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The Toxic Effect of Ketamine on the Central Nervous System - Potential Hazard or Safe to Use?

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The Neurotoxic Effect of Ketamine – Potential Hazard or Safe to Use? – A Systematic Review

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Zusammenfassung

Hintergrund: Immer mehr Studien der letzten Zeit weisen auf einen potentiellen neurotoxischen Effekt des NMDA Rezeptors Ketamin nach systemischer und/oder rückenmarksnaher Applikation hin. Ziel dieses Reviews ist es, einen Überblick über die präklinische und klinische Literatur der letzten Jahre zu geben, welche die potenziell neurotoxische Wirkung von Ketamin auf Nerven und Hirngewebe untersucht hat.

Methodik: Es wurden die Literaturdatenbanken Pubmed (von 1970 bis 2012) und Embase (von 1988 bis 2012) durchsucht. Die Datenerhebung wurde gemäß PRISMA-Statement in dessen aktueller Version durchgeführt.

Ergebnisse: Von 1171 Primärtreffern in der Literatursuche wurden 69 in dieses systematische Review eingeschlossen.

Tierstudien: Von 44 Studien zeigten 33 eine dosisabhängige Neurotoxizität von einzelnen oder wiederholten Gaben in einem Dosisbereich von 5-100 mg/kg systemisch, und 0.5-10 mg/kg intrathekal. Elf Studien konnten keine Neurotoxizität nachweisen.

Studien mit Zelllinien: In 16 von von 20 Studien wurde Neurotoxizität in Nervenzellen von jungen Tieren in einem weiten Dosisbereich von 0.001-21 Mm und Inkubationszeiten von 1-72 h festgestellt.Drei Artikel zeigten Neurotoxizität in menschlichem Gewebe. Zwei Studien konnten keine Neurotoxizität nachweisen.

Daten zum Menschen: Vier Case Reports zeigten Zeichen von Neurodegeneration. Eine randomisiert kontrollierte Studie konnte keine Neurotoxizität von 2 mg/kg i.v. Ketamin in Kindern zeigen.

Diskussion: Unsere Ergebnisse legen nahe, dass Ketamin im Tierversuch und bei Menschen nach systemischer oder rückenmarksnaher Verabreichung neurotoxische Effekte auslösen kann. Im Menschen und in Zelllinien ist diese Neurotoxizität abhängig von der Dosierung, der Verabreichungsdauer und dem Entwicklungszustand des Zentralnervensystems, wobei jüngere Individuen eine höhere Vulnerabilität gegenüber Ketamin induzierter Neurotoxizität aufweisen als ältere. Erstaunlicherweise gibt es darüber hinaus auch Hinweise für eine neuroprotektive Wirkung von Ketamin auf chemisch oder ischämisch geschädigtes Nervengewebe. Zukünftige Studien sollen diese Fragen klären.

Abstract

Background: Increasing evidence points to a potential neurotoxic effect of the NMDA receptor antagonist ketamine when administered systemically and/or neuraxially. This article aims to review recent preclinical and clinical literature investigating the potential neurotoxic effect of ketamine on nerve or brain tissue.

Methods: We searched Pubmed (1970 to 2012) and Embase (1988 to 2012). For data extraction, we followed the PRISMA-Statement in its current version.

Results: From 1171 primary hits, 69 records were finally included into this systematic review.

Animal studies: Out of 44, 33 showed a dose-dependent neurotoxicity of single or multiple doses ranging from 5-100 mg/kg systemically, and 0.5-10 mg/kg intrathecally (i.th.). Eleven studies failed to show significant neurotoxicity.

Cell line studies: Out of 20, 16 discovered significant neurotoxicity in neuronal cells of young animals in a wide dose range from 0,001-21 mM and incubation time varying from1-72 hours. Three articles revealed neurotoxicity in human cell lines. Two studies failed to present neurotoxicity.

Human Data: four case reports showed signs of neurodegeneration. One randomized controlled clinical trial showed no neurotoxicity of 2 mg/kg i.v. ketamine in children.

Discussion: Our results indicate that under certain conditions ketamine can exert neurotoxicity inanimals and humans when administered systemically or neuraxially. In animals and cell lines, neurotoxic events depend on ketamine's dose, exposure time and the developmental age of the central nervous system at the time of application, with young mammals being more susceptible to ketamine toxicity than older. In humans, three case reports show neurotoxic histological changes post mortem after intrathecal application. Controversially, some evidence reports neuropro-tective effects of ketamine when it is added to chemically or ischemically injured neuronal tissue. Further research needs to clarify these questions.

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Glossary and Abbreviations

2.3 DHBA	2.3 dihydroxybenzoic acid
AC3/C3A	Activated caspase-3
AIF	Apoptosis-inducing factor
Akt	A serine/threonine-specific protein kinase
Bax	Proapoptotic protein
Bcl-2	Protein family involved in apoptosis
BDNF	Brain derived neurotrophic factor
CA1, CA3	Hippocampal areas
CaMKII	Calcium/calmodulin dependent kinase
CaMKII	Calcium/calmodulin-dependent protein kinase II
CBF	Cerebral blood flow
CHD	Coronary heart disease
CMRO2	Cerebral metabolic rate of oxigen
CNS	Central nervous system
CYP3A4	Cytochrome P3A4
DAPI	Blue nuclear DNA stain
DG	Days of gestation
DIV	Days in vitro
E	Embryonic day
EC50	Half maximal effective concentration
ELFIA	Enzyme-linked fluorescent immunoassay
FADD	Fas associated protein with death domain
FJB	Fluoro-jade B
FJC	Fluoro-jade C
GABA	Gamma aminobutyric acid
GAP-43	Growth associated protein-43
GFP	Green fluorescent protein
HSP 70	Heat shock protein 70
i.m.	Intramuscular
i.p.	Intraperitoneal
i.th.	Intrathecal
i.v.	Intravenous

ICP	Intracranial pressure
JCR	Journal citation report
LDH	Lactate dehydrogenase,
MAP2	Microtubule-associated protein 2
μM	Micro Mol
mM	Milli Mol
MRI	Magnetic resonance imaging
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
NO	Nitric oxide
NOC-5	Nitric oxide releasing compound 5
ns	Not significant
P	Postnatal day e.g. P 7
p53	Tumor suppressor gene
pAkt	Phosphorylated Akt (serine/threonine kinase)
PC/RSC	Posterior cingulate/retrosplenial cortex
PCP	Phencyclidine
Perk	Phosphorylated extracellular signal-regulated protein kinase
PKA	Protein kinase A
PKC	Protein kinase C
PSANCAM	Polysialic acid neural cell adhesion molecule
PSA-NCAM	Polysialic acid neural cell adhesion molecule
s.c.	Subcutaneous
Src/Fyn	Protein tyrosine kinases
TNF	Tumor necrosis factor
TRADD	TNF receptor associated protein with death domain
TrkB	Receptor tropomyosin-related kinase B
TUNEL	Terminal dUTP nick-end labeling
v/v	Volume to volume
XTT	Tetrazolium hydroxide

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1 Introduction

Besides its wide use as an anesthetic in intensive care, emergency- and pain medicine, the NMDA antagonist ketamine recently caught great attention because of its toxicity to the developing brain in mammals when administered systemically or neuraxially. In contrast to other articles, which have discussed the use of different NMDA antagonists and their potential damage to the developing brain,[1] this systematic review focuses on ketamine solely.

Ketamine has been shown to be an excellent alternative adjuvant analgesic in situations of refractory pain.[2] But its good analgesic effects in conditions of chronic cancer pain or neuropathic pain are overshadowed by several case reports showing post mortem spinal cord damage after continuous intrathecal infusion.[3-5] Neuropathological findings in humans and preclinical studies reporting neurotoxicity in cell lines and rodents suggest a neurotoxic potential of this drug.[6,7] Controversially, some studies showed neuroprotective effects under certain conditions.

A recently performed meta-analysis showed clear benefits of ketamine as an adjunct to pediatric caudal anesthesia with prolonged postoperative analgesia and few adverse effects compared with local anesthetics alone.[8] Surveys undertaken in the United Kingdom confirm that ketamine is commonly used as an adjunct to local anesthetics in caudal and epidural pediatric anesthesia with very variable restrictions regarding the age of newborns and young children. According to these surveys, 32 % of the pediatric anesthesists added ketamine in a dose range from 0,25 to 1 mg/kg to local anaesthetic solutions in pediatric caudal anesthesia,[9] and 15 % used it as an adjunct in the intra- or postoperative period in a dose range from 0,5 -1 mg/kg.[10] In a survey conducted in Great Britain and Ireland, 37,5 % of pediatric anesthesists used ketamine as an additive to local anesthesia by using the caudal catheter technique in 43,6 %. Again, the upper age limit for performing this technique varied in a wide range from <3 month to <4 years.[11] The surveys may be interpreted as samples representative for the neuraxial use of ketamine in children in the western civilization. This widespread use together with an increasing number of preclinical studies indicating ketamines neurotoxic potential have already launched intense discussions about the safety of its use in children,[12] and lead to a decrease in the use of ketamine as an additive to caudal anesthesia in several european countries.[13] Once again, the question about neurotoxicity needs to be answered.

