CNS Drug Reviews
Vol. 11, No. 2, pp. 113–140

KTX 0101: A Potential Metabolic Approach to Cytoprotection in Major Surgery and Neurological Disorders

Sharon L. Smith[1], David J. Heal[1], and Keith F. Martin[2]

[1]*RenaSci Consultancy Ltd, BioCity, Nottingham, UK;*
[2]*KetoCytonyx Inc, Limeburner Lane, London, UK*

Keywords: Alzheimer's disease — Cardiopulmonary bypass — Cytoprotection – Epilepsy — Excitotoxicity — Ischemia — Ketones — KTX 0101 — Neuroprotection — Parkinson's disease — Sodium D-β-hydroxybutyrate — Status epilepticus — Stroke.

ABSTRACT

KTX 0101 is the sodium salt of the physiological ketone, D-β-hydroxybutyrate (βOHB). This neuroprotectant, which has recently successfully completed clinical Phase IA evaluation, is being developed as an intravenous infusion fluid to prevent the cognitive deficits caused by ischemic foci in the brain during cardiopulmonary bypass (CPB) surgery. KTX 0101 maintains cellular viability under conditions of physiological stress by acting as a "superfuel" for efficient ATP production in the brain and peripheral tissues. Unlike glucose, this ketone does not require phosphorylation before entering the TCA cycle, thereby sparing vital ATP stores. Although no reliable models of CPB-induced ischemia exist, KTX 0101 is powerfully cytoprotectant under the more severe ischemic conditions of global and focal cerebral ischemia, cardiac ischemia and lung hemorrhage. Neuroprotection has been demonstrated by reductions in infarct volume, edema, markers of apoptosis and functional impairment. One significant difference between KTX 0101 and other potential neuroprotectants in development is that βOHB is a component of human metabolic physiology which exploits the body's own neuroprotective mechanisms. KTX 0101 also protects hippocampal organotypic cultures against early and delayed cell death in an *in vitro* model of *status epilepticus*, indicating that acute KTX 0101 intervention in this condition could help prevent the development of epileptiform foci, a key mechanism in the etiology of intractable epilepsy. In models of chronic neurodegenerative disorders, KTX 0101 protects neurons against damage caused by dopaminergic neurotoxins and by the fragment of β-amyloid, $A\beta_{1–42}$, implying possible therapeutic applica-

Address correspondence and reprint requests to: Dr. S. L. Smith, RenaSci Consultancy Ltd, BioCity, Nottingham, NG1 1GF, UK.
Tel: +44 (115) 912-4260; Fax: +44 (115) 912-4263; E-mail: sharon.smith@renasci.co.uk

tions for ketogenic strategies in treating Parkinson's and Alzheimer's diseases. Major obstacles to the use of KTX 0101 for long term therapy in chronic disorders, e.g., Parkinson's and Alzheimer's diseases, are the sodium loading problem and the need to administer it in relatively large amounts because of its rapid mitochondrial metabolism. These issues are being addressed by designing and synthesizing orally bioavailable multimers of βOHB with improved pharmacokinetics.

INTRODUCTION

Many drug strategies have been developed in the laboratory for cytoprotection in acute and chronic neurological conditions, e.g., cognitive deficits incurred as a result of major surgery, stroke, head trauma and Parkinson's or Alzheimer's diseases, but in almost every case, they have not proven to be beneficial when evaluated in clinical trials. One common feature of these pharmacological approaches is the attempt to limit cellular damage by blocking one or more pathways in a proposed neurodegenerative cascade. Examples include the inhibition of excitatory neurotransmission and prevention of intracellular calcium entry, inflammation or free radical formation. One approach, which has received little attention until recently, is to deliver neuroprotection by exploiting the body's own systems for maintaining cellular viability. It is universally recognized that the mitochondrion is the "powerhouse" of all living cells and maintenance of ATP production by this organelle is essential for survival. Moreover, the brain is especially vulnerable to ischemic injury because it carries almost no energy reserves and, therefore, is totally reliant on a constant supply of oxygen and glucose from the bloodstream. What is much less appreciated is that the brain and other organs can metabolize ketone bodies, i.e., D-β-hydroxybutyrate (βOHB), acetoacetate and to a much lesser extent, acetone, as energy sources in the tricarboxylic acid (TCA) cycle. Moreover, the ketones generate ATP more efficiently as energy sources than glucose because they do not require phosphorylation before entering the TCA cycle (80; Fig. 1). This "ATP-sparing" mechanism is a critical advantage in situations where neurons and other cells are at risk due to either impaired mitochondrial function or the reduced availability of oxygen and glucose.

Ketone bodies (Fig. 2) have a key role in mammalian energy metabolism as intermediates in synthesis and breakdown of fat. During starvation, ketones are formed in large quantities by the oxidation of long-chain fatty acids in liver cell mitochondria (62). βOHB is the most abundantly produced ketone body, but normally it is present only at very low concentration (~0.05 mM) in human blood. However, its level can increase by 50- to 100-fold, to equal that of blood glucose, during starvation or ingestion of a ketogenic diet, which is composed of high fat and very low carbohydrates (24,89). Humans are distinguished from other mammalian species by their large brain, which accounts for ~20% of the resting oxygen consumption of the body, equivalent to ~100–150 g glucose metabolized each day. During fasting, ketone bodies are released from the liver into the bloodstream, and at high plasma concentrations, βOHB substitutes for glucose as a metabolic energy source in the human brain (12,61). Adult liver can synthesize ≤185 g ketones/day; they provide 2–6% of the body's energy requirements after overnight fasting and 30–40% after a 3 day fast (49). One largely unrecognized aspect of this process is that βOHB is a

