADP-ribose)ylation capacity of mononuclear blood cells correlates positively with life span of mammalian species. Here, we show that L-selegiline, an anti-Parkinson drug with neuroprotective activity and life span-extending effect in laboratory animals, can potentiate poly(ADP-ribose) formation in intact cells. COR4 hamster cells were incubated with L-selegiline (50 nM) for various time periods, followed by γ-irradiation (45 Gy). Quantification of cellular poly(ADP-ribose) levels at 10 min after starting the irradiation revealed significant increases (up to 1.8-fold) in cells preincubated with the drug for 8 h to 7 days compared with drug-free irradiated controls. There was no selegiline-induced change in poly(ADP-ribose) levels of unirradiated cells nor in basal or radiation-induced DNA strand breaks, respectively. Surprisingly, poly(ADP-ribose) polymerase-1 protein was down-regulated by L-selegiline treatment. Addition of L-selegiline to purified poly(ADP-ribose) polymerase-1 did not alter enzymatic activity. In conclusion, the results of the present study identify a novel intervention to potentiate the cellular poly(ADP-ribose)ylation response. We hypothesize that the effect of L-selegiline is due to modulation of accessory proteins regulating poly(ADP-ribose) polymerase-1 activity and that increased cellular poly(ADP-ribose)ylation capacity may contribute to the neuroprotective potential and/or life span extension afforded by L-selegiline.

Activation of the 113-kDa nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1; EC 2.4.2.30) represents one of the fastest responses of eukaryotic cells to DNA strand breaks as induced by alkylating agents, oxidants, or ionizing radiation (for recent reviews see Jacobson and Jacobson, 1999; Ha and Snyder, 2000; Shall and de Murcia, 2000; Bürkle, 2001b; Davidovic et al., 2001). PARP-1 catalyzes the covalent post-translational modification of nuclear proteins, including PARP-1 itself, with highly complex, branched chains of poly(ADP-ribose) (pADPr), using NAD⁺ as substrate. A few years ago, it was discovered that PARP-1 is apparently not the sole source of pADPr formation in living cells (Shieh et al., 1998), and several other polypeptides catalyzing DNA strand break dependent or independent formation of pADPr have been described since then (Ame et al., 1999; Berghammer et al., 1999; Johansson, 1999). However, under conditions of DNA strand breakage 75 to 97% of cellular pADPr formation in murine fibroblasts is due to activation of PARP-1 (Shieh et al., 1998).

Numerous studies have established the importance of poly(ADP-ribosyl)ation for the recovery of proliferating cells from DNA damage and the role of PARP-1 as a “survival factor” (Shall and de Murcia, 2000). PARP-1−/− mice are acutely sensitive to alkylating agents and γ-irradiation, in line with a role of PARP-1 in DNA base-excision repair, established in cell culture experiments (Trucco et al., 1998; Dantzer et al., 1999). In addition, PARP-1 acts as a negative regulator of DNA damage-induced genomic instability (Meyer et al., 2000; Bürkle, 2001c). In a systematic comparison of cellular poly(ADP-ribosyl)ation capacity of various mammalian species, the longest-lived species studied (i.e., human) displayed maximal enzyme activity at a level 5-fold that of the shortest lived (rat), despite identical PARP-1 protein levels in the two species (Grube and Bürkle, 1992). The observation of cellular poly(ADP-ribosyl)ation capacity being correlated with longevity of mammalian species is very much in line with the widely held view that DNA damage plays a major role in the ageing process (Bürkle, 2001a).