Therefore we decided to present a systematic review concerning ketamine-induced neurotoxicity.

1.1 Ketamine and its enantiomers

With the intention to create a new substance best suitable for human anesthesia, ketamine has been synthesized for the first time over 40 years ago and it soon became clear, that this agent holds unique characteristics incomparable to any other anesthetic drug that was known.[14] Ketamine is a derivative of aminocyclohexanone, whose chemical structure is related to phencyclidine. It has a chiral center and therefore exists as the two optical stereoisomers S (+) and R (-)-ketamine.[14,15] Though S (+) and R (-) ketamine have similar pharmacokinetic profiles, the right handed S(+)-isomer, which is available in Europe since 1998, is reported to be at least 4 times as potent as an analgesic compared to R(-)-ketamine in healthy volunteers and twice as potent as the racemic mixture. Without altering its anesthetic and analgesic potency, the clinical administered dose of S(+)-ketamine can be reduced to one half of the usual racemic dose, thus effecting also the quality of its side effects by reducing the unpleasantness of psychomimetic phenomena.[16,17] Ketamine can be given by many different routes including subcutaneous, intravenous, intramuscular, intranasal, oral, rectal, epidural or intrathecal administration.

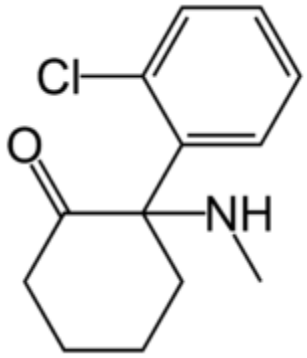

Fig. 1. Chemical structure of ketamine

1.1.1 Pharmacology and mechanisms of action

Ketamine exerts its effects by non-competitively blocking the NMDA receptor, an ion channel receptor activated by the excitatory amino acid L-glutamate.[18] There, it interacts with the phencyclidine binding site and inhibits the NMDA receptor activity.[19] Ketamine inhibits the peripheral reuptake of katecholamines like noradrenaline and dopamine on the synaptic end plate and potentiates katecholamine effects. This results in an increase of heart rate, cardiac output, centralvenous and arterial blood pressure.

Rapid Antidepressant effects have been described.[20]

Ketamine affects the cholinergic system in which it prevents the NMDAR-dependent acetylcholine release. It also inhibits other glutamate receptors and shows a weak agonist activity at opioid receptors as well as an affinity for GABA receptors.

1.1.2 Pharmacodynamics

Ketamine induces an anesthetic state known as “dissociative anesthesia”. This term means that *„though patients are anesthezised and do not respond to noxious stimuli, they are able*

to move, vocalize, keep their eyes open or may make ocular tracking movements".[15] Ketamine provides profound analgesia that continues into the postoperative period. In the central nervous system, ketamine increases the $CMRO_2$, CBF and ICP. In the respiratory system, ketamine leads to bronchodilatation and can therefore be useful in situations of bronchospasm. Because it causes increased salivation, it is generally administered together with anticholinergic substances.[15]

1.1.3 Pharmacokinetics

After an intravenous dose of 1-2 mg/kg, ketamine causes a rapid loss of consciousness. The redistribution half-life time is about 11-17 minutes. For parenteral administration, the bioavailability is approximately 95%. Ketamine is hepatically metabolized in to the less potent active metabolites norketamine and dehydronorketamine by N-demethylation primarily viaCYP3A4 cytochromes.[15] Norketamine and dehydronorketamine are further metabolized to an inactive glucuronide and finally excreted in urine and bile. Only a small fraction of the circulating ketamine is bound to plasma protein. Ketamine has a high hepatic extraction ratio and a high first-pass hepatic metabolism following oral administration.[14,15]

1.1.4 Side effects

The main side effects of ketamine administration are psychomimetic in nature e.g. hallucinations, nightmares, somnolence, drowsiness, dizziness, fatigue and nervousness. These effects are dose dependent and occur more frequently if doses of 1 mg/kg/h or higher are used. A combination of ketamine together with Midazolam or other benzodiazepines can help to reduce the incidence of these side effects.[15]

1.1.5 Clinical use

Since ketamine provides profound analgesia combined with narcotic and circuit stabilizing effects it is a commonly used drug for the induction of general anesthesia as well as intensive care and emergency medicine. Nevertheless, because of sympathicotonic effects, the use of ketamine in patients with coronary heartdisease (CHD) should be questioned critically. Ketamine also leads to a dose-dependent cerebral vasodilation with increased cerebral blood flow and should therefore not be used in patients with increased ICP.

For racemic ketamine, intravenous doses of 0.25-0.5 mg/kg are needed for analgesia and 1-2 mg/kg for the induction of general anesthesia. For S(+)-ketamine, these doses may be halved.

In Pain medicine, especially in palliative situations and cancer pain management, intravenous ketamine is used to achieve pain relief.[21] There is also evidence for benefits of intrathecal ketamine in patients suffering from neuropathic cancer pain. However, in such cases of refractory pain, ketamine serves as an alternative analgesic that should be considered as the last resort because there is no proof for the safety of ketamine when it is used intrathecally.[2]

Over the last years, perioperative low dose ketamine for the treatment of postoperative pain has been established in a variety of clinical settings including major abdominal surgery, radical prostatectomy, knee arthroscopy and several others.[22-27] One major benefit of intraoperative i.v. administered subanesthetic ketamine as an adjunct to general anesthesia is, besides pain relief, the reduction of postoperative opioid requirements.[28]

To further proof its ability to reduce postoperative opioid requirements and opioid induced side effects even at a minimal dose, there was a randomized controlled clinical trial undertaken recently at the department of anesthesiology and intensive care Graz, where S(+)-ketamine at either 0.125 mg/kg/h or 0.015 mg/kg/h (Miniket-study) was administered as a continuous infusion throughout major abdominal surgery until 48 h postoperatively.

1.2 The NMDA receptor complex

„NMDA receptors are heteromeric complexes composed of 4 subunits derived from 3 related families: NR1, NR2 and NR3".[29] Within these subunits, four different types of NR2 and 2 different types of NR3 are known.[18] The NR1 subunit is obligatory and forms together with either a NR2 or NR3 subunit the functional receptor. A typical NMDA receptor is build of 2 NR1 subunits containing the binding sites for glycine and 2 NR2 subunits which bind glutamate. The NMDAR is permeable to Ca^{2+} and its activation requires the binding of 2 molecules of glutamate and 2 molecules of glycine. The receptor channel is voltage gated by extracellular Mg^{2+} located in the channel pore. Other modulatory sites affect the receptor function including the polyamide site, Zn^{2+} site, proton-sensitive site, and redox modulatory site where NO produces S-nitrosylation. Non-competitive antagonists such as Ketamine, Amantadine, PCP, MK801 and Memantine bind to a specific binding site inside the ion channel (fig. 2).[29]

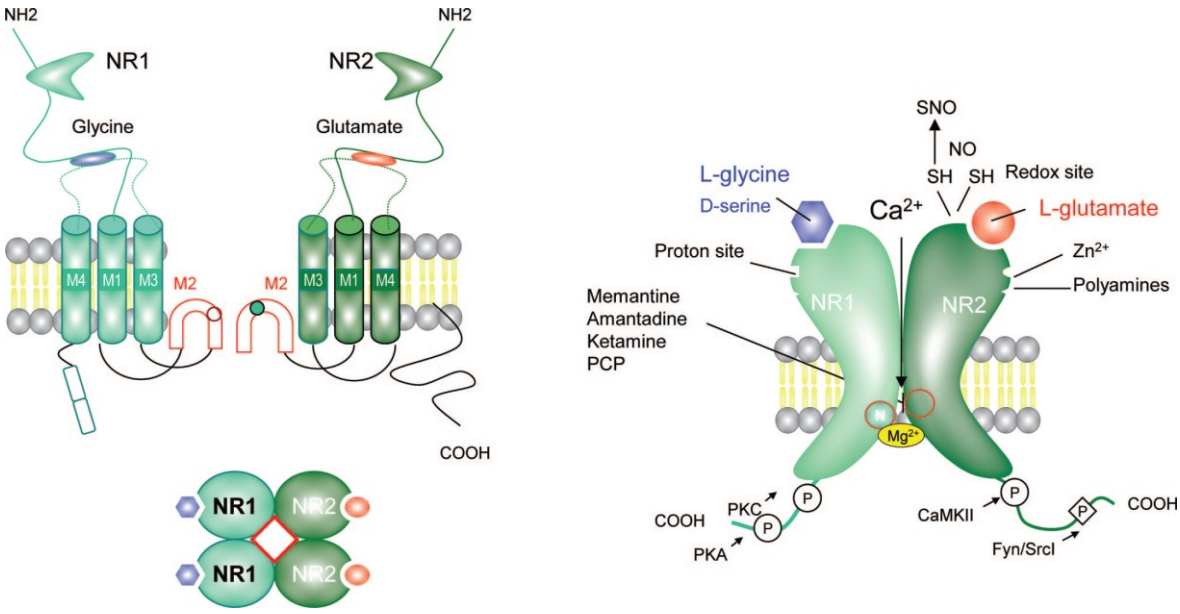

Fig. 2. Structure, gating and modulation of NMDA receptors
CaMKII = calcium/calmodulin dependent kinase; PKA = protein kinase A; PKC = protein kinase C; Src and Fyn = protein tyrosine kinases. From: Benarroch EE. NMDA receptors: Recent insights and clinical correlations. Neurology 2011;76:1750-7.