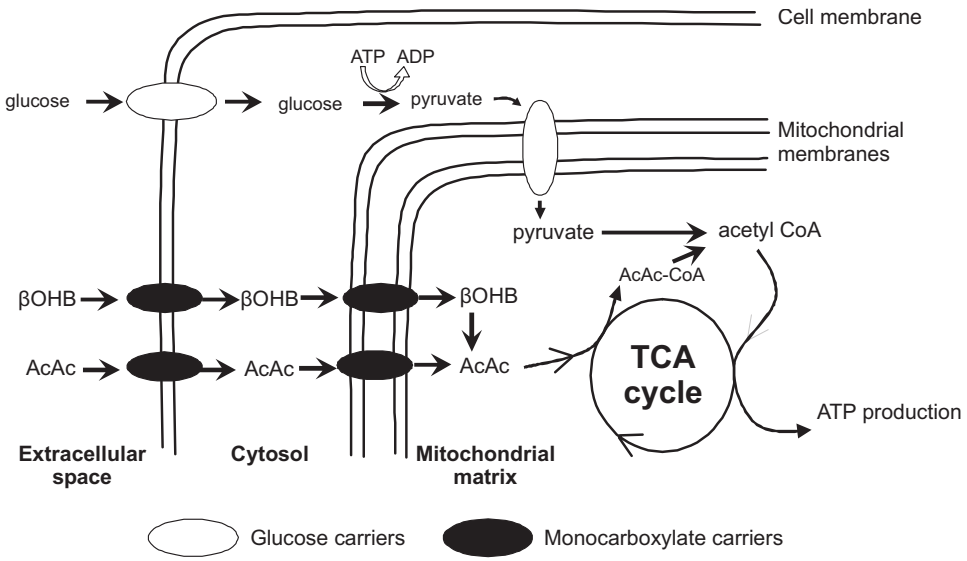

Fig. 1. ATP production by D-β-hydroxybutyrate (βOHB) and glucose in the TCA cycle. ATP production in the mitochondria is normally fuelled by glucose, but is more efficiently driven by βOHB and acetoacetate. AcAc, Acetoacetate; βOHB, D-β-Hydroxybutyrate; TCA, Tricarboxylic Acid Cycle.

more efficient energy source for the brain and other body tissues than glucose. βOHB has a 30% higher heat of combustion than pyruvate (the 3-carbon mitochondrial substrate formed as the end-product of glycolysis) because the former exists in a more reduced form (80). In the brain, >60% of the metabolic energy requirements can be supplied by ketones instead of glucose (61). Moreover, increased concentrations of ketones reduce glucose utilization in all tissues by inhibiting the enzymes, phosphofructokinase and hexokinase, and also by switching-off pyruvate dehydrogenase (19). These events markedly decrease the rate of glucose oxidation in the brain. However, a minimal level of glucose oxidation is still required for the optimum utilization of ketone bodies by the brain (35,88).

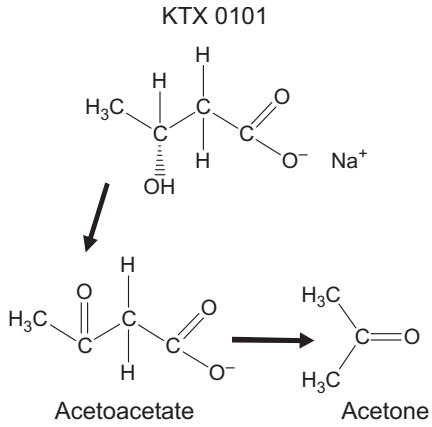

Fig. 2. Chemical structures of the ketone bodies. KTX 0101 is the sodium salt of βOHB.

For hundreds of years, it has been known that fasting, which markedly increases the circulating concentration of ketone bodies, can control seizures in epileptic individuals (75). However, fasting is not a viable approach for the long-term enhancement of ketone body production, so the "ketogenic diet" was developed and it has been successful in the treatment of drug-resistant epilepsy, particularly in children, since the 1920s (64,85,87). In fact, since the 1980's, it has become even more popular with some pediatric neurologists.

Consistent with an important role for βOHB in seizure control, anticonvulsant efficacy correlates well with plasma levels of this ketone (8,9,25). It is thought that these elevated levels of ketones provide the anticonvulsant properties and not the metabolic acidosis resulting from this regime (3). While 40% of patients on this regime experience a dramatic reduction in seizures (>90%), another 40% of patients show moderate benefit (50–90% reduction) (79). Ketogenic diets, of a mild form (i.e., low, rather than virtually no carbohydrates) have also recently become popular as weight-reducing regimes. The serum concentrations of ketones during these mild ketogenic diets reach only 0.3–0.4 mM. For the treatment of epilepsy, serum levels of ketone bodies need to range from 2–7 mM for a therapeutic effect. To achieve this endpoint, a very strict adherence to the diet must be followed and even small amounts of ingested carbohydrate will cause a marked decrease in the plasma levels of βOHB. The efficacy of βOHB in the prevention or reduction of seizures may arise from its use by the brain as an alternative energy source to glucose. In epileptic patients, there is a reduction of glucose uptake and metabolic activity in seizure foci (40). This, together with increased metabolic demand during a seizure, may exacerbate the neuropathological outcome (40). Recently, a study using ^{31}P magnetic resonance imaging was performed in patients with epilepsy (63). They found that after ingestion of a ketogenic diet, increased levels of high energy phosphates were present in the brain, thereby probably providing more neuronal stability. However, the long-term maintenance of patients on the ketogenic diet is not practical. Firstly, it is extremely unpalatable as it is composed of highly fatty foods, such as cream, cheese, butter, fatty meats, vegetable oils, etc. Moreover, adherence to the diet in children results in a range of unacceptable side-effects including, reduced growth rate, delayed puberty, obesity, deficiency of certain nutrients, depression, decreased bone density, elevated blood lipids and production of kidney stones (21,22). It is also potentially artherogenic, causing dangerous deposits of fat in blood vessels (13). To address these problems, the dietary substitution of medium chain triglycerides for long chain triglycerides since the 1970's has meant that ketogenic diets were possible without restriction of carbohydrates. However, this modified version of the ketogenic diet still produces unpleasant side effects including diarrhea, nausea and vomiting (67). This approach, therefore, would be entirely unsuitable for patients undergoing surgery, e.g., cardiopulmonary bypass (CPB) or coronary artery bypass graft (CABG) procedures or for the long-term treatment of neurodegenerative conditions, such as Parkinson's and Alzheimer's diseases.

