CNS Drug Reviews
Vol. 12, No. 1, pp. 77–90

Neuroprotective Activity of 3-Aminopyridine-2-Carboxaldehyde Thiosemicarbazone (PAN-811), a Cancer Therapeutic Agent

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Keywords: 3-AP — Brain ischemia — Free radical scavengers — Metal chelators — Neuroprotection — PAN-811 — Stroke — Triapine®.

ABSTRACT

3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) is a highly-hydrophobic small molecule that was originally developed for cancer therapy (Triapine®, Vion Pharmaceuticals) due to its ability to inhibit ribonucleotide reductase, a key enzyme required for DNA synthesis. 3-AP has a high affinity for divalent cations, chelating the Fe^{2+} at the R2 subunit of the enzyme and inhibiting formation of a tyrosyl radical essential for ribonucleotide reduction. We have demonstrated that 3-AP is also a potent neuroprotectant (as such, it is referred to as "PAN-811"). *In vitro* it completely blocks ischemic neurotoxicity at a concentration of 0.5 μM ($EC_{50} \approx 0.35$ μM) and hypoxic toxicity at 1.2 μM ($EC_{50} \approx$ ≈ 0.75 μM). Full protection of primary cortical and striatal neurons can be achieved with 3-AP when it is added to the medium at up to six hours after an ischemic insult. 3-AP also suppresses cell death induced by neurotoxic agents, including staurosporine, veratridine and glutamate, indicating activity against a central target(s) in the neurodegenerative process. 3-AP acts via neutralization of two important intracellular effectors of excitatory neurotoxicity; calcium and free radicals. Its reported ability to elevate anti-apoptotic proteins is likely to be a consequence of the suppression of excessive intracellular free calcium. In a rat model of transient ischemia, a single bolus delivery of 3-AP 1 h after the initiation of ischemic attack reduced infarct volume by 59% when administered i.c.v. (50 μg per rat) and by 35% when administered i.v. (1 mg/kg). In Phase I clinical trials in cancer therapy 3-AP had no cardiovascular, CNS or other major adverse effects. Thus, 3-AP has a

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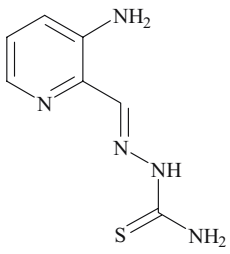

Fig. 1. 3-AP, a thiosemicarbazone, contains a pyridine ring with an amino group in position 3 and a carboxaldehyde thiosemicarbazone group in position 2.

high potential for development as a novel, potent neuroprotectant for the treatment of neurodegenerative diseases.

INTRODUCTION

3-Aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP/PAN-811) (Fig. 1), is a potent neuroprotectant belonging to a class of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones (HCTs), that were originally developed for the treatment of cancer. This class of compounds can suppress tumor growth by inhibiting the bioactivity of ribonucleotide reductase (RNR). RNR catalyzes the synthesis of deoxyribonucleotides from their ribonucleotide precursors and is as such required for DNA synthesis and repair (12,60). RNR is the rate-limiting enzyme in DNA synthesis due to its low abundance in normal cells. In comparison with several other key enzymes, RNR shows the greatest increase in activity in tumor cells (55,65), and, therefore, RNR is considered an important intracellular target for inhibiting cellular proliferation. In contrast to hydroxyurea (HU), which has a low affinity for RNR and a short half-life in humans (3,23,44), HCTs are strong RNR inhibitors. Several HCTs inhibit RNR at concentrations more than 1000-fold lower than those required for HU to achieve the same effectiveness (43). One of them, 5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (5-HP), manifested significant antineoplastic activity in animal models (13) but eventually showed disappointing results in clinical trials due to its rapid inactivation through glucuronidation and elimination in patients (32). New HCTs, that are not subject to such metabolic inactivation, have been synthesized and evaluated for antitumor activity; the most promising of them is 3-AP (37). 3-AP is a potent inhibitor of RNR with a broad spectrum antitumor activity in preclinical systems, effective in lung carcinoma, ovarian carcinoma, neuroepithelioma, leukemia, etc. (8,11,16). Several phase I clinical trials concluded that 3-AP produced no cardiovascular, central nervous system (CNS) or other major adverse effects at effective therapeutic concentrations (15, 22,45,62). 3-AP is currently manufactured under Good Manufacturing Practice (GMP) and undergoing phase II clinical trials (56) as a cancer chemotherapeutic agent.