ABBREVIATIONS: PARP, poly(ADP-ribose) polymerase; pADPr, poly(ADP-ribose); MAO-B, monoaminooxidase B; TCA, trichloroacetic acid; HPLC, high-performance liquid chromatography; PBS, phosphate-buffered saline; MOPS, 4-morpholinepropanesulfonic acid; FADU, fluorescence-detected alkaline DNA unwinding; TRI, total radioactivity input.
Over the past decade, a plethora of highly potent PARP inhibitors as well as a variety of molecular genetic approaches to inhibit or abrogate PARP-1 activity have been developed (Shall and de Murcia, 2000). In contrast, very little work has been invested so far to identify or develop substances that might potentiate cellular poly(ADP-ribosyl)ation. Here, we report that the anti-Parkinson drug l-selegiline (l-deprenyl) can potentiate poly(ADP-ribosyl)ation capacity of intact mammalian cells challenged with γ-irradiation. Selegiline has been used for the therapy of Parkinson’s disease on the basis of its monoamine oxidase B (MAO-B) inhibitory action (Gerlach et al., 1996). But in addition, it was observed that administration of selegiline at doses below MAO-B inhibition (<1 μM) can extend the life span of various animal species (Knoll et al., 1989; Freisleben et al., 1994; Ruehl et al., 1997; Stoll et al., 1997). Furthermore, neuroprotective effects have been proposed for selegiline, independently of MAO-B inhibition, both in vitro and in vivo (Semkova et al., 1996; Maruyama and Naoi, 1999; Klegeris and McGeer, 2000; Kitani et al., 2001; Ebadi et al., 2002). To date, the mechanisms underlying the life span-extending and neuroprotective properties of selegiline remain largely unclear, although an antiapoptotic function of selegiline (Naoi et al., 2000) as well as modification of the activity of endogenous antioxidant enzymes by selegiline (Kitani et al., 1999) have been discussed.

The results of the present study 1) identify a novel intervention to potentiate the cellular poly(ADP-ribosylation) response and 2) lead us to hypothesize that an increased cellular poly(ADP-ribosylation) capacity may contribute to the neuroprotective potential and/or life span extension afforded by selegiline.

Materials and Methods

Chemicals. l-selegiline (l-deprenyl) and 4,6-diamidino-2-phenylindol were purchased from Sigma Chemie (Deisenhofen, Germany). Trichloroacetic acid (TCA) was from Roth (Karlsruhe, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany).

Cell Culture. The simian virus 40-transformed embryonic hamster cell line COR4 (Meyer et al., 2000) was grown as a monolayer culture in Dulbecco’s modified essential medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Sigma Chemie). Cultures were incubated at 37°C with 5% CO2. For treatment with selegiline, the drug was dissolved in Millipore water, passed through a sterile filter, and added to standard medium at a final concentration of 50 nM. COR4 cells were exposed to the drug for time periods ranging from 2 h to 7 days. Every 2nd day, if applicable, cells were trypsinized and replated in fresh medium supplemented or not with selegeline, to prevent confluence.

HPLC-Based Quantification of Cellular pADPr Levels. COR4 cells were irradiated using a 147Cs γ-ray source (Gamma Cell 1000; Atomic Energy of Canada Ltd., Mississauga, ON, Canada) at a dose rate of 8 Gy/min at room temperature in phosphate-buffered saline (PBS; 137 mM sodium chloride, 2.7 mM potassium chloride, 1.5 mM potassium dihydrogenphosphate, and 8.1 mM disodium hydrogenphosphate). Ten minutes after starting the irradiation, cells were precipitated with 10% ice-cold TCA. After washing once in 20% ice-cold TCA and twice with 96% ethanol, cell pellets were dissolved in 1 M KOH and incubated at 60°C for 1 h. Quantification of pADPr was performed as described by Jacobson et al. (1984) with minor modifications. Briefly, ADP-ribose polymer was purified by a dihydroboronate chromatography step and enzymatically digested to nucleosides, followed by fluorescent derivatization. Fluorescence-based detection and quantification of pADPr-specific nucleosides was carried out using reversed-phase HPLC. Statistical analysis of the data was performed using Student’s t test. Results were considered significant at p < 0.05 and highly significant at p < 0.001.