1.2.1 Distribution of NMDA receptors

NMDA receptor expression patterns change during the development of the CNS, and it is therefore difficult to provide an exact mapping of the different subtypes located in the human brain. From animal studies, however, it can be concluded that the main types of the NR1 receptors (NR1 and NR1-2) are expressed more or less homogeneously throughout the brain and spinal cord, while several isoforms (NR1 b, NR1-3, NR1-4) are restricted to specific parts of the CNS including cortex, hippocampus, caudate, thalamus, cerebellum, colliculi and hippocampus. NR2 and NR3 subtypes respectively, are located in CNS regions e.g. hippocampus, cerebellum as well as in the brainstem.[18] NMDA receptors are also expressed by cortical interneurons, spinal cord neurons and glial cells.[29]

1.2.2 NMDAR mediated effects

The NMDA receptor complex is involved in central pain sensitization and in the development of the neuropathic pain syndrome. Prolonged excitations of the NMDAR lead to an opening of its ion channel and result in the development of opiod tolerance and opiod-induced pain.[30]

NMDA receptor antagonist mediated neurotoxicity has first been questioned over 20 years ago when Olney et al.[31] described pathomorphological changes in brain neurons of adult

rats after subcutaneous administration of PCP and related agents in relatively low doses. Still, despite several hypothesis that are trying to explain this phenomenon, the exact mechanisms of this neuronal damage remain unclear.

1.3 Mechanisms of cellular injury and cell death

Noxious agents lead to complex alterations of cellular homeostasis, loss of cell function or cell death. These alterations are characterized by specific morphological cellular changes. Cellular injuries may be caused by hypoxia, pathogens, chemical substances, physical, immunological, genetic or nutritional factors[32].

The extent of damage also depends on the interaction between cell and noxious agent. A cell has several options to repair damage or to make damaging agents harmless (biotransformation, phagocytosis, degradation, excretion etc).The extent of damage thus depends on the oxygen supply and the nutritional status of a cell. Highly active cells are often more susceptible to damage than less active[32].

1.3.1 Apoptosis

Apoptosis is programmed cell death. It can be stimulated from outside the cell or caused by internal cell processes. It is an active process and part of the cellular metabolism. Thus, the cell is able to organize its own death and perish without damaging surrounding cells. Specific proteolytic enzymes, called caspases, play a crucial role in the cascade resulting in apoptosis.Apoptosis is an important physiological process involved in the development of many organisms. In humans for example, apoptosis is required to reduce unnecessary or potentially harmful cells of the immune system and plays a role in the development and maintenance of the plasticity of the CNS.

The two major ways of how apoptosis is induced are the Extrinsic and the intrinsic pathway. The extrinsic or receptor pathway begins with ligand binding at a receptor of the TNFα family. These receptors have a so called death domain at the cytoplasmic side of the cell membrane. There, TNF-related apoptosis-inducing ligand (TRAIL) binds followed by Fas associated protein with death domain (FADD) which binds to TRAIL. Pro-caspase 8 binds to the death effector domain of FADD and finally, autocatalytic activation of caspase 8 induces the caspase-cascade leading to cell death.[32,33]

The intrinsic or mitochondrial pathwaycan be triggered by a variety of including irradiation, hypoxia, free radicals, toxins or chemotherapeutic agents and involvethe release of Cytochrome c and other pro-apoptotic factors such as AIF from the mitochondrial intermambrane space into the cytoplasm.[33] Cytochrome c release depends

on the ratio of apoptosis promoting factors to such that inhibit apoptosis and can be caused by activation of p53 and consecutively by the expression of members of the Bcl-2 family e.g. Bax. Caspase 9 finally initiates the apoptosis cascade. Then, similar to the extrinsic pathway, the proteolytic caspase 3 and others lead to apoptotic cell death.[32]

1.3.2 Necrosis

Necrosis, in contrast to apoptosis, is the premature death of cells inliving tissue. It is marked by denaturation of proteins or enzymatical liquidation of cells and tissue. Necrosis can be classified due to its morphological patterns. This classification includes coagulative necrosis, liquefactive-, fibrinoid-, caseous-, haemorrhagic- and ischemic necrosis.[32]

Changes during necrotic cell death include nuclear shrinkage (pyknosis), disintegration (karyolysis) and breaking of the nucleus into debris (karyorhexis). Necrosis is morphologically characterized by early cell swelling, vacuolation and swelling of cell organells e.g. endoplasmatic reticulum and mitochondria. Disintegration of the cell releases enzymes inducing an inflammatory reaction which is mainly caused by neutrophilic granulocytes.[32] Unlike apoptotic, necrotic material cannot be easily located by phagocytes which results in a buildup of dead cells and debris all around the site of the cell death. Therefore, healthy tissue near the necrotic area may also die.

1.3.3 Neurotoxicity

Neurotoxicity is defined as the ability of a drug or chemical substance to damage brain or nerve tissue including transient injury and loss of cell functions. Dependent on the nature, intensity and duration of neuronal damage, cells can either fully recover from a transient injury, lose their function irreversibly or die after induction of apoptosis or necrosis via several intracellular cell death pathways.

2 Aims and Hypothesis

The aim of this systematic review was to identify studies, which intended to show a significant direct cytotoxic and/or apoptosis inducing effect of isolated ketamine administration.We hypothesize that ketamine-induced neurotoxicity depends on dosage, the route ofadministration and the developmental stage of the CNS with greater sensitivity in the developing brain, both in animals and humans.

3 Material and Methods

This review follows the recommendation of the PRISMA statement for preferred reporting items for systematic reviews and meta-analyses in its current version.[34]
Neurotoxicity in the context of this article shall be defined as a transient damage to the nervous system or the destruction of neuronal tissue including every process leading directly or indirectly to neuronal degeneration.

3.1 Systematic literature search

Database search was performed in Pubmed (from 1970 to 2012) and Embase (from 1988 to 2012). For this search, "ketamine", "neurotoxicity", "neurotoxic", "apoptosis", "neurodegeneration", and "neuronal cell death"were used as the free text and MeSH terms. Language was restricted to English, German and French. Review articles, meta-analyses, conference papers, abstracts, correspondence articles, comments, letters, notes, discussions, practice guidelines and editorials were excluded from the search. Additional articles were identified by hand search. Three authors were contacted to obtain additional data.[35-37] The last electronic search was performed in March 2012.
Two independent authors screened all hits. After initial identification and removal of duplicates, all remaining articles were first title, than abstract screened. All articles considered as "not relevant to the topic" or "not meeting inclusion criteria" were excluded. Finally, the full texts of the remaining articles were assessed for eligibility.

3.2 Inclusion and exclusion criteria

I searched studies in animals, in animal or human neuronal cell cultures to investigate the neurotoxic effects of ketamine on the developing or mature central nervous system. Additionally, human case reports and studies were considered.
We included controlled studies using racemic or S (+)-ketamine with an intravenous, subcutaneous, intraperitoneal or intrathecal route of administration as well as studies using cell lines incubated with ketamine. As adequate control, either saline or no treatment was considered. Only studies presenting methods to identify survival of cells, neuronal injury, neuroapoptosis or related effects were included. I excluded any articles using toxic or ischemic models to induce neuronal injury previously, simultaneously or after ketamine application. All animal studies with other medication than ketamine or anesthetics were excluded. A ketamine treatment for longer than one month was considered as a chronic treatment and therefore these studies were excluded.

3.3 Data extraction

For data extraction and statistics, the following information was collected: species, tissue, age, route of administration, dosage, time of incubation or exposure to the drug.
Information was extracted regarding Ketamine dosage (normalized to mg/kg in animal studies and μM or mM in cell line studies), route and time of administration, and the age of individuals and cultures.