The mechanism underlying deactivation of RNR by 3-AP involves iron chelation. RNR provides deoxyribonucleotides which are the building blocks for DNA replication in all living cells (Fig. 2, for a review see ref. 31). RNR is a multisubunit enzyme, composed of two identical R1 subunits and two identical R2 subunits. The R1 subunits carry two catalytic and four allosteric sites, which play a role in binding ribonucleotide di- or triphosphates (NDPs or NTPs) and reducing those substrates to deoxyribonucleotides di- or tri-

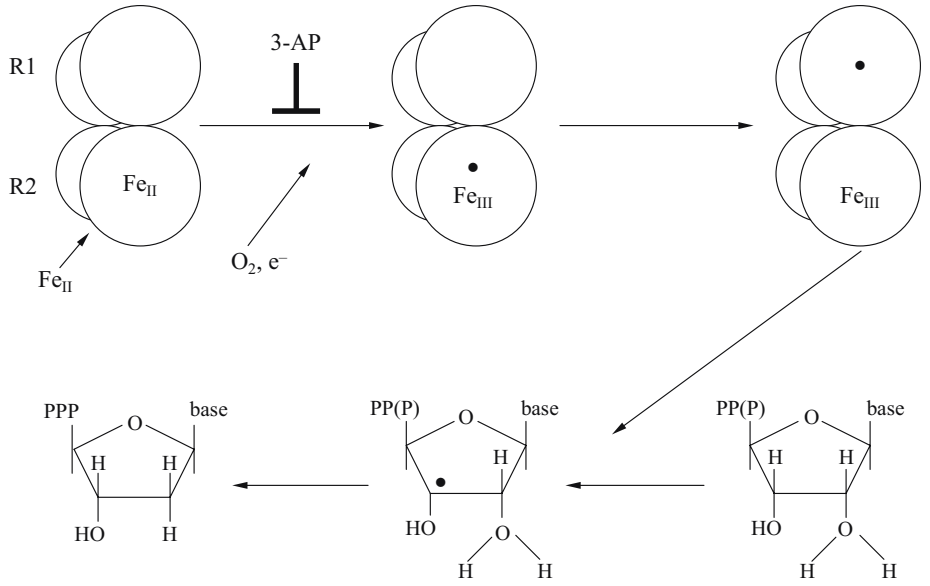

Fig. 2. Bioactivity of RNR is Fe_{II}-dependent and can be suppressed by 3-AP. The flowchart is drawn based on refs. 30,53,57. RNR is composed of R1 and R2 subunits. When the metal binding center at R2 subunit combines with Fe_{II}, a tyrosyl free radical (•) and Fe_{III} are generated in the presence of O_2 and e^-. A coupled electron/proton (H• radical) is transferred via a conserved hydrogen-bonded pathway from the tyrosyl radical to the catalytic center in R1 and further to the ribonucleotide. The ribonucleotide is consequently reduced to deoxyribonucleotide. The Fe-chelator (3-AP) complex destructs the tyrosyl radical, blocks deoxyribonucleotide formation, and consequently DNA synthesis.

phosphates (dNDPs or dNTPs) by catalyzing the replacement of an OH group of a NDP or NTP with hydrogen in a protein radical-dependant manner (58,60). The R2 subunits contain two oxygen-linked diferric clusters and tyrosyl radicals. A long-range protein-mediated radical transfer pathway appears to be responsible for the delivery of the radical from the tyrosine in R2 to the substrate on R1 (49,54). The tyrosyl free radical is stabilized by iron (59). The iron chelators, aroylhydrazones, including 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone and its analogs (14,19,40,51,52) tachpyridine (*N*,*N*′,*N*″-tris(2-pyridylmethyl)-*cis*,*cis*-1,3,5-triaminocyclohexane; 1,61), and 3-AP (16, 17), demonstrate substantial antiproliferative activity. Inhibition of tumor growth by these iron chelators is mediated by the suppression of RNR activity (16). The Fe-chelator complex destroys the tyrosyl radical, blocking dNDP or dTDP formation and consequently DNA synthesis (Fig. 2) (53,57). HCTs also chelate other divalent metals, such as copper (II) and Zinc (II) (5,6).

The intracellular accumulation of free calcium (Ca^{2+}) and free radicals plays an important role in acute neuronal injury, such as ischemic stroke, as well as in chronic neurodegeneration, such as Alzheimer's disease (AD) (2,20,24,50). After middle cerebral arterial occlusion (MCAO) necrotic cell death predominates in the central core of brain infarct. This necrotic cell death is coincident with an overall increase in intracellular free calcium ($[Ca^{2+}]_i$) and is reflected by a rapid and long-term decrease of extracellular calcium ($[Ca^{2+}]_e$). In contrast, in the penumbral area, where mainly a delayed apoptotic cell

death is observed, the $[Ca^{2+}]_e$ decreases only transiently (33,36,47). Free radical levels significantly increase in the central core during both, the ischemic insult and reperfusion period, while in the penumbra they increase only during reperfusion (38). Thus, it is clear that the neurodegenerative cascade resulting in necrosis in the central core and apoptosis in the penumbra is differentially regulated by the intracellular mediators, Ca^{2+} and free radicals.