Fluorescence-Spectrophotometric Assay for the Determination of DNA Concentrations. Fluorescence-spectrophotometric determination of the DNA content of the samples prepared for HPLC-based quantitation of cellular pADPr was performed essentially as described by Brunk et al. (1979). Briefly, after dissolving ethanol-washed cell pellets (see above) in 1 M KOH, 30 μl-aliquots were taken out and mixed with 30 μl of 2 M MOPS, respectively, resulting in a pH of 7.0. The basal fluorescence of the dilution buffer (10 mM Tris-HCl, pH 7.0, 100 mM NaCl, 10 mM EDTA, and 0.5 μM/l 4,6-diamidino-2-phenylindole) was measured using a fluorescence spectrophotometer (M2000; Hitachi, Tokyo, Japan), with an excitation wavelength of 360 nm and an emission wavelength of 450 nm. Ten microliters of the sample was added to the reaction buffer, followed by recording of the increased fluorescence due to the presence of DNA. This procedure was repeated five times. Then 10 μl of Tris-EDTA buffer containing COR4 DNA at a known concentration (“DNA standard”) was added, followed by measurement of the fluorescence. Again, the procedure was repeated five times. Linear regression analysis was performed on the data points obtained from the two DNA solutions, respectively, yielding excellent correlation coefficients (about 0.999). The DNA concentration of the test sample was deduced from the ratio of the slopes and the known concentration of the DNA standard.

SDS-Polyacrylamide Gel Electrophoresis and Western Blotting. This was performed essentially as described previously (Meyer et al., 2000). Briefly, proteins were extracted from COR4 cells by incubating whole cells in lysis buffer (62.5 mM Tris-HCl, pH 6.8, 6 M urea, 10% glycerol, 2% SDS, 5% β-mercaptoethanol) at 95°C. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane (Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK). The membrane was incubated overnight at 4°C with a monoclonal antibody C-Ⅱ-10 directed against PARP-1 (kindly provided by G. G. Poirier, Health and Environment Unit, Laval University Medical Research Center, Centre hospitalier Universitaire de Quebec, Faculty of Medicine, Laval University, Quebec, Canada), diluted 1:5 in PBS, 0.05% Tween 20, and 5% dry milk, followed by incubation for 1 h at room temperature with a peroxidase-linked goat anti-mouse antibody diluted 1:1000 in PBS, 0.05% Tween 20, and 5% dry milk, followed by incubation for 1 h at room temperature with a peroxidase-linked goat anti-mouse antibody diluted 1:1000 in PBS, 0.05% Tween 20, and 5% dry milk. Membranes were processed for chemiluminescence plus reaction (Amersham Pharmacia Biotech, Piscataway, NJ) was carried out, the membrane was exposed in a chemiluminescence imager (LAS 100, Fuji; Raytest, Straubenhardt, Germany). Band intensity was quantified using the Aida software, version 2 (Raytest).

Fluorescence-Detected Alkaline DNA Unwinding (FADU). This was done using the FADU procedure (Birnboim and Jeyvak, 1981) in a recently developed automated format (R. Pfeiffer, A. Leake, M. Müller, T. B. Kirkwood, and A. Bürke, manuscript in preparation). Briefly, 7 × 104 cells/well of a 96-well plate were lysed in a detergent/urea buffer at 0°C, followed by partial unwinding of the DNA, starting from DNA strand interruptions, under controlled alkaline conditions for 10 min at 10°C and measurement of the fraction of DNA that had undergone double stranded, using the fluorescence generated by the DNA intercalating dye SYBR Green as a readout. Assays were done in 12-fold parallel determination. T-samples (high control) were not exposed to alkaline pH, i.e., no unwinding occurred at all. P0 samples (untreated cells) and P samples (treated cells) were exposed to alkaline pH. Therefore, in P0 samples unwinding did occur, starting from chromosome ends and any spontaneous internal strand breaks, and also in P samples, starting additionally from damage-induced strand breaks. The fluorescence intensity of P samples is inversely related to the number of DNA strand breaks present at the time of lysis.