In summary, βOHB is not a novel pharmacological agent, but a normal component of human metabolic physiology. Importantly, it is a more energy efficient fuel for cells than glucose in circumstances where oxygen levels are low (13). In view of the facts that ketogenesis has a range of potential therapeutic applications, but the ketogenic diet has severe limitations, this physiological approach for neuroprotection has been adopted by KetoCytonyx as the technology platform for developing KTX 0101 (the sodium salt of βOHB) and a range of ketone-based new chemical entities (NCEs). KTX 0101 is being developed for the potential treatment of various acute neurodegenerative conditions, particularly the prevention of cognitive deficits resulting from major surgery, whilst the orally active NCEs with longer biological half-lives will be evaluated for treating various chronic neurological disorders, including Parkinson's and Alzheimer's diseases. It is important here to emphasize that KTX 0101 is not being developed to treat patients who have suffered a major thromboembolic stroke, but as a neuroprotectant pretreatment for individuals undergoing surgery, such as CPB or CABG, to prevent cognitive impairments

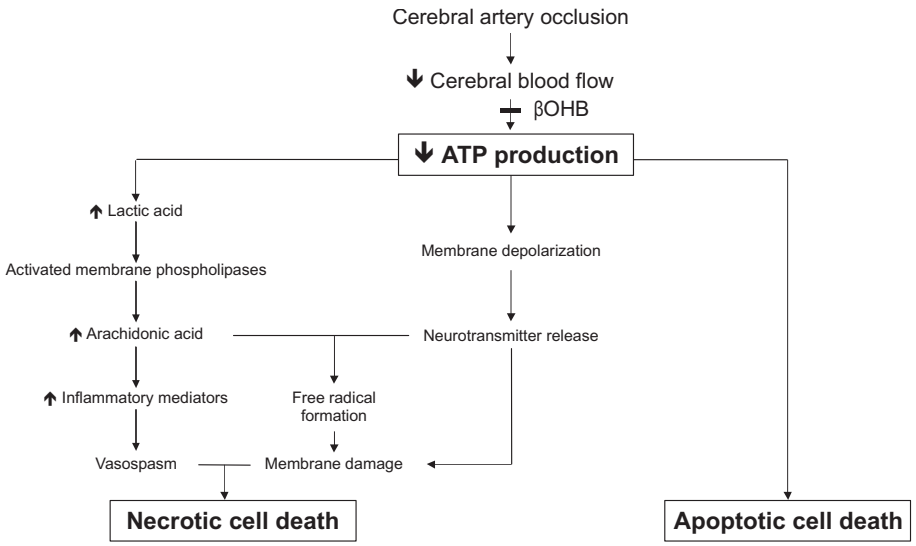

Fig. 3. Summary of the ischemic cascade showing the point at which D-β-Hydroxybutyrate (βOHB) provides neuroprotection.

due to ischemia caused by multiple micro-emboli arising from these procedures. Experiments with KTX 0101 have determined its role in maintaining the viability of neuronal tissues under various experimental conditions. In describing the studies, we have used the term KTX 0101 when experiments have been performed by KetoCytonyx and its research collaborators, in all other cases the compound is referred to under its generic name, sodium βOHB.

NEUROPROTECTION IN CARDIOPULMONARY BYPASS

As a consequence of the increased prevalence of coronary artery disease over the last 40 years, CPB is now a common surgical procedure with 800,000 operations worldwide, including 300,000 in the USA (56). Mortality after this procedure has gradually fallen in recent years, but adverse effects including coma, delirium, focal strokes and more subtle cognitive impairment remain of prime concern (28,53). The occurrence of brain injury due to ischemia during the procedure may leave the sufferer with persistent motor, sensory, behavioral or intellectual deficiencies. Approximately 10% of patients suffer permanent cognitive impairment and as many as 80–90% show a substantial decrease in function in the period after CPB (20). Cognitive deficit is thought to result primarily from multiple small emboli, which occlude blood flow to the brain, thereby causing ischemia leading to cell death (Fig. 3). No neuroprotective drugs have received regulatory approval for the prevention of cognitive impairment after CPB but not because they have not shown any evidence of efficacy. The antiepileptic drug, remacemide, provided a modest improvement in the learning ability of patients in a clinical trial in CPB surgery (4). Similarly, lidocaine

has been reported to reduce the short-term deficit in cognitive function following CPB surgery (83), although a more recent study in patients undergoing cardiac surgery did not find a positive outcome with this drug (30). An evaluation of chlomethiazole in this indication just failed to show a statistically significant improvement ($P < 0.06$) in CPB patients (45). Cognitive impairment in CPB and CABG surgery is of considerable importance and clinical trials in these indications have a significant advantage compared with stroke because the neuroprotective intervention can be given prior to the ischemic episode. Although there are currently no proven animal paradigms to model cognitive deficits in CPB, sodium βOHB (generic KTX 0101) has nonetheless been demonstrated to maintain cellular viability in much more severe ischemic situations. Recent findings have shown that KTX 0101 provides neuroprotection during hypoxia *in vitro* (58), in models of global and focal cerebral ischemia in rats (73,74), cardiac ischemia (90), and in lung hemorrhage (2,46).

Experimental Evidence for Cytoprotection in the Brain

Experiments with KTX 0101 have determined its role in maintaining the viability of neuronal tissues under conditions of hypoxic or ischemic insult.

In vitro evidence of cytoprotection

KTX 0101 has been demonstrated to protect rat primary hippocampal cells against the effects of hypoxia (58). Cells were incubated with 4 mM KTX 0101 and immediately exposed to hypoxic conditions for up to 6 h. KTX 0101 significantly improved neuronal survival for the 6-h period of hypoxia. After 2 h, KTX 0101 reduced the number of neurons showing acute cell death, indicated by trypan blue permeability. After 4 h of hypoxia, propidium iodide uptake showed that KTX 0101 decreased the numbers of neurons with condensed nuclei, indicative of apoptosis. In addition, the mitochondrial transmembrane potential was maintained for up to 2 h of hypoxia in cells treated with KTX 0101, while that in the control group was decreased. Further evidence of the protective effect of KTX 0101 was demonstrated by decreased caspase-3 activation, reduced poly (ADP-ribose) polymerase (PARP) cleavage and reduced cytochrome C release during the first 2 h of exposure to the hypoxic insult.