3.4 Statistics

Because of the heterogeneity of the data, especially the different methods for assessing neuronal cell death, it was not possible to perform a meta-analysis.
The statistical analysis was computed with SPSS (version 17) and Microsoft Excel. For the effect size calculation, statistically nominal and ordinal data were extracted, and fixed and random statistical models were processed. As the literature delivered a huge diversity of statistical methods, the extracted data scale had to be reduced finally to nominal levels. As a main coefficient, the r-variable was extracted and computed over the extracted data.[38-40]

4 Results

4.1 Systematic literature search

From 1171 primary hits, 1102 were subsequently excluded and 69 finally included into this systematic review (Fig. 1): 64 preclinical studies, 4 case reports and 1 randomized controlled trial. Specific information about each of the studies included is presented in Table 1 and 2. If not stated otherwise, racemic ketamine was used in the studies. All included articles were written in English.

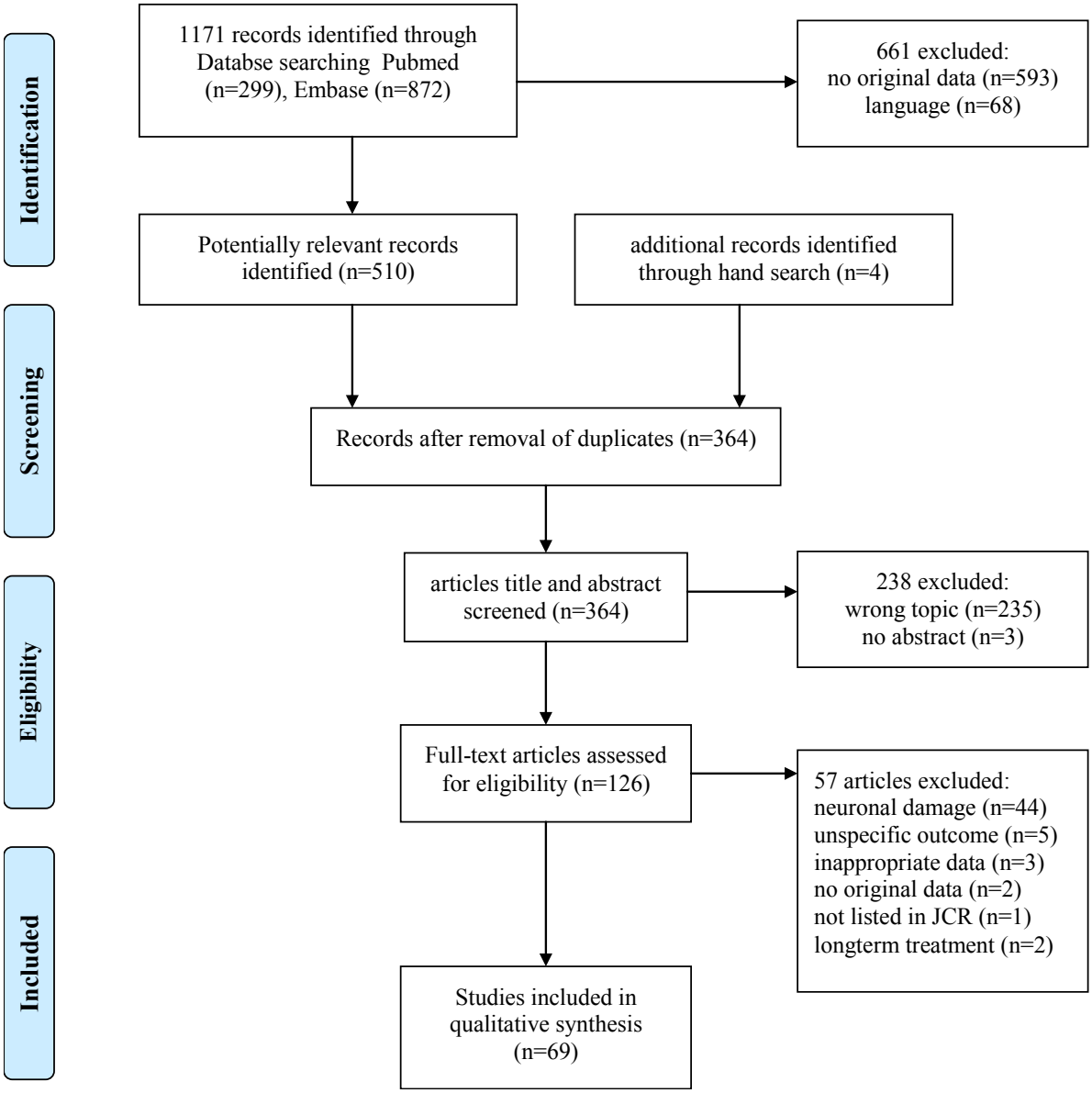

Fig. 3. Literature reviewing process according to the PRISMA statement

4.2 Animal studies

The majority of articles (n=23) investigated neurodegenerative effects of ketamine in rats. Seven studies used mouse pups, 4 used rabbits, three adult dogs and another three used rhesus monkeys. One study investigated ketamine effects in pigs. Out of 44 studies, 33 studies showed a dose-dependent neurotoxicity of single or multiple doses ranging from 5-100 mg/kg systemically,[7,36,37,41-67] and 0.5-10 mg/kg intrathecally (Table 1a).[6,68-70]Additionally, the younger the animal, the more vulnerable were cells in even lower doses. Eleven studies failed to show significant neurotoxicity.[71-81]

4.3 Cell line studies

Twenty studies analyzed the effects of ketamine in cell cultures (Table 1b). In 12 studies rat neuronal cultures were used, 3 used human cell lines, 4 neuronal tissue from mice, onefrom rhesus monkeys, and one investigated ketamine effects on the developing brain in zebrafish.

Sixteen studies observed significant neurotoxicity in neuronal cells of young animals in a wide dose range from 0.001-21 mM and incubation time varying from1-72 h. In most studies, either a dose or time dependent relation was reported, in some even both.[7,82-95] All 3 human studies revealed neurotoxicity. A dose dependent, significant increase of apoptosis in human neuroblastoma and T-lymphoma cells was reported after 24 hours of incubation with 1-12 mM S(+)-ketamine,[96,97] or after 48 hours with 0.42 -21 mM ketamine, respectively.[85] Two studies failed to detect neurotoxocity after incubation for up to 24 hours in doses from 10-100 μM.[98,99]

4.4 Human data

Four case reports and one clinical trial were identified through database search (Table 2). Three case reports in cancer patients described neuropathological findings post mortem after intrathecal ketamine. Two report used racemic ketamine containing the preservative benzethoniumchlorid,[3,4] one used S (+)-ketamine.[5] One report showed MRI signs of neurodegeneration after systemic ketamine for a maximum of 72 hours.[100] One randomized controlled trial revealed no neurotoxic events after 2 mg/kg ketamine i.v. in children younger than one year.

Table 1a: Animal Studies

Reference	Animals	Route	Ketamine Dosage	Methods	Neurotoxicity/Outcome
Ullah et al.[65] 2012	P 7 rats	s.c.	40 mg/kg single shot	AC3-, TUNEL- and FJB staining, Bax expression	Increased neuronal cell death in cortex and thalamus
Turner et al.[66] 2012	P7 rats	Not reported	20 mg/kg single shot, 4 injections over 4 h	AC3 immunostaining	Significant increase of apoptosis
Brambrink et al.[67] 2012	GD 120, P 6 rhesus monkeys	i.v.	20 mg/kg i.m. followed by 20–50 mg/kg/h i.v. cont. infusion for 5 h	AC3 immunostaining	Significant increase of apoptosis; fetal monkeys > neonates
Liu et al.[42] 2011	P 7 rat pups	s.c.	5, 10, or 20 mg/kg in one, three or six injections at 2-h intervals	Electron microscopy, in situ hybridization, micro array analysis and gene expression profiling	6 x 20 mg/kg ketamine significantly increased neuronal cell death in frontal cortex, while lower doses and fewer injections did not
Zhang et al.[41] 2011	P 7 rat pups	s.c.	6 x 20 mg/kg at 2-h intervals	Micro PET images following injection of [(18)F]-DFNSH, DNA fragmentation measured by ELISA	↑ neuronal apoptosis, uptake of [(18)F]-DFNSH significantly increased in frontal cortex
Gomez et al.[68] 2011	adult dogs (n = 16)	i.th.	0.5 and 0.7 mg/kg S(+)-ket single shot	light microscopic spinal cord examination	Alterations in ketamine groups including gliosis, axonal edema, central chromatolysis, lymphocyte infiltration and thickening of dura mater
Shi et al.[43] 2010	P 7 rat pups	s.c.	6 x 20 mg/kg at 2-h intervals	TUNEL staining, RNA isolation and gene expression microarray analyses, Q-PCR and in situ hybridization	Significantly ↑ neuronal cell death in ketamine group. 32 of the differentially expressed genes associated with cell death or differentiation and NMDA receptor activity
Walker et al.[6] 2010	P 3, P 7, P 21 rat pups	i.th.	3, 10, 15 mg/kg	FJC and AC3 satining, examination of histopathological change and glial activation	↑ neuronal apoptosis and microglial activation predominantely in the dorsal horn at P 3, slight increase at P 7, not statistically significant
Soriano et al.[7] 2010	P 7 rat pups	i.p.	5, 10, and 20 mg/kg at 90-min intervals over 6 h	Western blot analysis and caspase-3 immunohistochemistry	Dose- and time-dependent increase in expression of cell cycle proteins (D1, cdk4) and AC3
Gutierrez et al.[44] 2010	P 7, P 14, P 21 rat pups	s.c.	2, 5, 10, 20 and 40 mg /kg; 4 injections at 1-h intervals	AC3 staining and western blot analysis	Dose dependent ↑ of AC3 at P 7 in somatosensory cortex, no significance at P14, P21
Zhang et al.[46] 2009	P 7 rat pups	s.c.	6 x 20 mg/kg at 2-h intervals	FDG and F-annexin V uptake assessed using mirco PET imaging, Bax and β-actin expression via western blot	F-annexin V uptake significantly increased in left frontal cortex, significantly increased Bax protein expression
Ibla et al.[48] 2009	P 7 rat pups	i.p.	7 x 20 mg/kg at 90 min intervals over 9 h	Cupric-silver and AC3 staining, BDNF and TrkB cDNA products and protein levels	↑ number of silver stained neurons in retrosplenial and temporal cortex regions, ↑ AC3 positive cells in cortical and thalamic brain regions