AD is a chronic neurodegenerative disease occurring most commonly in aged individuals. This indicates an important contribution of accumulated age-related risk factors to the initiation and development of the disease. Recently, excessive accumulation of divalent metals, such as calcium and iron, and reactive oxygen species (ROS) have been suggested to play a role in the etiology of AD (20,24,50). Memantine, which blocks the influx of extracellular free calcium, has been shown in phase III clinical trials to be effective in the treatment of AD (50) and has recently been approved by the FDA for this purpose. Scavengers of ROS, such as vitamin E and ginkgo biloba, are known to protect neurons against Aβ-induced cell death (2,10,67).

Since 3-AP is a robust divalent ion chelator with a consequent function of free radical scavenging, it was hypothesized that this compound could protect against neuronal cell death due to acute or chronic insults (25–27). The research data generated over the past three years demonstrated a potent neuroprotective activity of 3-AP *in vitro* and *in vivo*. 3-AP prevented neurotoxicity caused by hypoxia, hypoglycemia/hypoxia (H/H), glutamate-, veratridine (a sodium channel opener related Ca^{2+} influx; 4,29), hydrogen peroxide, or staurosporine (apoptosis inducer; 21,46) — (9,25–28). As a neuroprotectant 3-AP was assigned a code number PAN-811 (28). This article reviews evidence for the neuroprotectant activity of 3-AP, its mechanism of action, its *in vivo* distribution, its toxicity, and relevant clinical studies.

CHEMISTRY

3-AP was originally synthesized by Sartorelli's group at Yale University School of Medicine in 1992 (37). Its chemical structure is shown in Fig. 1; its empirical formula is $C_7H_9N_5S$ with a molecular weight of 195.24 Da. It has a melting point of 232°C. The white to pale yellow powder of this compound is highly hydrophobic, soluble at 0.1 mg/mL H_2O, 1.25 mg/mL ethanol, and 15 mg/mL of polyethylene glycol-300 (PEG-300) or 7:3 (v/v) PEG-300:ethanol.

PHARMACOLOGY

Neuroprotective Efficacy and EC_{50} of 3-AP *in Vitro* and *in Vivo*

Neuronal cell death in the central core and surrounding penumbral area of an infarct following a cerebral ischemic attack seems to be induced by different insults. Extreme ischemia (or H/H) occurs in the central core but that in the penumbral area is much

less pronounced. In contrast, hypoxia affects the penumbral area for a longer period of time after resolution of the arterial occlusion. During 2-h MCAO in the rat, glucose concentration is greatly reduced in the central core of the infarct, from 2.12 down to 0.21 mmol/kg, but only mildly (to 1.42 mmol/kg) in the penumbra. It recovers to a normal level in both areas (2.65 mmol/kg in the central core and 2.69 mmol/kg in the penumbra) at 1 hour after reperfusion (18). Reperfusion also restores interstitial oxygen tension (pO_2) in the central core to its preischemic value but penumbral pO_2 recovers only partially (39). To facilitate drug screening we attempted to mimic local environment in the area between central core and penumbra during ischemia and after reperfusion *in vivo*, and established *in vitro* H/H (1.2 mM glucose and 0% pO_2, insulted for 6 hours) and hypoxia only models (0% pO_2, insulted for 18 h) of neurodegeneration. 3-AP at concentrations of 0.63 μM and 0.45 μM fully blocked H/H-induced mitochondrial dysfunction and LDH release with an EC_{50} of 0.2–0.35 μM. At 1.0 to 1.2 μM 3-AP completely inhibited hypoxia-induced mitochondrial dysfunction and LDH release (28). We also determined therapeutic windows in the H/H insult model: as 6-h during the H/H insult, and 48-h during recovery period subsequent to the H/H insult. Results were quantified with the LDH assay and cells were evaluated morphologically. Pretreatment with 3-AP showed only minimal protection, while neurons that received 3-AP during or especially after H/H insult were well protected. Thus, effective neuroprotection by 3-AP does not require pretreatment (28). An *in vivo* study in the MCAO model demonstrated therapeutic benefit when rats were treated with 3-AP during the ischemic insult (28,41). 3-AP was administered intracerebroventricularly (i.c.v.) at a dose of 50 μg per rat at 1 h after arterial occlusion. Staining of consecutive brain sections demonstrated that 3-AP greatly reduced the infarct size. Computer-assisted quantitative analysis revealed a 59% reduction in total infarct volume for 3-AP-treated rats. We also investigated the effect of a single intravenous (i.v.) bolus injection of 3-AP at a dose of 1 mg/kg at 1 h after arterial occlusion to determine whether the compound remained effective when delivered systemically. Again, 3-AP significantly reduced the infarct size, showing a 35% decrease in total infarct volume (41). Thus, 3-AP can reduce ischemic neurodegeneration *in vivo*. By systemic delivery, it has maximal efficacy at a dose of 1 mg/kg. By i.c.v. administration the maximal effect of 3-AP is achieved at a dose of 50 μg/rat within tested dose range (41).