In vivo evidence of cytoprotection

In studies *in vivo*, rats were subjected to a global ischemic insult (bilateral carotid artery occlusion) and given a constant intravenous infusion of KTX 0101 (10, 30, or 100 mg/kg/h) starting at initiation of the ischemic episode (74). When killed 6 h later, the KTX 0101-treated animals showed decreased edema and sodium content in their cerebral hemispheres compared with the saline-infused group (74). This neuroprotection evoked by KTX 0101 was significant at all doses (74). The reduction of edema was maintained even when administration of KTX 0101 (30 mg/kg/h) was delayed until 3 h after the onset of vessel occlusion (74). KTX 0101 infusion (30 mg/kg/h) also increased ATP and decreased lactate content in the cerebral hemispheres of animals killed 3 h post-insult (Fig. 4). These investigators also determined the neuroprotective effect of KTX 0101 in animal models of permanent and temporary focal cerebral ischemia (73). Rats were subjected to permanent focal ischemia by occlusion of the middle cerebral artery (76) and were given either an intravenous infusion of KTX 0101 (30 mg/kg/h) or saline vehicle

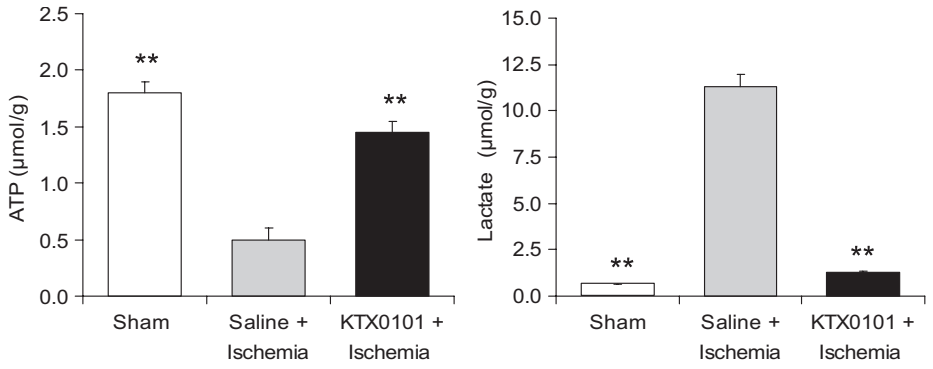

Fig. 4. KTX 0101 maintains metabolic viability during global ischemia. Global ischemia was produced in rats by bilateral common carotid artery occlusion. KTX 0101 was administered at 30 mg/kg/h i.v. commencing at the time of occlusion. Biochemical measurements were made 3 h post-occlusion. $^{**}P < 0.01$ vs. saline + ischemia ($n = 8$). Data from ref. 74.

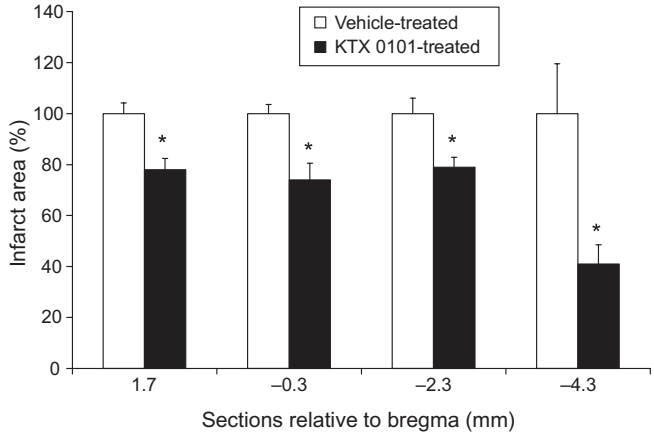

Fig. 5. Neuroprotection provided by KTX 0101 after permanent middle cerebral artery occlusion in rats. Rats were subjected to permanent arterial occlusion and were killed, 24 h later, KTX 0101 was infused at 30 mg/kg/h i.v. starting at the time of occlusion. Cerebral infarct area was obtained by histological staining. $^{*}P < 0.05$ compared with vehicle-treated group ($n = 11$–13). Data from ref. 73.

starting at the time of the insult. When the animals were killed, 24 h later, KTX 0101 had significantly reduced the area of the brain lesion (Fig. 5). This neuroprotective effect was not found at 72 h post-occlusion, however (73). The findings were extended to ischemia/reperfusion injury. Temporary focal ischemia was induced in rats by insertion of a nylon suture into the external carotid artery to occlude the origin of the middle cerebral artery for 2 h (54). KTX 0101 (30 mg/kg/h) or saline was infused intravenously commencing at the time of occlusion. When measured 24 h later the infarct area was significantly reduced in this ischemia/reperfusion injury model (Fig. 6A). KTX 0101 was also neuroprotective when its infusion was delayed until 1 h after arterial occlusion (Fig. 6B).

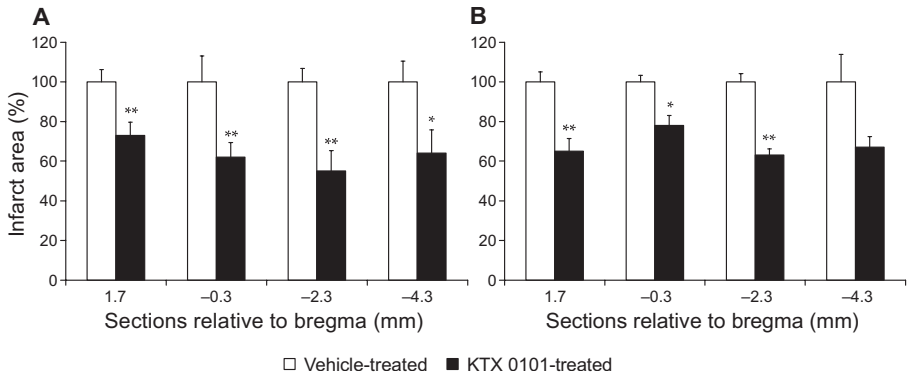

Fig. 6. Reduction of cerebral infarct area by KTX 0101 after temporary middle cerebral artery occlusion in rats. Vehicle or KTX 0101 (30 mg/kg/h i.v.) were infused (A) immediately after arterial occlusion (n = 13–15) or (B) 1 h after the surgical insult (n = 10–12). Rats were killed 24 h after arterial occlusion. Cerebral infarct areas were determined by TTC staining. *$P < 0.05$, **$P < 0.01$ compared with Vehicle-treated rats. Data from ref. 73.