Straiko et al.[47] 2009	P 5 mouse pups	s.c.	40 mg/kg single shot	Phosphorylation of ERK1 assessed via western blot analysis, AC3 staining	Supression of ERK and Akt phosphorylation, significant ↑ in AC3-positive profiles
Zou X et al.[45] 2009	P7 rat pups	s.c.	single or multiple (3 or 6) injections of 5, 10, 20 mg/kg at 2 h intervals	AC3 immunostaining, ELISA, FJC staining, light and electron microscopy	Significant ↑ of AC3 and neurodegeneration in cells of the frontal cortex after 6 injections of 20 mg/kg, no effect of single shot or 3 injections
Zou X et al.[37] 2009	P 5, P 6 rhesus monkeys	i.m./i.v.	20 mg/kg i.m. followed by 20–50 mg/(kg h) i.v. cont. infusion for 3, 9 or 24 h	AC3 immunostaining, FJC and silver staining	Ket infusions for either 9 or 24h significantly ↑ neuronal cell death in frontal cortex, no significance after 3 h
Yaksh et al.[69] 2008	8–24 month old dogs	i.th.	10 mg/day cont. Infusion over 28 days	histopathological spinal cord examination	minor local catheter reaction, mild histopathologic change i.e. inflammation within meninges and parenchyma
Viberg et al.[49] 2008	P 10 mouse pups	s.c.	5 or 25 mg/kg single shot	Western blot analysis for expression of GAP-43 and CaMKII	significant ↑ in CaMKII and GAP-43 levels in hippocampus after 5 or 25mg/kg; no differences in CaMKII levels in cortex between ket and controls; significant decrease in GAP-43 level in cortex of mice treated with 25 mg/kg ket
Majewski-Tiedeken et al.[51] 2008	adult mice	i.p.	5 x 5mg/kg	Silver staining and AC3 immunostaining	increased cell death in hippocampal area CA3
Rovnaghi et al.[50] 2008	neonatal rats	s.c.	2 x 2.5 mg/kg	Western blot analysis, FJB staining and Fos immunohistochemistry	No sign. differences in Caspase-3, Bax, and Bcl-2 expression between groups; ↑ fos-expression in piriform and dorsal endopiriform cortex in ket group; slightly ↑ glial activation in ket group
Zuo et al.[52] 2007	adult mice	i.p.	50 mg/kg single shot (acute group) 50 mg/kg/day over 7 days (chronic group)	basal hydroxyl radical (*OH) levels in PC/RSC measured using microdialysis	Both acute and chronic ket significantly ↑ basal *OH levels
Slikker et al.[36] 2007	GD 122, P 5, P 35 rhesus monkeys (n = 18)	i.m./i.v.	20 mg/kg i.m. followed by 20–50 mg/kg/h i.v. cont. infusion for 3 or 24 h	AC3, FJC and silver staining, TUNEL assay, electron microscopy, analysis of NR1 subunit expression	↑ of AC3 and FJC positive cells in frontal cortex at GD122 and P 5, but not at P 35, neurotoxicity was also exposure time dependent, NR1 subunit mRNA upregulated in P 5 monkeys treated for 24 h
Vranken et al.[70] 2006	White new zealand rabbits (n = 18)	i.th.	0.7 mg/kg/d pres.-free S (+)-ket for 7 consecutive days	Histopathologic spinal cord examination	Mild to severe gray and white matter damage
Rudin et al.[54] 2005	P 7 mouse pups	s.c.	1.25 mg/kg, 2.5, 5, 10, 20, and 40 mg/kg single shot	Silver staining, TUNEL assay	Dose dependent apoptotic damage detected 72 h post-injection at doses ranging from 5 to 40 mg/kg
Jevtovic-Todorovic et al.[55] 2005	6, 18, 24 month old female rats	i.p.	20, 40, 60, 80, 100 mg/kg single shot	Light microscopically counting of vacuolated neurons in PC/RSC	Significant neurotoxicity at doses from 40-100 mg/kg peaking at 3h and more severe in old compared to young rats

Young et al.[53] 2005	P 7 mouse pups	s.c.	10, 20, 30, 40 mg/kg single shot	Caspase-3 immunohistochemistry, silver staining and quantitative cell counts, combined light and electron microscopy	Dose-dependent significant increase in the rate of neuroapoptosis in caudate/putamen and cerebral cortex
Fredriksson et al.[58] 2004	P10 male mouse pups	s.c.	50 mg/kg single shot	Flouro Jade staining	↑ neurodegeneration in hippocampus and parietal cortex
Fredriksson et al.[57] 2004	P 10 mouse pups	s.c.	50 mg/kg single shot	FJB staining	↑ neurodegeneration in parietal cortex
Scallet et al.[56] 2004	P 7 rat pups	s.c.	7 x 10 or 20 mg/kg at 90 min. intervals vs. 20 mg/kg single shot or saline control	FJB-, DAPI-, silver- and AC3 immunostaining	Ket blood levels close to an anesthetic level in humans did not produce neurodegeneration. 7 x 20 mg/kg ket increased number of degenerating neurons in dorsolateral thalamus
Hayashi et al.[60] 2002	P 7 rat pups	i.p.	25, 50, 75 mg/kg single shot or 7 x 25 mg/kg at 90 min intervals over 9 h	Silver staining	Significant increase in degenerating neurons in rat pups treated with seven ketamine doses, no effect of single shot
Ma et al.[59] 2002	female rats (no age described)	s.c.	25, 50, 100 mg/kg single shot	Measurement of c-Fos expression in the PC/RSC	Dose dependent increase of c-Fos positive neurons in the PC/RS
Jevtovic-Todorovic et al.[61] 2001	P 8 and 1, 2, 3, 8 month old rats of both sexes	s.c.	20, 40, 50, 60, 80 mg/kg single shot	Histological quantitative assessment of number of vacuoled neurons in the RSC	Dose, age and sex dependent neurotoxicity with females being more sensitive at any age
Jevtovic-Todorovic et al.[62] 2000	Adult female rats	i.p.	20, 30, 40, 60, 80 mg/kg single shot	Histological counting of vacuolated neurons	Dose dependent neurotoxic reaction (sign. from 60-80 mg/kg)
Kim et al.[63] 1999	Adult female rats	s.c.	50 mg/kg single shot	PC/RSC-Ach output measured via in vivo microdialysis	Ketamine significantly elevated Ach output to 300-400% of baseline
Näkki et al.[64] 1996	P 10-90 day old female rats	i.p.	20-100 mg/kg single shot	Number of HSP70-immunoreactive neurons in the PC/RSC measured	Significantly ↑ number of HSP70 positive cells at 60-100 mg/kg, age dependency of HSP70 ↑
Animal Studies without significant neurotoxic Effects					
Ribeiro et al.[71] 2012	Adult male mice	i.p.	25 mg/kg and 75 mg/kg ketamine single shot	AC3 immunostaining, BDNF expression	No differences in cell death compared to controls
Rojas et al.[72] 2012	16 Dogs		1 mg/kg S(+)-ketamine single shot	Light microscopical spinal cord examination	No alterations compared to controls
Lyall et al.[74] 2009	P 6 rat pups	s.c.	50 mg/kg single dose	AC3 immunostaining	Ketamine (in contrast to MK 801) did not induce signifcant apoptosis in inferior collicolus and surrounding brain stem sections compared to vehicle controls