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Determination of PARP-1 Activity in Vitro. This was done essentially as described previously (Beneke et al., 2000). Two micrograms of purified recombinant human PARP-1 (Beneke et al., 2000) was combined with reaction buffer (100 mM Tris-HCl, pH 8.0, 10 mM MgCl₂, 1 mM dithiothreitol, 40 μg/ml histone type IIa (Sigma Chemie), 50 μg/ml of the “activator” oligonucleotide GGAATTCC (Berger and Petzold, 1985), 0.2 mM β-NAD⁺ (grade V; Sigma Chemie), 370 kBq/ml [32P]NAD⁺ (PerkinElmer Life Sciences, Boston, MA) and L-selegiline at the concentrations indicated in a final volume of 100 μl. Reactions were run for 10 min at 37°C and stopped by adding 100 μl of ice-cold 20% TCA. Samples were vacuum-aspirated on GF-Whatman filters (Whatman, Maidstone, UK) and washed with ice-cold 20% TCA and then with 70% ethanol. PARP-1 activity was quantified by β-scintillation counting of acid-insoluble radioactivity, this value being expressed as a percentage of total radioactivity input (%TRI). Statistical analysis of the results was performed using the Mann-Whitney U test. Results were considered significant at p < 0.05 and highly significant at p < 0.001.

Results

pADPr Levels in Selegiline-Exposed Cells after γ-Irradiation. The experimental design for the determination of cellular pADPr levels is depicted in Fig. 1A. After culturing COR4 cells in standard medium supplemented with 50 nM selegiline for indicated times varying from 2 h to 7 days, cells were irradiated with 45 Gy. γ-Radiation was deliberately chosen as DNA-damaging treatment to prevent problems with toxicokinetics or drug-drug interactions that could emerge when using chemical compounds. In all cases, pADPr synthesis was allowed to take place for a fixed time (10 min) at room temperature before cells were precipitated using ice-cold TCA. Subsequently, determinations of both cellular pADPr and cellular DNA content were carried out as described under Materials and Methods. After irradiation of

Fig. 1. pADPr levels in γ-irradiated COR4 cells pretreated or not with L-selegiline. A, schematic of the experimental protocol. COR4 cells were incubated in standard growth medium containing or not 50 nM L-selegiline for varying times from 2 h up to 7 days as indicated. Cells were subsequently irradiated with 45 Gy and cellular pADPr levels were determined 10 min after starting the irradiation as described under Materials and Methods. B, time course of 2- to 24-h preincubation with L-selegiline. Given are mean values (± S.E.M.) of the fold-increase in cellular pADPr levels relative to selegiline-free irradiated controls (n, number of samples; p, significance versus control). C, time course of 3- to 7-day preincubation.
nonpretreated (i.e., control) COR4 cells with 45 Gy, pADPr levels showed a dramatic, typically around 40-fold, increase compared with unirradiated control cells, as expected. Within each set of experiments this level was defined as 1.0 (Fig. 1, B and C, control). This radiation-induced increase in pADPr levels proved significantly higher (up to about 1.8-fold) in cells pretreated with 50 nM selegiline for times ranging from 8 h to 7 days (Fig. 1, B and C), whereas shorter duration of pretreatment did not lead to statistically significant effects (Fig. 1B). A highly significant enhancement of pADPr accumulation by 1.47-fold was detected in cells exposed to selegiline for 3 days (Fig. 1C; \( p = 0.0001 \)). Interestingly, radiation-induced pADPr levels tended to be significantly lower after 7 days of preincubation with selegiline compared with 3 days (Fig. 1C, \( p \) (3 day versus 7 day) = 0.0068), while still remaining significantly higher than in irradiated controls (Fig. 1C, \( p \) (7 day versus control) = 0.014).