In addition to reducing infarct size, KTX 0101 also significantly improved functional outcome assessed by scoring of postural reflexes (73). KTX 0101 reduced cerebral edema and sodium content, but did not affect malondialdehyde concentrations (73) indicating that KTX 0101 did not reduce oxygen free radical production in this experiment. KTX 0101 had no effect on physiological measurements, i.e., blood pressure, PCO_2, PO_2, blood pH or body temperature, in experiments defining its effects in models of global ischemia or permanent or temporary focal ischemia (73,74), showing a direct neuroprotective role for this ketone.

Neuroprotection afforded by ketones has also been demonstrated for neonatal hypoxia-ischemia in rats (17). Plasma βOHB levels were moderately, but highly significantly, increased following administration of dexamethasone (0.1 mg/kg i.p.) 22 h prior to the surgical insult. After 3 days of recovery, the neuropathological scores for brain sections taken from the dexamethasone treated rats were profoundly decreased compared with those given vehicle (17).

Experimental Evidence for Peripheral Cytoprotection

In addition to providing neuroprotection in the brain, sodium βOHB (generic KTX 0101) has also been demonstrated to protect peripheral tissues from the detrimental effects of ischemia. Zou et al. (90) intravenously infused rats with 30 mg/kg/h sodium βOHB for 1 h prior to occluding the left coronary artery for 30 min, followed by 120 min of reperfusion. Four groups of rats were used: two groups of rats were fasted for 84 h before surgery (to elevate blood ketone levels), whilst the other two were maintained on a normal diet. Plasma βOHB levels at the end of the experiment are shown in Table 1. Infarct size determined by TTC (triphenyltetrazolium chloride) staining was markedly decreased in the fasted animals given sodium βOHB compared with all other groups (Table 1). Myocardial apoptosis, as defined by single-strand DNA staining, was similarly reduced in the "fasted + sodium βOHB-treated" group of rats (Table 1), and concentrations of ATP in the heart were also significantly higher in this group (Fig. 7). This study

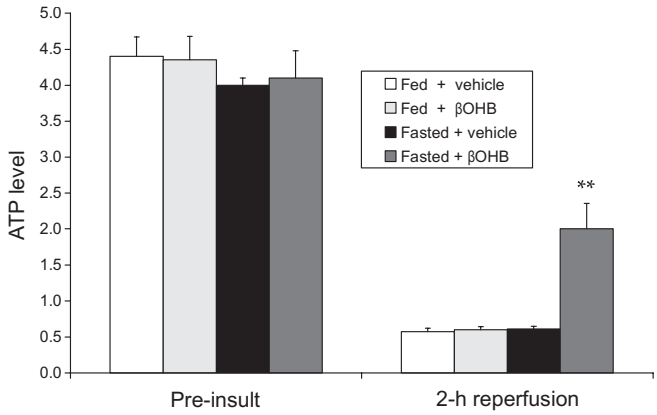

Fig. 7. Effect of sodium βOHB on myocardial ATP levels after ischemia-reperfusion in rats. Sodium βOHB was administered at 25 μmol/kg/min (i.v.) 1 h before coronary artery occlusion. Ischemia was induced for 30 min and animals were killed after 2 h of reperfusion. ATP levels were measured using a bioluminescence luminometer. $^{**}P < 0.01$ compared with all other groups ($n = 6$). Data from ref. 90.

demonstrates that high concentrations of βOHB reduce myocardial infarct size and apoptosis during ischemia/reperfusion.

Sodium βOHB has been shown to reduce lung cell damage in animal models of hemorrhagic shock. Alam et al. (2) subjected rats to hemorrhage (27 mL/kg) over 10 min using a femoral vein catheter, followed by a continuous bleed of 8 mL/kg for 75 min. Resuscitation fluids were then infused through the venous catheter for 45 min. During this period, a third bleed of 8 mL/kg was performed through a femoral, arterial catheter. The animals were killed 1 h after resuscitation and lung tissue damage was measured by the concentrations of bax (pro-apoptotic) and bcl-2 (anti-apoptotic) proteins. Resuscitation with lactate-Ringer's solution resulted in significantly increased pulmonary apoptosis compared with the sham hemorrhage group (2). However, animals resuscitated with ketone-Ringer's solution (containing 28 mmol/L sodium βOHB; equal to 3 volumes of lost blood) showed

TABLE 1. Protection by sodium D-β-hydroxybutyrate (βOHB) against cardiac ischemia in rats

Group	Infarct area (%)	Apoptosis (%)	βOHB plasma levels (μM)
Fed + Vehicle	72 ± 3	39 ± 6	388 ± 31
Fed + NaβOHB	75 ± 5	37 ± 5	851 ± 91††
Fasted + Vehicle	70 ± 5	34 ± 3	802 ± 86††
Fasted + NaβOHB	26 ± 4**	9 ± 2**	1735 ± 161**

Note. Animals were given sodium βOHB (25 μmol/kg i.v.) for 1 h before coronary artery occlusion (30 min) with reperfusion for 2 h. Some groups were food deprived for 84 h before the intervention. Infarct size was determined histologically at the end of the reperfusion period. Apoptosis in the area of risk was measured by ssDNA staining. Positive myocyte nuclei were counted in histological sections. $^{**}P < 0.01$ compared with all other groups ($n = 8$), $^{††}p < 0.01$ compared with the fed + vehicle group. Data from ref. 90.

no such increase in bax protein levels (2). Similar positive results for the cytoprotective effect of βOHB were demonstrated by the reduced ratio of bax/bcl-2 protein expression on western blots in comparison to the group given lactate-Ringer's solution (2). A subsequent study by this group (46) extended these findings. The same hemorrhagic model was employed, the only difference being that the rats were killed 2 h after resuscitation. These investigators confirmed that ketone-Ringer's infusion (28 mmol/L sodium βOHB; equal to 3 volumes of lost blood) decreased pulmonary apoptosis as revealed by the bax/bcl-2 protein ratios on western blots (46). They did not, however, find any difference of ATP levels in comparison to the group resuscitated with lactate-Ringer's solution (46). On this occasion, plasma levels of βOHB were markedly increased to 120 ± 49 mM at the end of the resuscitation period compared with 0.16 ± 0.03 mM at the start of the hemorrhagic hypertension (46). These experiments demonstrate that ketone bodies are also promising candidates for use as additives in resuscitation fluids.