Winkelheide et al.[73] 2009	adult rats	i.v.	0.75 and 1 mg/kg/min S(+)-ketamine infusion	Histopathological examination of CA1	No damage in either group observed
Lopez-Galindo et al.[75] 2008	2 month old rats (n = 20)	i.p.	0.9% saline followed by 100 mg/kg ket single shot	Light microscopy	No differences in cell counts in ketamine only group vs. control
Anand et al.[77] 2007	neonatal rats	s.c.	2 x 2,5 mg/kg	FJB staining and Fos immunohistochemistry	No significant increases in FJB positive cells in ketamine group vs. control
Fredriksson et al.[76] 2007	P 10 mouse pups	s.c.	25 mg/kg single shot	Flouro-Jade staining	Ketamine alone did not elicit any significant increase in apoptosis
Errando et al.[78] 1999	Landrace large white pigs of both sexes (n = 20)	i.th.	0.25 mg/d racemic ketamine 5 % or pres.-free ketamine 5 % over 7 days vs. benzethonium chloride	light microscopic histopathological spinal cord examination	No neurotoxic effect of preservative free ketamine, discrete neurotoxic effect of racemic ketamine, moderate effect of preservative alone i.e. neuronal loss, gliosis, spongiosis
Borgbjerg et al.[79] 1994	White new zealand rabbits (n = 14)	i.th.	1.5 mg/kg 1 % pres. free ket once a day for 14 consecutive days	light and electron microscopic spinal cord examination	No differences between spinal cords of ketamine and control
Malinovsky et al.[80] 1993	White new zealand rabbits (n = 40)	i.th.	1.1 mg/kg 1 % pres.-free ketamine single shot vs. 1 % D(-)-ketamine	Light and flourescence microscopic spinal cord examination	Not ketamine, but chlorobutanol induced significant severe spinal cord lesions
Malinovsky et al.[81] 1991	White new zealand rabbits (n = 40)	i.th.	1.1 mg/kg 1 % ket single shot	Light and flourescence microscopic spinal cord examination	No neurocellular differences in spinal cord examinations between ketamine and controls

Table 1b: Neurotoxicity in Cell Lines

Reference	Culture	Time	Ketamine Dosages	Methods	Outcome
Sinner et al.[101] 2011	E 19 rat hippocampal neurons	24h	S(+)-ketamine at 3µM (ns), 10 µM (ns) 25 µM	TUNEL staining, AC3 staining, western blot	Significant increase of apoptosis after 25 µM ketamine, not after 3 or 10 µM
Campbell et al.[84] 2011	P 0-2 and P7-8 mice cortical neurons and glia	6 h 12 h	100 µM (ns), 1 mM, 3 mM 100 µM (ns)	MAP2 immunoreactivity (ELFIA), LDH levels in culture media	No significant neurotoxic effects of 100 µM ketamine even after 12 h exposure, significant neurotoxicity at supraclinial concentrations (1-3 mM)
Fu et al.[83] 2011	P1 rat forebrain culktures	12 h	1 µM (ns), 10 µM, 20 µM	Cell viability (MTT assay), Caspase-3 activity, NR2B expression using Western Blot	Significant decrease in cell viability, increase in caspase-3 activity and apoptosis. NR2B expression down-regulated by ketamine

Kanungo et al.[82] 2011	Zebrafish embryos	2 h 20 h	0.5 mM (ns), 2.0 mM (ns) 0.5 mM (ns), 2.0 mM	morphological changes examined by visually monitoring, GFP expression in motor neurons	Prolonged exposure (20 h) to ketamine at 2.0 mM resulted in reduction in GFP expression intensity in motor neuron population and in significant reduction in axon length. No differences at 0.5 mM.
Soriano et al.[7] 2010	E18 rat primary neurons	6 h 24 h	10 μM (ns) 0.1 μM (ns), 10 μM and 1mM	western blot analysis and caspase-3 immunohistochemistry	dose- and time-dependent increase in expression of cell cycle proteins (D1, cdk4) and AC-3
Braun et al.[97] 2010	Human T-lymphoma, Neuroblastoma cells P2 rat astrocytes	24 h	S(+)-ketamine 2 mM and 4 mM alone and with 5μM benzethonium	flowcytometry, mitochondrial activity (XTT) assay	Increase of percentage of apoptotic and necrotic cell death to almost 3 fold with Ketanest S and S(+)-Ketamine containing benzethonium. LD 50 of S(+) ket: 4.7 mM in primary rat astrocytes
Braun et al.[96] 2010	Human T-lymphoma cells, cells deficient of caspase-8, FADD deficiant cells, neuroblastoma cells	24 h	S(+)-ketamine and racemic ketamine at doses ranging from 0.5 mM to 12 mM	flowcytometry, mitochondrial metabolic activity and C3A	Dose dependent ketamine induced apoptosis in lymphocytes and neuroblastoma cell lines, protection against ket induced apoptosis in cell lines with alterations of the mitochondrial pathway
Mak et al.[85] 2010	human neuroblastoma (SH-SY5Y), fibroblasts (NIH-3T3), cultured human neurons	48 h	racemic ketamine from 4.2 μM up to 21 mM	MTT assay, western blot, caspase 3 activity, Bax/Bcl-2 ratio	Significant cell death in all culture types. Undifferentiated cells and fibroblasts were more susceptible at lower doses than differentiated neuronal cells. No difference in caspase-3 activities between treated and untreated undifferentiated SHSY5Y cells.
Wang et al.[86] 2008	newborn rat (not specified) forebrain cells	2 - 24 h	racemic, 1 μM (ns), 10, 20 mM	MTT assay, expression, PSA-NCAM expression, LDH release	10 and 20 μM significantly decreased cell viability, ketamine at 10 μM caused increase in DNA fragmentation and marked reduction in PSA-NCAM expression
Vutskits et al.[87] 2007	P0 rat differentiated and undifferentiated GABAergic interneurons	up to 72 h	racemic, 0.042 mM to 21 mM	quantitative analysis of dentritic arbours and β-tubulin isotype III positive cells, parameters of dendritic shape and extent	Significant cell loss of differentiated neurons 24 h after ketamine treatment at ≥ 84 mM and at later time points using ≥ 168 mM. Significant cell loss detected from the 1st day post-exposure in cultures treated with ≥ 42 mM
Shang et al.[88] 2007	P1 rat primary cortical neuronal cultures	24 h	0.1 μM 10 μM 30 μM	Cell viability (MTT assay), TUNEL assay, C3A western blot	Ketamine from 0.1 μM to 30 μM significantly decreased cell viability. Ketamine at 10 μM increased number of TUNEL positive cells, reduced pAkt levels and induced a time dependent increase in caspase-3-like proteinase activities

Vutskits et al.[90] 2006	P 0 rat immature GABAergic interneurons	1 – 96 h	increasing concentrations ranging from 0.042 µM to 168 µM	TUNEL assay, quantitative analysis of dendritic arbors, determination of parameters of dendritic shape and extent	Dose- and time dependent apoptosis, Reduced dendritic arborization after 4 h treatment with 21 µM.
Takadera et al.[91] 2006	DG18-19 rat cultured cortical neurons	48 h	100 µM	cell staining and flourescent microscopy, gel electrophoresis, western blotting and C3A	Time-dependent decrease in number of neurons and increased apoptotic cell death at 100 µm
Wang et al.[89] 2006	P3 rhesus monkey frontal cortical cultures	24 h	racemic, 1µM (ns), 10µM, 20 µM	fragmented DNA detection by ELISA, LDH release, mitochondrial function, western blot analysis and electrophoretic mobility shift assay	Ketamine produced apoptosis at 10 and 20 µM, no significant neurotoxic effects at 1 µM
Wang et al.[92] 2005	P1 rat forebrain cultures	2 -48 h	racemic, 0.1 µM (ns), 1 µM (ns), 10 and 20 µM	ELISA, MTT assay, LDH release, western blot, TUNEL assay	increased cell death at 10 and 20 µM, significance starting at 6 h exposure time
Lucas et al.[93] 1991	E 14 mice spinal cord cultures	24 h	100 µM to 6 mM	erythosine B determination of cell viability	dose dependency of neurotoxicity, maximum non toxic dose of ketamine: 400 µM
Lucas et al.[94] 1990	E 14 mice spinal cord cultures	24 h	100 µM to 4.5 mM	erythosine B determination of cell viability	4.5 mM resulting in 100% cell death
Shahar et al.[95] 1989	P17 rat spinal cord slices	24 h	0,25 mM, 0.5 mM, 1 mM	electron microscope observation	Dose dependent, morphological changes. Exposure of 1 mM ketamine to cultures caused transient injury to neuronal elements and myelin sheath
Cell line Studies without any significant neurotoxic Effects					
Desfeux et al.[98] 2010	P 2 mouse pups Cerebral slices	6 h	10 (ns) or 100 µM (ns)	Measuement of LDH level and caspase-3-activity	10 or 100 µM of ketamine induced a small but nonsignificant decrease in LDH activity
Shibuta et al.[99] 2006	E 16 rat cortical neurons	24 h	racemic ket 50 µM	photomicorgraphs before and after treatment	No cytotoxic effect of ketamine on survival rate of neurons compared to controls