3-AP also prevents glutamate-, veratridine (a sodium channel opener related to Ca^{2+} influx; 4,29)-, and staurosporine (apoptosis inducer; 21,46)-induced neuronal cell death (9). These effects may be due to its calcium chelating activity. Glutamate neurotoxicity is mediated by activation of NMDA receptors on the membrane of neurons, which causes influx of $[Ca^{2+}]_e$ and accumulation of $[Ca^{2+}]_i$. Excessive $[Ca^{2+}]_i$ induces ROS overproduction in the mitochondria leading to neuronal cell death (35,42,66). Inhibition of calcium influx with a NMDA receptor antagonist, such as MK-801 or memantine, can also block glutamate-induced neuronal cell death (63,64). Treatment with 10 μM 3-AP reduced 100 μM glutamate-induced neuronal cell death by 89% (9). Staurosporine is typically used as an apoptotic inducer. It has been shown to increase $[Ca^{2+}]_i$ and ROS, release of cytochrome c from mitochondria, and to activate caspases (7,21,34,48). In cortical neurons 3-AP, at 10 μM for 24 h prior to and during the insult, reduced neurotoxicity of 1 μM staurosporine by 47% (9). Veratridine causes depolarization of excitable cells by binding to voltage-dependent Na^+ channels and by maintaining Na^+ channels in an open state for a prolonged period of time (4). Veratridine also induces neuronal apoptosis by triggering calcium

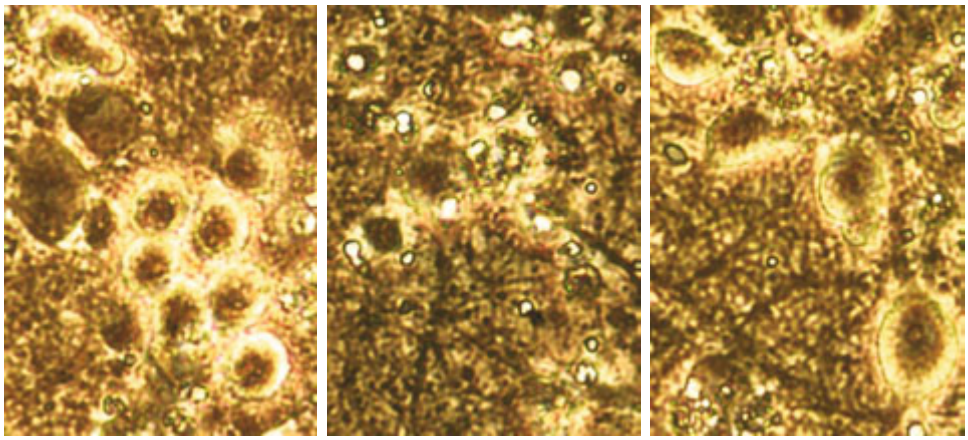

Fig. 3. 3-AP blocks excitatory neurotoxicity. Neurons at culture age of 14 days were co-treated for 2.5 h with 25 μM glutamate and 1:12,500 PEG:EtOH (vehicle) or 2 μM 3-AP. The cultures were photographed 6 days after insult at magnification of 300×.

influx, ROS production, and caspase activation (30). 3-AP suppresses veratridine-induced neuronal cell death by 42% (9). The blockade of glutamate (25 μM)-induced excitatory neurotoxicity by 3-AP is illustrated in Fig. 3. In the presence of 3-AP, both the neuronal soma and neuritic processes were well preserved.