KTX 0101 as a Potentially Neuroprotective Infusion Fluid for Use in Cardiopulmonary Bypass

In our initial discussion of ketogenesis as a therapeutic approach, it was evident that neither adherence to a strict ketogenic diet, nor starvation is viable for enhancing βOHB blood levels to evoke neuroprotection. The above overview provides good experimental evidence to demonstrate that in rodents maintained on a normal diet, infusion of sodium βOHB is neuroprotective in both global and focal ischemia with or without reperfusion injury. In all of the above experimental studies, with the exception of Zou et al. (90), KTX 0101 or sodium βOHB delivers cytoprotection under conditions of normal physiological energy metabolism, i.e., in the presence of glucose as an energy source, providing the plasma levels of βOHB are sufficiently great. It is only during the ketogenic diet, when carbohydrates intake needs to be abolished to force the body into ketogenesis, where fasting or dietary restriction has to be applied. This is one of the major shortcomings of the ketogenic dietary approach to long-term therapy. It is also the reason why it cannot be used for acute surgical approaches because the patients would need to be starved for ~3–5 days to reach therapeutically useful plasma levels of βOHB. This would not be achievable with a simple presurgical overnight fast. As βOHB itself is not freely soluble in water, it is not suitable for use as a neuroprotective infusion fluid. On the other hand, KTX 0101 is highly water soluble and can be formulated as a concentrated solution for dilution to an isotonic infusion fluid for intravenous administration to patients undergoing surgery, such as CPB or CABG. Thus, a priming load of KTX 0101 can be administered to CPB patients followed by a sustained infusion for as long as is required to maintain a therapeutically effective circulating concentration of βOHB during and after surgery. Currently, the standard sodium-based infusion fluid used in cardio thoracic surgery contains two organic molecules, acetate and gluconate, providing 33% of the anions, while the other 67% is chloride. Substitution of KTX 0101 for the acetate and gluconate is more relevant, because acetate and gluconate are not physiological metabolic fuels. Experimental research has also shown that glucose-based infusion fluids can produce harmful effects in the ischemic brain (51). In contrast, KTX 0101 is a highly efficient fuel under experimental ischemic conditions (73,74), maintaining neuronal function with the lowest possible demand on precious oxygen availability. Preserving the metabolic status of neurons and

other brain cells during transient ischemia caused by the microemboli generated during CPB surgery could reduce, and possibly even prevent, the adverse neurological and psychiatric consequences of this life-saving procedure.

NEUROPROTECTION DURING *STATUS EPILEPTICUS*

Most patients with epilepsy achieve complete seizure control by therapeutic intervention with antiepileptic drugs, but as many as 25 to 30% of epilepsy sufferers remain intractable to current anticonvulsant treatment (1).

In vitro evidence of neuroprotection

Intractable epilepsy is thought to develop following an initial seizure event early in life, such as a febrile seizure or *status epilepticus* (5,72). This initial seizure activity leads to neuronal cell death and subsequent epileptogenesis that develops eventually into pharmaco-resistant epilepsy. In turn, intractable epilepsy is itself known to cause neurodegeneration and cognitive deficits (7). In view of the postulated role for βOHB in seizure control and its reported neuroprotective properties, we have studied whether KTX 0101 (sodium βOHB) was neuroprotective in an *in vitro* model of *status epilepticus* (33,38). Kainic acid is a glutamatergic neurotoxin that causes cell death through activation of AMPA and kainate receptors. Kainic acid induced *status epilepticus* leads to the development of repeated spontaneous epileptiform-like discharges with a characteristic pattern of neuronal cell death in the hippocampus, similar to that observed clinically (6,39). In this *in vitro* model of *status epilepticus*, kainic acid (1 μM) was added to hippocampal organotypic slice cultures for 24 h. Cell survival in the CA1 region was determined by confocal microscopy assessment of propidium iodide uptake at 24 h and 7 days. Thirty minutes of *status epilepticus* is believed to demarcate the transition point at which seizure-induced neuronal injury takes place in patients (55). Therefore, KTX 0101 was administered to the cells 30 min after kainic acid. The concentrations of KTX 0101 employed, i.e., 0.3, 1, or 3 mM, were selected based on the finding that seizure control was observed in patients with blood concentrations of βOHB <6 mM (81). Addition of KTX 0101 to the culture medium produced a concentration-dependent reduction in neuronal cell death at 24 h (Fig. 8A). Although the percentages of neuronal cell loss in the presence of KTX 0101 were not significantly lower than those obtained in the kainic acid-treated control cultures, they were also not significantly higher than in the untreated controls. However, incubation with KTX 0101 did evoke significant reductions in neuronal cell loss 7 days after the induction of kainic acid-induced *status epilepticus* (Figs. 8B, 9). Neuroprotection was maximal at all concentrations of KTX 0101, which reduced cell death to the control levels.

Differences in the ability of KTX 0101 to inhibit cell death 24 h and 7 days after the induction of *status epilepticus* can be attributed to the prevention of neuronal cell death caused by two distinct cellular mechanisms, ie necrosis and apoptosis. Both apoptotic and necrotic-mediated cell death have been extensively reported after kainic acid application *in vivo* and *in vitro* (14,26,65). Systemic injection of kainic acid to rats to induce *status epilepticus* caused DNA fragmentation (an indicator of apoptosis) in the hippocampus as early as 8 h and it reached a maximum at 48 h (44). Necrosis, however, is detected on a

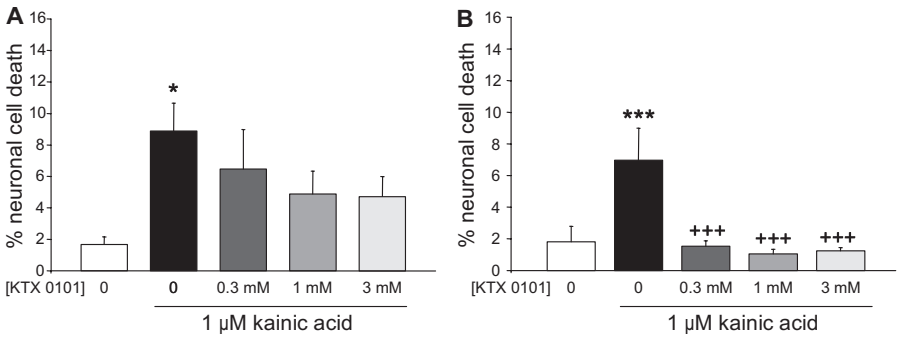

Fig. 8. KTX 0101 prevents immediate and delayed hippocampal neuronal cell damage in a model of *status epilepticus*. Hippocampal organotypic cultures were incubated with kainic acid (1 μM) for 24 h and KTX 0101 for either (A) 24 h or (B) 7 days. $^{*}P < 0.01$, $^{***}P < 0.001$ vs. control, $^{+++}P < 0.001$ vs. kainic acid alone (n = 12–13). Data from ref. 33.