s.c. = subcutaneous, i.m. = intramuscular, i.v. = intravenous, i.p. = intraperitoneal, i.th. = intrathecal, DIV = days in vitro, MAP2 = microtubule-associated protein 2, ELFIA = enzyme-linked fluorescent immunoassay, LDH = lactate dehydrogenase, FJC = fluoro-jade C, XTT = tetrazolium hydroxide, PSA-NCAM = polysialic acid neural cell adhesion molecule, AC3/C3A = activated caspase-3, CA1, CA3 = hippocampal areas, TUNEL = Terminal dUTP nick-end labeling, FJB = fluoro-jade B, DAPI = blue nuclear DNA stain, PC/RSC = posterior cingulate/retrosplenial cortex, N2O = nitrous oxide, NR1 = NMDA receptor subunit, P = postnatal day e.g. P 7, v/v = volume to volume, pERK = phosphorylated extracellular signal-regulated protein kinase, Akt = a serine/threonine-specific protein kinase, BDNF = brain derived neurotrophic factor, TrkB = receptor tropomyosin-related kinase B, CaMKII = calcium/calmodulin-dependent protein kinase II, GAP-43 = growth associated protein-43, 2,3 DHBA = 2,3 dihydroxybenzoic acid, XTT = tetrazolium hydroxide, PSANCAM = polysialic acid neural cell adhesion molecule, NR2B = NMDA receptor subunit 2 B, E = embryonic day, DG = days of gestation, GFP = green fluorescent protein (transgenic protein), pAkt = phosphorylated Akt (serine/threonine kinase), ns = not significant

Table 1. Neurotoxicity of ketamine in preclinical studies

Neurotoxicity of Ketamine - Case Reports					
Reference	**Individual**	**Route of Administration**	**Ketamine Dosage**	**Methods**	**Neuropathological Findings**
Vranken et al.[5] 2005	52-year-old Caucasian female	i.th.	S(+)-ketamine up to 50 mg/kg/d for 28 days	Post mortem light microscopically spinal cord examination; immunohistochemistry	Histopathological abnormalities in spinal cord and nerve roots including central chromatolysis, nerve cell shrinkage, neurophagia, microglial upregulation and gliosis
Ubogu et al.[100] 2003	44-year-old man	i.v.	2 mg/kg initial bolus, followed by 2 mg/kg/h infusion with titration up to 7,5 mg/kg/h after 48 h (72 h in total)	Cranial MR image with and without gadolinium contrast	Mild to moderate cortical volume loss, significant generalized cerebellar atrophy without contrast enhancement
Stotz et al.[4] 1999	72-year-old Caucasian female	i.th.	Racemic ketamine (containing benzethonium chloride) 67,2 mg mean daily dose over 7 days	Post mortem light microscopically examination of spinal cord and leptomeninges	Focal lymphocytic vasculitis in the medullary tissue, in the nerves, and in the leptomeninges of the thoracic and lumbar spinal cord close to catheter injection site; No signs of necrosis, hemorrhage, demyelinization or vacuolization found
Karpinsky et al.[3] 1997	51-year-old Caucasian male	i.th.	Racemic 0,25 % ketamine (with 0,1 mg/ml benzethonium chloride) 5 mg/d over 3 weeks	Post mortem light microscopy, immunohistochemical staining to glial fibrillary acidic protein (GFAP), electron microscopic brain and spinal cord examination	Mild to moderate subpial vacuolation of the thoracic and lumbar spinal cord + astroglial cell reaction, mild leptomeningeal fibrosis in sections of neocortex; collagen deposition and crystal formation within interstitial spaces in vacuolated areas
RCTs without Ketamine induced Neurotoxicity					
Bhutta et al.[102] 2011	24 infants < 1 year	i.v.	2 mg/kg before cardiopulmonary bypass surgery	Plasma markers of inflammation and central nervous injury incl. neuron specific enolase measured by ELISA at end of surgery and 6, 24 and 48 h after surgery. Magnetic resonance and spectroscopy before surgery and at time of hospital discharge	No significant differences in expression of cytokines, chemokines, S 100, neuron specific enolase between cases and controls. MR with spectroscopy showed that ketamine administration led to a significant decrease in choline and glutamate plus glutamine/creatine in frontal white matter

Table 2. Human data

5 Discussion

To the best of our knowledge, this is the first systematic review focusing on the neurotoxic effects of ketamine on neuronal tissue and cell lines, in humans and animals, respectively. Some older reviews, like the one from Mellon et al.[1] have presented some aspects concerning ketamine-induced neurotoxicity, but none of them fully focused on ketamine's effect on all kinds of human and non-human neuronal tissue systematically like in this article.

5.1 Ketamine's dosage, exposure time and route of administration

Emphasizing our hypothesis that neurotoxicity induced by ketamine is dose dependent, we found an increasing number of studies supporting us.[7,44,53,54,61,62,84,89,92,96,101]

Soriano et al.[7] reported an increase of pro-apoptotic enzymes and activation of cell cycle proteins in P7 rats after 5 injections of either 5, 10 or 20 mg/kg ketamine, though significance was reached only with concentrations of 10 and 20 mg/kg. Similar effects were observed after treatment of primary neurons with ketamine at 10 and 1000 μM, but not with a dose as small as 0,1 μM. In primary neurons, the activation of cell cycle proteins was highest at 48 hours of treatment with 10 μM ketamine compared to those treated for shorter periods and controls, suggesting that the time of exposure to ketamine may play a role just as important as dosage for the induction of neurodegeneration.[7,37]

Neurotoxic effects also appear to be cumulative since there is evidence that repeated doses of ketamine result in greater cell death than after one single-shot administration.[42,45,60] When Liu et al.[42] tested incremental doses of ketamine, one or three injections of 5, 10 or 20 mg/kg did not increase neuronal cell death in the frontal cortex of P7 rats, while six injections of 20 mg/kg ketamine resulted in enhanced apoptosis. Cumulation may also be the reason why several studies investigating the effects of a single dose of ketamine systemically failed to evoke neurotoxicity.[56,74-76]

To further confirm the hypothesis of dose dependency, neuraxial ketamine in dose ranges from 1 to 1,5 mg/kg did not result in neurocellular damage.[78,79,81] Spinal cord lesions were caused by chlorobutanol or benzethonium chloride though, suggesting that discrete, but non-significant neurotoxic effects of ketamine may be induced by racemic mixtures containing these preservatives.[78,80] The study of Yaksh et al.[69] demonstrated histopathological changes in the spinal cord after 28 days of i.th. treatment with 10 mg/kg ketamine in dogs, which is consistent with human case reports referring to post mortem

spinal cord damage after ketamine treatment for the management of cancer pain.[3-5] More recently published preclinical studies report that doses as low as 0.5 and 0.7 mg/kg S(+)-ketamine appear to be sufficient to induce spinal cord alterations after intrathecal administration.[68,70] Considering these results as well as the capability of ketamine to rapidly cross the blood brain barrier,[103] the route of administration must be taken into account when discussing potential neurotoxic effects in humans. Therefore, the risk benefit ratio of the neuraxial use of ketamine should always be kept as small as possible especially when it is administered to children.

5.2 Cells

Ketamine exerts neurotoxicity in many types of cells of the central nervous system including neurons, primary neurons, GABAergic interneurons, T-lymphoma- and neuroblastoma cells, and spinal cord cultures.[7,83,88,90,93,95-97] Braun et al.[97] demonstrated that incubation with 4.7 mM ketamine for 24 hours reduced the mitochondrial activity of astrocytes to 50%. Campbell et al.[84] found an increase of LDH levels after treatment with 1 and 3 mM ketamine indicating that not only neurons, but also glial cells are susceptible to ketamine induced damage. Vutskits et al.[90] demonstrated that a treatment with ketamine at 10 μM over one hours was sufficient to trigger apoptosis in immature GABAergic interneurons, whereas at lower doses, cell death was only induced when the drug was present for 48 hours or longer. In a second study, they showed that although ketamine concentrations of 20 μg/ml or higher are required to induce cell death in differentiated neurons, incubation for more than 24 hours at a concentration as low as 0.01 mg/ml, while not affecting survival, altered dentritic arbor architecture.[87] Considering dentritic arborization as one important premise for proper neuronal interaction,we conclude that chronic administration of subanesthetic doses of ketamine could possibly influence also long-term neuronal network functions. Desfeux et al.[98]revealed that ketamine, even at low concentrations, induced an increase of Caspase 3 activity and stimulation of Bax expression in P2 cortical slices of mice, albeit these results were not significant compared to controls. Inconsistently with these data, Shibuta et al.[99] found no effects of 50 μM ketamine alone on the survival rate of E16 rat cortical neurons using ketamine as a control to compare it to other groups in which the drug was co-administered together with either NMDA or a nitric oxide-releasing compound (NOC-5).