Intracellular Targets of 3-AP

The potent neuroprotective activity of 3-AP may be due to its effects, in excitatory neurotoxic pathway, on both intracellular targets — excessive free calcium and ROS accumulation. The following four observations support this hypothesis. First, 3-AP inhibits both ischemic and hypoxic neuronal cell death, indicating that it is likely to suppress accumulation of intracellular free calcium as well as ROS. The results obtained from double staining and DNA gel electrophoresis demonstrated that H/H induced a mixture of necrotic and apoptotic neuronal cell death, where necrosis is dominant and the required insult duration is only 3–6 h. In contrast, a hypoxic insult typically induces apoptotic cell death and the duration of insult needed to cause cell death is at least 18 h (28). These results correlate well with the *in vivo* findings that stroke leads to a rapid cellular necrosis in the central core of the infarct and initiates delayed neuronal cell death in the penumbral area (33,36,38,47). The intracellular signaling molecules for H/H- and hypoxia-induced cell death seem to be different (28). MK-801, a NMDA receptor antagonist with a function of blocking calcium influx suppresses at a concentration of 5 μM H/H-induced neurotoxicity by 83% while memantine at an equivalent dose or green tea (GT, containing antioxidative chemicals) has little effect. In contrast, MK-801, at 5 μM, only slightly affects hypoxia-induced neuronal cell death, while memantine, at the same concentration, pro-

vides 50% protection and GT, at a dilution of 1:200, prevents hypoxic neurotoxicity. These findings suggest that intracellular accumulations of free calcium and ROS are likely to mediate ischemic and hypoxic neuronal cell death respectively. 3-AP, at a 2.5-fold lower concentration than MK-801, completely blocks ischemia- and hypoxia-induced neurotoxicity. This implies that 3-AP is able to suppress accumulation of excessive intracellular free calcium as well as ROS.

Second, PAN-811 has been found to suppress both intracellular free calcium and ROS by Fura Red and DHR123 analyses (28). $[Ca^{2+}]_i$ levels in hypoxic or H/H- insulted neurons were measured using the fluorescent dye Fura Red. Intracellular Ca^{2+} levels increased 19-fold due to H/H assault whereas hypoxic assault resulted in only a marginal, statistically insignificant enhancement of intracellular calcium. In contrast, H/H and hypoxia caused 1.85- and 1.4-fold enhancements in the intensity of DHR-123 fluorescence, respectively. It appears that MK-801 efficiently blocks H/H-induced calcium influx and neuronal cell death, but does not significantly reduce the fluorescent strength of DHR123. Therefore, it is more likely that $[Ca^{2+}]_i$ plays a significant role in H/H-induced neuronal cell death, whereas ROS predominantly mediates hypoxic neurotoxicity. 3-AP reduces both $[Ca^{2+}]_i$ and mitochondrial ROS, and, therefore, inhibits both H/H- and hypoxia-induced neurotoxicity (28).

Third, PAN-811 inhibits glutamate-induced elevation of intracellular free calcium. Ischemic neuronal cell death is predominantly mediated by excitatory neurotoxicity. Glutamate is an agonist at the NMDA receptor, playing an important role in excitatory neurotoxicity. Therefore, the capability of 3-AP to suppress $[Ca^{2+}]_i$ was also examined in a model of glutamate-insult (Fig. 4). Neurons treated with 20 μM glutamate display 2.8-fold elevated $[Ca^{2+}]_i$ levels. 3-AP, at 5 μM, fully suppresses this effect.

Finally, 3-AP can directly chelate free calcium and scavenge free radicals in a cell-free environment (28). 3-AP is capable of direct chelation of free calcium. Co-incubation of 3-AP, 2 μM, with 1 μM Ca^{2+} in a cell free environment, chelates over 50% of free calcium (28). 3-AP also directly scavenges free radicals and this effect is independent of its calcium-binding function. At 10 μM 3-AP scavenges about 60% of 500 μM diphenylpicrylhydrazyl (DPPH), a stable free radical, in a cell-free and metal free environment (28).

3-AP also inhibits the H/H- and hypoxia-induced down-regulation of Bcl-2 and Bcl-XL. This effect is likely to be dependent on the chelation of calcium by 3-AP, since MK-801 also preserves the levels of Bcl-2 and Bcl-XL (9,28). In general, 3-AP independently affects at least two intracellular targets — excess free calcium and ROS (Fig. 5), which play important roles in acute and delayed (or chronic) neurodegeneration, respectively.

The ability of 3-AP to kill cancer cells while preserving neurons is likely to be determined by the mitotic status of each cell type. For example, RNR shows a greater increase in activity in tumor as compared to normal cells (55,65). Thus 3-AP exerts a greater effect in suppressing tumor growth. Neurons are terminally-differentiated cell types. It is conceivable that RNR becomes less important for neuronal cell survival and, therefore, 3-AP exerts little or no effect on this cell type under normal conditions. In contrast, 3-AP protects from excitatory neurotoxicity or from hypoxic neuronal cell death by acting upon different intracellular targets, chelation of $[Ca^{2+}]_i$ and scavenging of free radicals.