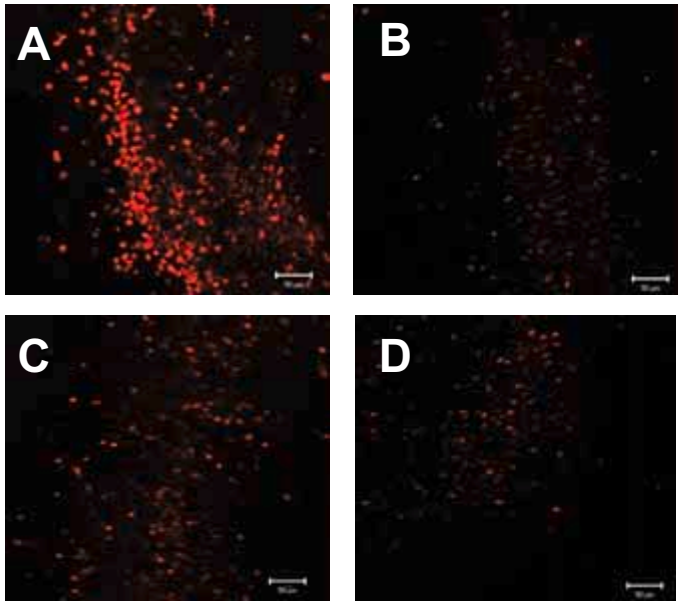

Fig. 9. Neuroprotection by KTX 0101 against kainic acid-induced excitotoxicity. Propidium iodide uptake into hippocampal CA1 region in organotypic cultures. (A) kainic acid treated cultures 7 days post treatment (1 mM for 24 h); (B) kainic acid (7 days post treatment) + KTX 0101 (0.3 mM for 7 days); (C) kainic acid + KTX 0101 (1.0 mM for 7 days); (D) kainic acid and KTX 0101 (3.0 mM for 7 days). Data from ref. 33.

different temporal scale than apoptosis. Acidophilic neurons, characteristic of necrosis were detected as early as 40 min after *status epilepticus* induced by kainic acid injections in rats (26). Thus, the neuroprotective benefit of KTX 0101 in this kainic acid-induced *status epilepticus in vitro* is likely to have been the result of a reduction in apoptosis as well as necrosis (33).

NEUROPROTECTION DURING EXCITOTOXICITY

Excitotoxicity-induced neurodegeneration has been widely implicated in ischemia, epilepsy, and Alzheimer's disease.

In vitro evidence of neuroprotection

In a model of glutamate-mediated excitotoxicity, Massieu et al. (57) investigated the ability of another ketone body, acetoacetate, to protect hippocampal cells, after acute and chronic treatment with the glycolysis inhibitor, iodoacetate. Primary cultures of hippocampal neurons taken from 17–18 day old embryos were exposed to 50 μM iodoacetate for 0.5 h. Following this treatment, 5 mM acetoacetate was added to the cultures for the next 24 h. Measurements of cell viability by immunohistochemistry using anti-microtubule associated protein (MAP-2) and anti-glial fibrillary acidic protein (GFAP) antibodies showed that the addition of 5 mM acetoacetate significantly increased the number of surviving neurons (57).

In vivo evidence of neuroprotection

Massieu and co-workers (57) have also shown that acetoacetate reduces excitotoxic brain damage *in vivo*. Rats were peripherally administered iodoacetate (15 mg/kg/day) for 3 days, followed by 500 nmol of the glutamate transporter blocker, L-trans-pyrrolydine-2,4-dicarboxylate (PDC), given directly into the hippocampus. The protective effect of acetoacetate was evaluated both acutely and chronically. All animals were killed 24 h after the last intervention and neuronal damage in the CA1 hippocampal layer was defined by standard histological staining. Administrations of PDC + iodoacetate induced damage in the CA1 hippocampal layer compared with PDC given alone. Treatment with acetoactetate attenuated this damage irrespective of the route of administration, i.e., subcutaneously, intraperitoneally or intravenously (Fig. 10). Determination of blood levels showed that acetoacetate was increased 2.5-fold compared with control values 30 min after intravenous infusion of 100 μmol of this ketone (57).

NEUROPROTECTION IN CHRONIC NEUROLOGICAL DISORDERS

Parkinson's Disease

Parkinson's disease is a progressive neurodegenerative disorder resulting from the death of cells containing the neurotransmitter, dopamine, in specific regions of the brain, i.e., the substantia nigra and nigrostriatal neuronal pathways, responsible for the fine control of movement. The loss of dopaminergic function results in a motor syndrome of bradykinesia (slow movements), dyskinesia (abnormal movements), akinesia (rigidity), resting tremor and postural instability. Frequently, the disease also causes depression, dementia, personality changes and speech deficits. The symptoms of Parkinson's disease