**pADPr Levels in Unirradiated Selegiline-Exposed Cells.** To see whether drug treatment per se could lead to increased pADPr accumulation under the chosen conditions, pADRr levels were determined in unirradiated COR4 cells after incubation with 50 nM L-selegiline for 3 days. As expected, the levels in unirradiated cells not exposed to selegiline were very low (<1 pmol/100 \( \mu \)g of DNA; Fig. 2B). No significant difference between selegiline-treated cells and control cells was observed (\( p = 0.54 \)), thus ruling out that the results depicted in Fig. 1 might be due to an additive effect of selegiline and \( \gamma \)-irradiation.

**Spontaneous and \( \gamma \)-Radiation-Induced DNA Strand Breakage in Selegiline-Exposed COR4 Cells.** Because PARP-1 is directly activated by DNA strand breaks, the above-mentioned effect of selegiline (Fig. 1) might hypothetically be due to increased numbers of breaks introduced by the standard dose of \( \gamma \)-radiation applied. We therefore determined the number of DNA strand breaks in selegline-exposed COR4 cells after \( \gamma \)-irradiation at various doses, using the FADU procedure (Birnboim and Jevcak, 1981) in a recently developed automated format (R. Pfeiffer, A. Leake, M. Müller, T. B. Kirkwood, and A. Burke, manuscript in preparation). The \( P_0/T \) ratios, reflecting the level of spontaneous DNA strand breaks (see Materials and Methods), were 88.7% for unirradiated controls and 89.4% for unirradiated selegline-treated cultures (not significant). Upon \( \gamma \)-irradiation, there was a dose-dependent reduction of \( P_0/T_0 \) ratios (Fig. 3, A and B), as expected, but again the level of DNA strand breakage in selegline-treated cells was very similar to that of unirradiated controls

![Fig. 2. Basal pADPr levels in L-selegiline-exposed and control COR4 cells.](image)

A, schematic of the experimental protocol. COR4 cells were incubated in standard growth medium containing (+Sel) or not (−Sel) 50 nM selegline for 72 h. Cellular pADPr levels were determined as described under Materials and Methods. B, pADPr levels are given as mean values ± S.E.M. of triplicates. No significant differences were observed (\( p = 0.54 \)).

![Fig. 3. DNA strand breakage in \( \gamma \)-irradiated COR4 cells pretreated or not with L-selegline.](image)

Cells were incubated in standard growth medium supplemented or not with 50 nM L-selegline for 3 days, followed by \( \gamma \)-irradiation on ice at various doses as indicated. DNA strand breaks were determined by fluorescence-detected alkaline DNA unwinding in 12-fold parallel determination as described under Materials and Methods. A and B, given are the mean \( P_0/T_0 \) values ± S.E.M., representing the fraction of DNA remaining double-stranded during the alkaline DNA unwinding procedure in irradiated samples (dose ×) relative to unirradiated controls, as a measure that is inversely related to the number of radiation-induced DNA strand breaks. B, statistical evaluation by Student’s t test of differences between higher versus lower irradiation dose and of selegline-incubated cells versus controls, respectively. Note that with every doubling of irradiation dose, the numbers of DNA strand breaks increase significantly, whereas at any irradiation dose used the values from selegline-incubated cells are very similar to those of selegline-free cells.
of control cells at any irradiation dose tested (Fig. 3, A and B). Therefore, a putative modulation of the DNA strand breakage by selegiline preincubation can be dismissed as an explanation for the drug's effect on radiation-induced pADPr levels (Fig. 1).