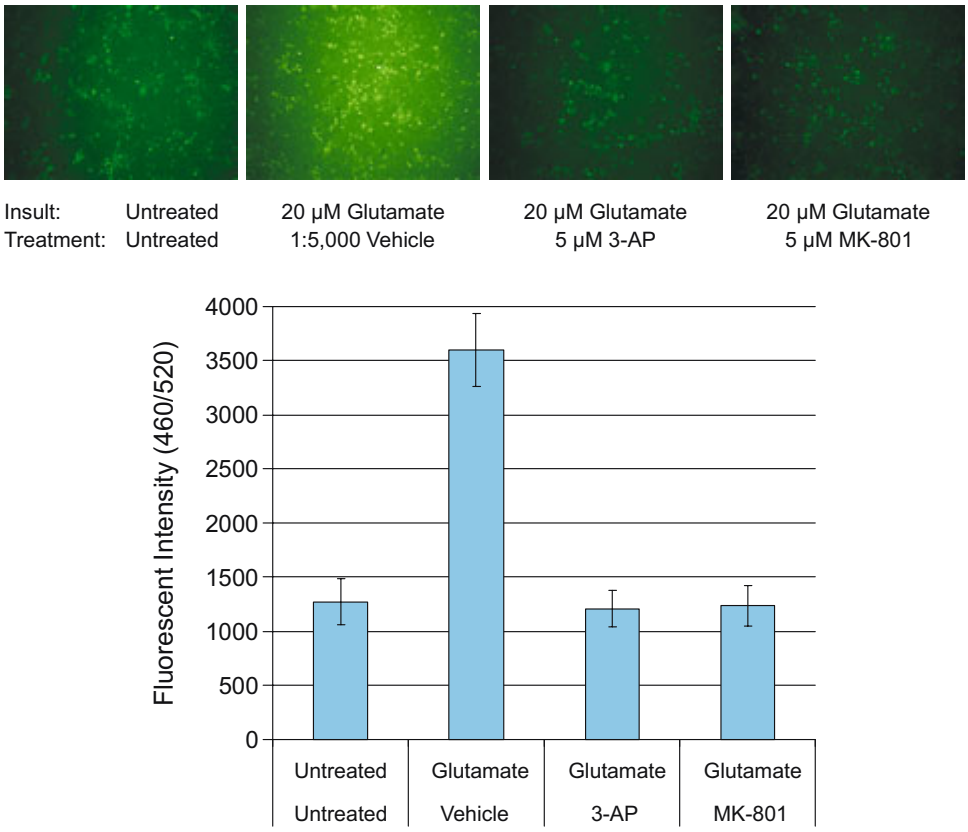

Fig. 4. 3-AP suppresses intracellular free calcium. Neurons were cultured for 16 days, treated with 20 μM glutamate together with 1:5000 vehicle, 5 μM 3-AP or MK-801 for 30 min and further co-incubated for 30 min with 10 μM Calcium Green-1. The fluorescent intensity was examined under an inverted fluorescent microscope and quantified with a FluoStar plate reader at excitation 460 nm and emission 520 nm. Data are expressed as mean values ($n = 6$) ± S.D.

HUMAN PHARMACOKINETICS

The pharmacokinetics of 3-AP has been investigated in several Phase I clinical trials. Using a single intravenous dosing schedule (infusion for 8 h), the data generated from the serum and urine of 27 patients with advanced cancer demonstrated that the serum concentration-time curve (AUC) and peak serum concentration (C_{max}) are linear with 3-AP dose up to 105 mg/m^2. At lower doses of 3-AP (<60 mg/m^2), the elimination half-life ($t_{1/2}$) ranged from 30 to 120 min with a median value of approximately 1 h. 3-AP was detected in urine during 8 h after drug administration. The cumulative urinary recovery averaged 2–5% of the administered dose. At the higher doses (60 and 80 mg/m^2), the following pharmacokinetic data were obtained: $t_{1/2} = 66 \pm 32$ min, body clearance (Cl) 0.79 ± 0.47 mL/min per m^2, volume of distribution at steady state (V_{dss}) 59.9 ± 6.6 L/m^2, C_{max} 0.97 ± 0.32 μg/mL, and urinary excretion 1.4 ± 0.3% of dose (15). In a separate regimen, 3-AP was administered at a dose of 96 mg/m^2 by 2-h i.v. infusion, daily for 5 days on on