5.3 Young brains are especially vulnerable

Our data indicates that the developmental age of the central nervous system exposed to ketamine plays a crucial role in the presence and the extent of neuroapoptosis. Therefore, regardless of the breed, younger animals were more susceptible to neurotoxicity than older ones.[6,36,44] The highest amount of neurotoxic events over all animal studies was noticed at P7. Remarkably, most of the studies (approx.50 %) also investigated neurotoxicity at P7. Thus, compared to the mature central nervous system, the brain seems to be highly susceptible in the developmental stage known as "brain growth spurt", a period characterized by rapid synaptogenesis and reorganization of neuronal structures. This underlines the hypothesis that ketamine too, leads to a degenerative process similar to physiological apoptosis primarily mediated by blockade of the NMDA receptor like it has been shown for other NMDA antagonists such as MK-801.[104]Not all studies showed this clear age dependency. In contrast with other preclinical research, Jevtovic-Todorovic et al.[61]reported a total insensitivity of P8 rats to ketamine neurotoxicity. Näkki et al.[64] showed that heat shock proteins (HSP 70), which serve to protect cells from injury, were expressed in neurons of 30-90 day old rats, but not in younger animals treated with ketamine. One limitation to using HSP 70 as a selective apoptosis criterion in this study seems to be the inability of very young animals toproduce this protein, expecting that the immature NMDA receptors cannot respond with full protein expression, therefore HSP 70 cannot clearly reflect neurotoxicity.

5.4 The paradox of ketamine-induced neuroprotection

Complicating the discussion about ketamine induced neurotoxicity, we also found an increasing body of evidence for a neuroprotective effect of ketamine, when added to models of neurotoxin- or ischemia-induced brain injury.[77,105-124]

Ketamine effects are mainly based on a NMDA receptor blockade, consequently neuroprotective effects appear to be similarly dose dependent as the neurotoxic ones.[105,107,108,112,123-127] Wang et al.[105] showed that a ketamine concentration of 0.1 mM attenuated the increase of apoptosis after stimulation of neuronal PC12 cells with glutamate, whereas maximal neuroprotection was reached at 1 mM. In the study of Berman et al.[113,116] cerebellar granular neurons were protected from glutamate and brevetoxin induced injury in a concentration dependent manner. 7.02 µM ketamine in the glutamate study respectively 9 µM in the brevetoxin study were needed to protect half of the neurons from damage (EC_{50} values) showing a sigmoidal curve. Similar effects in these studies

were shown for the selective, high affinity NMDA receptor antagonist Mk-801 at very small doses.

As an examples for neuroprotective effects after cerebral ischemia, Engelhard et al.[109] investigated the effect of 1 mg/kg S (+)-ketamine prior to carotid occlusion in rats. They found that the concentration of the pro-apoptotic protein Bax was 250% higher in controls compared with S (+)-ketamine, indicating that ketamine may be beneficial in the situation of insult. Other research demonstrated that ketamine significantly reduced necrosis,[120] apoptosis and brain edema[106] following traumatic brain injury in rats, especially in the early posttraumatic phase. Although in these animal models very high systemic doses were used, these results suggest that ketamine could prevent posttraumatic neuronal cell loss. Interestingly, ketamine only induced neuroprotective effects in models of chemical injury when potent neurotoxic substances were added. One research team showed that S(+)-ketamine up regulates the expression of proteins associated with regeneration[128] and increases cell survival and axonal regeneration[115] following glutamate injury in rat hippocampal neurons. Rovnaghi et al.[50] showed that systemic ketamine is able to attenuate the cell death resulting from inflammatory pain in cortical and hippocampal areas of neonatal rats. Notably, cell death in this study correlated with glial activation, not with the expression of apoptotic proteins. Taking all this into consideration we conclude that in the condition of strong or neuropathic pain and in some particular clinical conditions the neuroprotective effect of ketamine (within a normal dose range)could potentially outweigh its neurotoxic effect.

Since this article was not set up to review ketamine's neuroprotective effects, the results presented here are incomplete. To fully elucidate this effect, it should be the topic of a separate review.

5.5 Neurotoxicity in the human CNS

Human case reports show spinal cord damage such as nerve cell shrinkage, chromatolysis, lymphocytic vasculitis and subpial vacuolation after intrathecal long-term administration of ketamine for the treatment of chronic cancer pain[3-5]. In these cases, however, high daily doses of up to 67.2 mg were administered as a continuous infusion. Several animal studies suggest that histopathological findings in some of these cases may also be related to racemic ketamine containing preservatives such as benzethonium chloride and chlorobuthanol.[78,80] On the other hand, more recently published reports showed MRI signs of neurodegeneration in the brain and spinal cord in humans after treatment with S(+)-

ketamine and racemic ketamine without preservative.[5,100] Therefore, other factors such as metastatic infiltration, radiation therapy or adjuvant chemotherapy in terminal cancer patients may also be responsible for neurotoxicity.[3,5] Since human data on this topic is rare, only one randomized clinical trial investigated both neurotoxicity and neuroprotection of systemically administered ketamine in children younger than one year.By determination ofplasma markers of central nervous system injury e.g. neuron-specific enolase after treatment with 2 mg/kg ketamine i.v. and magnetic resonance imaging with spectroscopy before and after cardiopulmonary bypass, Bhutta et al.[102] found no evidence for either a neurotoxic or neuroprotective effect of ketamine i.v. Considering the low power of this pilot study due to its low sample size, future research with larger study populations may help to fully elucidate the hazardous power as well as the neuroprotective potential that ketamine holds in certain clinical situations.

5.6 Limitations of this review

Due to the inconsistency of the methods used to determine neurotoxic effects in studies it was not possible to perform a meta-analysis for that issue. In respect of the cell lines used, studies showed a great heterogeneity making it unable to pool the data and compare between the groups. We therefore summarize our findings descriptively. The study of Rovnaghi et al.[50] which shows protective effects of ketamine in a chronic inflammatory pain model in rats was included into this review although it looks like a double publication when comparing to the study conducted by Anand et al.[77] published one year earlier.

5.7 Summary

Ketamine induced neurotoxicity is dependent on many factors including time of exposure, dosage, age of individuals and the route of administration in animals. In human neuronal cell lines, all cell lines showed signs of apoptosis. In cancer patients post mortem, intrathecal application evoked neurotoxic effects in the spinal cord. The overall picture leads to the conclusion that this NMDA antagonist definitely holds a neurotoxic potential. The question that remains to be answered is whether the doses used in clinical practice and/or the route of administration - especially in pediatric anesthesia - are sufficient to trigger these effects in the human CNS. Future research with more randomized controlled trials and greater study populations investigating ketamine effects in humans needs to answer these questions.

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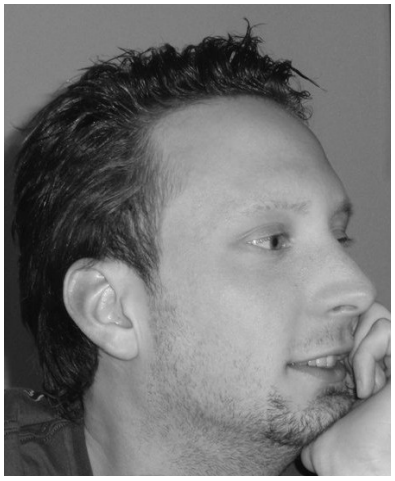
7 Curriculum vitae



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2011/01-02	Unterassistent an der Abteilung für Innere Medizin am Universitätsspital Zürich

2011/04-05	Praktikum an der Abteilung Akutpsychiatrie der Landesnervenklinik Sigmund Freud Graz
2011/06	Famulatur am Schizophreniemodul der Klinik für Psychiatrie und Psychotherapie der Charite Berlin
2012/06	Posterpräsentation am ESA (Euroanesthesia)-Kongress in Paris, Frankreich

Persönliche Fähigkeiten und Kompetenzen

Muttersprache:	Deutsch
Sonstige Sprachen:	gute Englischkentnisse, Italienisch, Französisch
Hobbies:	Malerei, Karate, Lesen, Filme