**PARP-1 Protein Levels in the Presence of Selegiline.** Western blotting of whole-cell extracts was performed to investigate the pattern of PARP-1 expression in COR4 cells exposed to 50 nM selegiline for 3 days (Fig. 4A). Note that cells were not irradiated before Western blot analysis. Blots showed a single band migrating at 113 kDa specific for PARP-1 (Fig. 4A). As expected from the absence of any toxic effect of selegiline treatment, no apoptosis-related proteolytic cleavage product was observed. Quantification of the intensity of the bands at 113 kDa surprisingly revealed a 40% reduction in extracts of selegiline-incubated cells compared with controls (Fig. 4B). A parallel gel stained with Coomassie Blue demonstrated equal loading of extracts from selegiline-exposed cells and controls (Fig. 4C). The possibility that PARP-1 might be extensively automodified after selegiline treatment and therefore might not migrate into the gel is ruled out by the data shown in Fig. 2, where no increase in cellular poly(ADP-ribose) levels was detected in unirradiated cells. Thus, under the given experimental conditions, incubation of COR4 cells with 50 nM selegiline down-regulated cellular PARP-1 protein levels.

**Activity of PARP-1 in Vitro in the Presence of Selegiline.** To investigate whether selegiline can influence PARP-1 activity in vitro, purified recombinant human PARP-1 (Beneke et al., 2000) was incubated with selegiline at various concentrations (50 nM, 500 nM, and 5 μM) in a reaction buffer comprising 32P-labeled β-NAD⁺ as a substrate and histones serving as “acceptor” proteins. PARP-1 activity was stimulated by saturating concentrations of a double-stranded oligonucleotide, which is recognized by the enzyme as double strand breaks (Berger and Petzold, 1985). Enzyme activity was expressed as the %TRI converted into acid-insoluble material (Table 1, top row). The results did not reveal any significant change in PARP-1 activity at any concentration of selegiline tested (Table 1, bottom row).

**Discussion**

The process of poly(ADP-ribosyl)ation has been shown to be important for the repair of DNA damage in proliferating cells and for the maintenance of genomic stability, which is the basis for the role of PARP-1 as a cytoprotective survival factor (Jacobson and Jacobson, 1999; Shall and de Murcia, 2000; Bürkle, 2001a,b). The previously observed positive correlation between cellular poly(ADP-ribosyl)ation capacity and species-specific life span (Grube and Bürkle, 1992) is in perfect agreement with such cellular function of poly(ADP-ribosyl)ation.

Several laboratories have observed life span-extending and neuroprotective properties of the anti-Parkinson drug l-selegiline (Knoll et al., 1989; Freisleben et al., 1994; Semkova et al., 1996; Ruehl et al., 1997; Stoll et al., 1997; Kitani et al., 1999; Maruyama and Naoi, 1999; Klegeris and McGeer, 2000; Naoi et al., 2000; Kitani et al., 2001; Ebadi et al., 2002). These effects were recorded at concentrations below MAO-B inhibition (≤1 μM), implying they were mediated by alternative mechanisms. We hypothesized that up-regulation of cellular poly(ADP-ribosyl)ation capacity might be a candidate mechanism.

![Fig. 4.](image-url)Western blot analysis of PARP-1 in COR4 cells exposed or not to l-selegiline. A, Western blot of whole-cell extracts from COR4 cells that had been incubated or not with 50 nM selegiline for 72 h. The blot was developed with monoclonal antibody C-II-10 specific for PARP-1. Extracts were loaded at three different dilutions, respectively. B, quantification of intensities of the PARP-1-specific bands at 113 kDa. Note that there was about 40% reduction of band intensity as a result of selegiline treatment compared with controls. C, Coomassie Blue staining of a parallel gel, confirming equal loading of lanes.
The context of other projects (our unpublished data) or in mitochondrial forms of PARP. However, immunofluorescence possibility that selegiline might up-regulate DNA strand damaging treatment of cells (Du et al., 2003), raising the dose tested (Fig. 3).