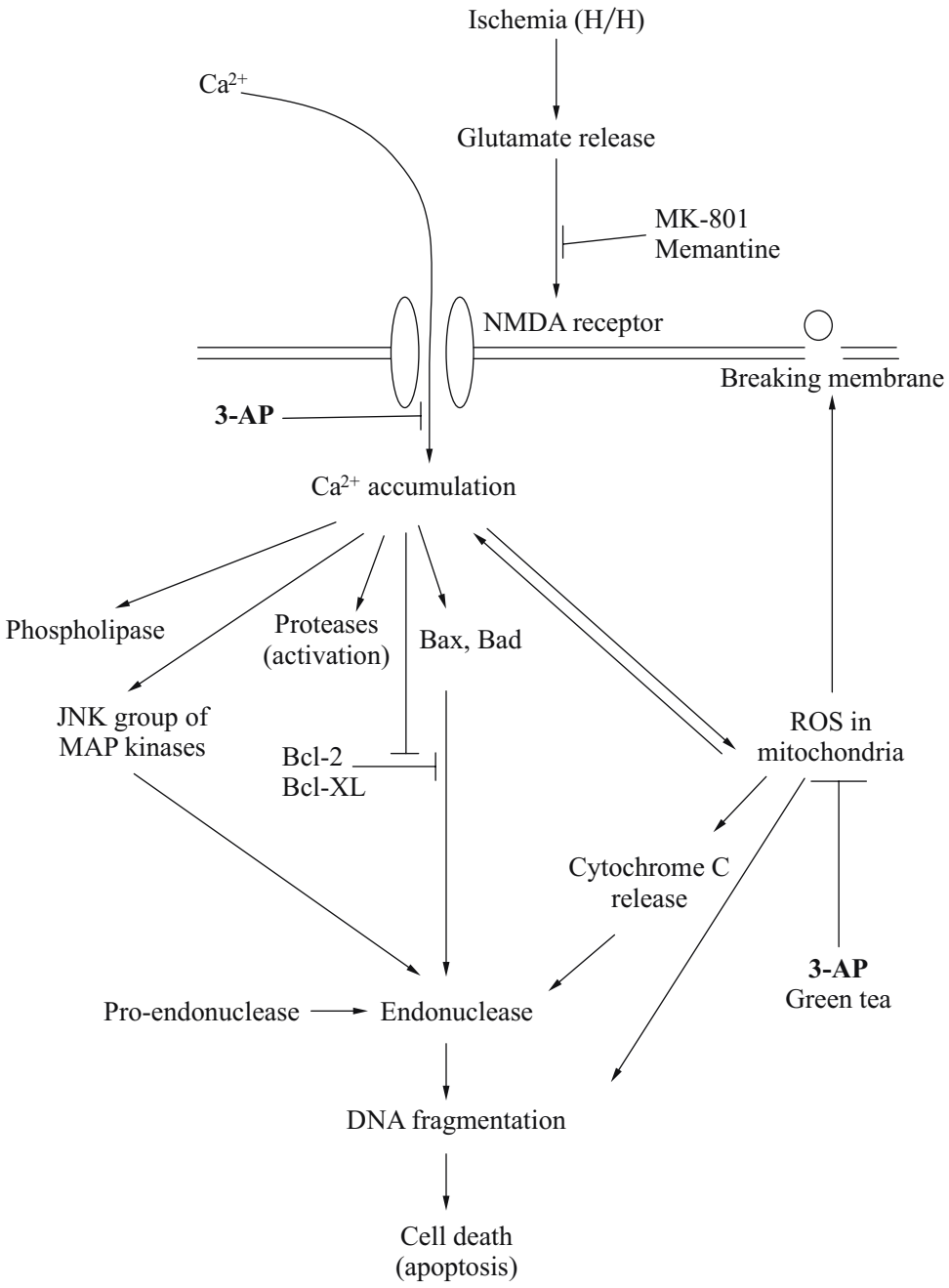

Fig. 5. Hypothetical intracellular targets for 3-AP. Excitatory neurotoxicity is mediated by overproduction of glutamate, which further activates NMDA receptor on the neuronal membrane and causes an influx of extracellular calcium. Excessive intracellular calcium induces accumulation of ROS in the mitochondria and downregulation of antiapoptotic proteins Bcl-2 and Bcl-XL. 3-AP chelates intracellular calcium and scavenges free radical, blocking excitatory neurotoxicity.