The results of the present study provide clear evidence in favor of this assumption. The γ-irradiation-induced formation of pADPr in living hamster cells in culture was significantly potentiated when cells were preincubated with 50 nM selegiline for a minimum of 8 h. The effect of selegiline on cellular poly(ADP-ribo)sylation capacity was still present after 3, 5, or 7 days of preincubation with selegiline. However, by 7 days some decline in the potentiation effect was observed, with radiation-induced polymer levels being significantly lower compared with 3 days of preincubation, while still significantly higher than irradiated controls. This indicates that the potentiation of cellular poly(ADP-ribo)sylation capacity induced by selegiline may be a transient phenomenon.

In principle, a variety of enzymes involved in pADPr metabolism might mediate the above effect, i.e., the various pADPr polymerases and also pADPr glycohydrolase, the main enzyme catalyzing pADPr catabolism. However, in view of the fact that PARP-1 is carrying out the bulk of pADPr formation under conditions of DNA breakage (Shieh et al., 1998), we focused our attention on this enzyme. Because PARP-1 is activated by DNA strand breaks, the question arose whether the mere incubation of the cells with selegiline will lead to DNA damage or potentiate the damaging effects of γ-radiation, which would provide a trivial explanation for the observed effect on pADPr levels. However, cellular pADPr levels in unirradiated COR4 cells were unaffected by the presence of selegiline (Fig. 2), thus making it very unlikely that selegiline itself causes DNA damage. This observation is in keeping with the absence of DNA strand break formation by the mere incubation of cells with selegiline (Fig. 3) and also with the absence of any cytotoxic effect on COR4 cells during incubation with 50 nM selegiline (data not shown), because DNA damage typically leads to cell growth arrest or cell death.

To definitely exclude the rather remote possibility that selegiline might act as a radiation sensitizer and potentiate the number of DNA strand breaks forming after γ-radiation, the level of DNA strand breakage was directly determined using the FADU technique. This assay yielded very similar results in selegiline-free and pretreated cells at any radiation dose tested (Fig. 5).

Very recently, it was shown that not only nuclear but also mitochondrial poly(ADP-ribo)sylation can occur upon DNA-damaging treatment of cells (Du et al., 2003), raising the possibility that selegiline might up-regulate DNA strand breakage specifically in mitochondria, thereby activating mitochondrial forms of PARP. However, immunofluorescence analyses of γ-radiation-induced poly(ADP-ribose) formation in COR4 cells (or parental CO60 cells) we have performed in the context of other projects (our unpublished data) or in HeLa cells (Alvarez-Gonzalez et al., 1999) consistently revealed dose-dependent formation of poly(ADP-ribose) exclusively in the nuclei, whereas we have never observed any polymer-specific signals in the extranuclear compartment. Thus, we believe that in the COR4 cell system any putative selective action of selegiline on mitochondrial polymer formation is very unlikely to account for the effect we describe here.

To address the mechanisms underlying the observed effect of selegiline on cellular pADPr levels, PARP-1 expression was studied after a 3-day incubation of the cells with 50 nM selegiline. Our previous experiments with transfected hamster cell cultures had shown that overexpressing PARP-1 can lead to a large increase in cellular poly(ADP-ribo)sylation capacity (Meyer et al., 2000). On the other hand, it should be noted that naturally occurring differences in maximal PARP-1 activity of mononuclear blood cells from different mammalian species (Grube and Bürkle, 1992) or in lymphoblastoid cell lines from centenarians and controls (Muiras et al., 1998) could not be explained by differences in PARP-1 protein levels, indicating that the regulation of poly(ADP-ribo)ylation capacity does not exclusively depend on the regulation of PARP-1 protein expression. Along these lines, Western blot analysis of selegiline-exposed cells (Fig. 4) revealed down-regulation of PARP-1 protein levels by about 40%, rather than up-regulation. Therefore, the observed increase in cellular poly(ADP-ribo)sylation capacity induced by selegiline cannot be explained by any putative increase in PARP-1 protein levels. On the contrary, one might even speculate that the increased cellular poly(ADP-ribo)sylation capacity may activate some negative feedback mechanism leading to a down-regulation of cellular PARP-1 protein levels. Such a scenario might also underline the observed waning of the selegiline effect after 7 days of preincubation.