every-other-week schedule. In this trial 3-AP also displayed linear pharmacokinetic behavior. At the 96 mg/m^2/day peak plasma levels of 3-AP averaged 8 μM and $t_{1/2}$ ranged from 35 min to 3 h, with a median value of ~1 h. Cumulative urinary recovery averaged 1–3% of the administered dose (45). In a long-term intravenous infusion (96 h beginning on day 1 and day 15 or day 1 and day 8 of the trial), the median steady-state plasma concentrations of 3-AP for patients receiving 140 and 160 mg/m^2 per day were 0.68–0.75 and 0.93–1.05 μM, respectively. The $t_{1/2}$ associated with the 3-AP elimination rate at the end of the infusion ranged from 1.67 to 2.30 h (22). In summary, 3-AP had a $t_{1/2}$ of 1 h in the serum, and there was no drug accumulation under multiple-dosing schedule (45). 3-AP is excreted by the kidneys and is also metabolized. Importantly, 3-AP has been demonstrated to cross the blood-brain barrier and to inhibit the growth of L1210 leukemia cells in the brain by 95% (16). Its ability to enter the brain is likely to benefit its potential use in the treatment of neurodegenerative diseases.

ADVERSE EFFECTS IN CLINICAL TRIALS

3-AP has been administered by single 2-h infusion every 4 weeks in 46 courses of therapy. It was well tolerated at doses up to 105 mg/m^2 and a dose limiting toxicity was not identified. Hematological toxicity was uncommon. One patient developed grade 4 thrombocytopenia at the lowest dose level, and one patient had grade 3 anemia. Two patients developed grade 3 coagulation abnormalities. Mild (grade 1) nausea and diarrhea occurred in a couple of patients at the highest dose levels (60 and 80 mg/m^2). The only other adverse effects of more than grade 1 occurring in more than 10% of the patients were fever and asthenia (15). A separate trial indicated that the most common nonhematological adverse effects of 3-AP were asthenia, fever, nausea and vomiting, mucositis, decreased serum bicarbonate, and hyperbilirubinemia. These effects were predominantly grade 1–2 in severity and were rapidly reversible. Adverse hematological effects on the every-other-week schedule were leucopenia and anemia. Thrombocytopenia was less common. The conclusion from this trial was that 3-AP administered at a dose of 96 mg/m^2 by 2-h i.v. infusion, five times on an every-other-week schedule, had an acceptable safety profile (45).

CONCLUSION

3-AP represents a novel approach to the blockade of excitatory neurotoxicity, including that associated with an ischemic stroke. Unlike other antagonists of NMDA or AMPA receptors and ion channels, 3-AP chelates the intracellular free calcium at a site that is downstream from the receptors. Under acute conditions, when multiple receptor and calcium channel openings occur, an antagonist blocking one receptor or channel may not be sufficient to fully block the neurotoxic pathway. 3-AP has, therefore, an advantage over available NMDA or AMPA antagonists. In addition, 3-AP scavenges free radicals and reduces intramitochondrial ROS. 3-AP not only blocks the excitatory neurotoxic pathway at

multiple sites but also suppresses ROS induced by upstream signals other than intracellular free calcium. The maximal neuroprotective effect of 3-AP can be achieved at blood levels that are much lower than *in vitro* drug concentrations that can still be tolerated by neurons. This should allow the use of 3-AP in a wide dose range needed to achieve optimal therapeutic efficacy. Promising results have been seen with 3-AP in a MCAO animal model where 3-AP was administered at a dose of 50 μg per rat (the highest dose tried) by a bolus i.c.v. administration 1 h after artery occlusion. 3-AP reduced infarct volume by 59%. Furthermore, the effective window for administration of 3-AP, during and/or after ischemic insult, should be adequate for stroke therapy. Since 3-AP is currently in Phase II clinical trials for cancer therapy, its human pharmacokinetic and toxicologic properties are known. This knowledge is expected to shorten the time required for the development of 3-AP as a neuroprotective drug. At present, the only drug for stroke therapy that is approved by the FDA is tissue plasminogen activator (tPA), a thrombolytic drug. The development of neuroprotective drugs has a priority in stroke therapy, since neurodegeneration continues for hours or even days following reopening of an occluded artery. Some questions relevant to the use of 3-AP as a neuroprotectant remain, however. They include optimal delivery route, treatment duration, and quantitative assessment of the ability of 3-AP to pass the BBB. After resolution of these issues, 3-AP will be on its way to enter clinical trials in neuroprotection.

Acknowledgments. The authors are grateful to National Institute of Neurological Disorders and Stroke Grant 1 R43 NS048694-01 and Vion Pharmaceuticals (New Haven, CT, USA) for 3-AP.

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