In the present work, the mechanism of selegiline-induced potentiation of radiation-induced cellular pADPr levels could not yet be established. We currently speculate that cofactors (e.g., accessory proteins) regulating PARP-1 activity might be involved. Down-regulation of poly(ADP-ribose) glycohydrolase activity seems to be a less likely explanation, because basal pADPr levels were not increased upon 3-day exposure of cells to the drug.

This is the first report linking l-selegiline, i.e., a clinically used anti-Parkinson drug with neuroprotective and life span-extending activity, with poly(ADP-ribo)sylation, i.e., a highly conserved cellular reaction known to be involved in cytoprotection and to be correlated with mammalian life span. Although the underlying mechanism still remains to be elucidated, our present findings may have a number of implications.

First, it will be important to directly address whether the neuroprotective effects of selegiline are mediated by up-regulation of cellular pADPr levels in neuronal cells under (sub-)lethal stress. If so, this might define a novel therapeutic strategy, which may seem surprising and paradoxical at first glance, in view of the present mainstream tendency to concentrate on the benefits of PARP-1 inhibition under various pathophysiological conditions in a attempt to rescue (potentially lethally) damaged neurons, muscle fibers, or pancreatic islet cells. The latter paradigm is based on the fact that the poly(ADP-ribo)sylation system plays an important pathogenetic role in a number of diseases such as diabetes mellitus type 1, ischemia-reperfusion damage in brain, heart, kidney, and bowel, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-in-

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<th>Selegiline Concentration</th>
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duced acute Parkinsonism, hemorrhagic and septic shock, and chronic inflammation of the bowel (Bürkle, 2001b). In all of these disease states, accumulation of DNA damage, as induced by massive release of reactive oxygen species, leads to overactivation of PARP, which subsequently results in depletion of NAD⁺, and as a consequence, of ATP pools and failure of energy metabolism. In contrast, moderate activation of PARP-1 without alteration of cellular NAD⁺ levels can have beneficial effects under conditions of stress. For example, it has been reported in a rat model of mild and transient global cerebral ischemia that PARP activation significantly contributed to survival of hippocampal neurons after reperfusion (Nagayama et al., 2000). A similar conclusion was reached in another study showing that PARP-1 mRNA transiently increased in the dentate gyrus after a brief period of global ischemia in gerbil brain or after injection of the glutamate agonist kainic acid into rat brain, suggesting a role for PARP-1 in DNA repair after mild brain injury (Liu et al., 2000). Together, there seems to exist a spectrum of PARP-mediated biological responses, ranging from protection under conditions of mild damage to enhanced cytotoxicity under conditions of severe damage (e.g., prolonged ischemia). Therefore, our present findings fit well into the global picture of the role of poly(ADP-ribose)ylation as it has emerged to date. We speculate that pharmacological enhancement of the poly(ADP-ribosyl)ation system using selegiline, in the absence of overt pathophysiological conditions associated with cytotoxic PARP overactivation, may prove an interesting new therapeutic option that may produce beneficial effects at the level of preventive medicine.

In addition, it will be equally interesting to investigate whether the life span-extending effect of selegiline depends on up-regulation of cellular pADPr levels. Last, our previous studies on transfected cells with increased cellular pADPr levels have revealed improved maintenance of genomic stability in proliferating cells under genotoxic stress (Meyer et al., 2000; Bürkle, 2001c). If the same could be achieved by selegiline treatment, then this drug might become an interesting candidate as an adjunct to cytotoxic chemotherapy or radiotherapy, as it would “freeze” the potentially dangerous process of genomic instability, which can be induced in malignant and/or normal cells by cytotoxic chemo-/radiotherapy and is a driving force of tumor cell progression toward ever higher levels of malignancy (Bürkle, 2001c).

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