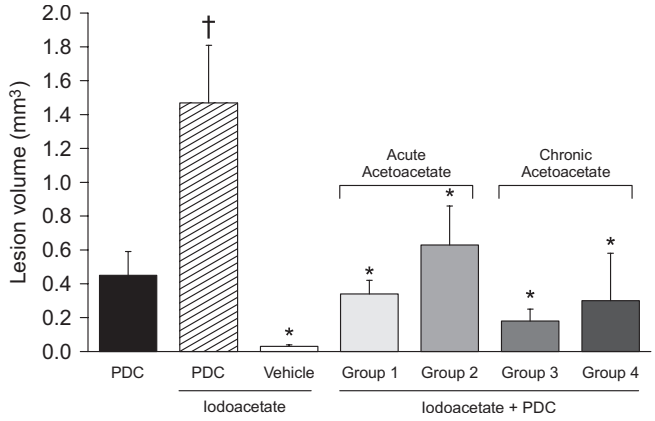

Fig. 10. Protection by acetoacetate against excitotoxic damage in the CA1 hippocampal cell layer. Rats were given iodoacetate systemically (15 mg/kg/day) for 3 days, then 500 nmol PDC (i.c.v.). Various doses of acetoacetate were given by different routes of administration (Group 1, i.v.; Groups 2 + 4, i.p.; Group 3, s.c.). Animals were killed 24 h after the last intervention. Neuronal damage in the CA1 hippocampal region was determined histologically. $^{\dagger}P < 0.05$ compared with PDC group, $*P < 0.05$ compared with PDC + iodoacetate group (PDC and PDC + iodoacetate groups, $n = 12–15$; all other groups, $n = 5–6$). Data from ref. 57.

gradually become more severe with time leading to almost total motor incapacity. The disease is slightly more prevalent in men than women and the average age of onset is around 60. There are currently 1 to 1.5 million Americans with Parkinson's disease and tens of millions of sufferers worldwide. With all developed countries having an increasingly aged population, an important fact is that 1 to 2% of people over the age of 65 years will become victims of Parkinson's disease.

Although drugs or other therapeutic interventions to delay or halt the progression of Parkinson's disease are needed, for more than 50 years drug therapy has focused on the symptomatic relief of the disease. These symptomatic relief drugs include levodopa (the metabolic precursor of dopamine), selective dopamine receptor subtype agonists, e.g., ropinerole, and inhibitors of monoamine oxidase type B, e.g., seligiline, or catechol-*O*-methyltransferase, e.g., entacapone. They provide help, but their medium to long-term use is associated with severe side effects, i.e., "on-off" periods, dyskinesias and drug-induced psychosis. These drugs do not halt or appreciably slow the progression of Parkinson's disease and it is a matter of some concern that levodopa therapy is believed to exacerbate dopamine cell loss through free radical formation (15,71).

Recently, impaired mitochondrial function due to inhibition of the pyruvate dehydrogenase complex (50) and an alteration in the antioxidant protection system, most notably reduced levels of copper/zinc dependent superoxide dismutase (SOD-1) (47), has been postulated to be an important factor contributing to the loss of dopamine-containing neurons in the substantia nigra (36,41). A reduction in the function of pyruvate dehydrogenase has important consequences for the viability of neuronal cells in the brain, which under normal metabolic conditions are totally dependent on glucose for their energy production. First, there is a lowered efficiency of energy production, a decrease in both acetyl coenzyme A (acetyl CoA) and the metabolites of the first third of the TCA cycle, and also a deficiency in mitochondrial NADH (66). This decrease in the energy of ATP hydrolysis

leads to a second phase when intracellular Na^+ and Ca^{2+} increase and there is a loss of cellular K^+ that leads ultimately to cell death. In view of the evidence above that βOHB and other ketone bodies can maintain ATP production in cells under conditions of poor oxidative function, and in addition, are neuroprotective, there is a strong scientific rationale to investigate the ability of KTX 0101 (sodium βOHB) to provide symptomatic relief and to slow the progression of dopaminergic neurodegeneration in Parkinson's disease.

In vitro evidence of neuroprotection

KTX 0101 has been shown to protect mesencephalic neurons *in vitro* against the neurotoxic actions of the dopamine neurotoxin, MPP^+ (43). Cells from 14-day-old embryonic rat brains were cultured with 4 mM KTX 0101 for 48 h before addition of 1–10 μM MPP^+. After a further 48 h, neuronal survival was determined by immunostaining with anti-tyrosine hydroxylase (TH) antibody. Incubation with MPP^+ reduced mesencephalic cell count of TH-positive cells at all concentrations (Fig. 11). Preincubation with 4 mM KTX 0101 improved the survival of TH-positive cells at all concentrations of MPP^+ (Fig. 11). Ketone addition to the culture medium also reversed the effect of MPP^+ to decrease the outgrowth of neurites (Fig. 11).

A further study by this group (37) demonstrated the neuroprotective effect of KTX 0101 against the dopaminergic neurotoxins, rotenone and MPP^+, in differentiated dopaminergic neuroblastoma cells. Addition of 4 or 8 mM KTX0101 to the cell culture medium significantly reduced cell death at every dose of rotenone (60–500 nM) or MPP^+ (0.5–3 mM) used. This was found to be the case when cell viability was assessed using three different methods, i.e., histological staining, MTT and lactate dehydrogenase-cytotoxicity assays.

In vivo evidence of neuroprotection

The above described *in vitro* findings have been extended by the recent reports that constant subcutaneous infusion of sodium βOHB (generic KTX 0101; 0.4, 0.8, or 1.6 mmol/kg/day) to mice prevents motor deficits and protects dopaminergic neurons in the substantia nigra from destruction by the MPP^+ precursor, MPTP (18 mg/kg i.p. × 4) (77). Sodium βOHB was given 24 h before the MPTP injections and was continued for a further 7 days. The 1.6 mmol/kg/day dose provided a stable plasma level of ~0.9 mM. At the end of the experiment, brains were examined for anti-TH immunoreactivity. The number of TH-positive cells was counted in the substantia nigra and TH density was determined in the striatum as indices of the viability of dopaminergic innervation of this area. Animals treated with the highest dose of sodium βOHB had an increased number of nigral TH-positive staining neurons, indicating increased cell viability (Table 2); this effect was complemented in the striatum by an increased density of TH-positive staining (Table 2).

In addition to measuring dopaminergic neuroprotection immunohistochemically, animals were also assessed for motor performance after MPTP administration (77). Seven days after MPTP or saline injections, the subcutaneously implanted osmotic minipumps (delivering 1.6 mmol/kg/day sodium βOHB or vehicle) were removed. The mice were allowed to recover for a further 7 days before being assessed on rotarods. Marked motor deficits were observed in the MPTP-treated animals. Sodium βOHB alone did not affect performance in saline-injected mice, but significantly attenuated the MPTP induced motor

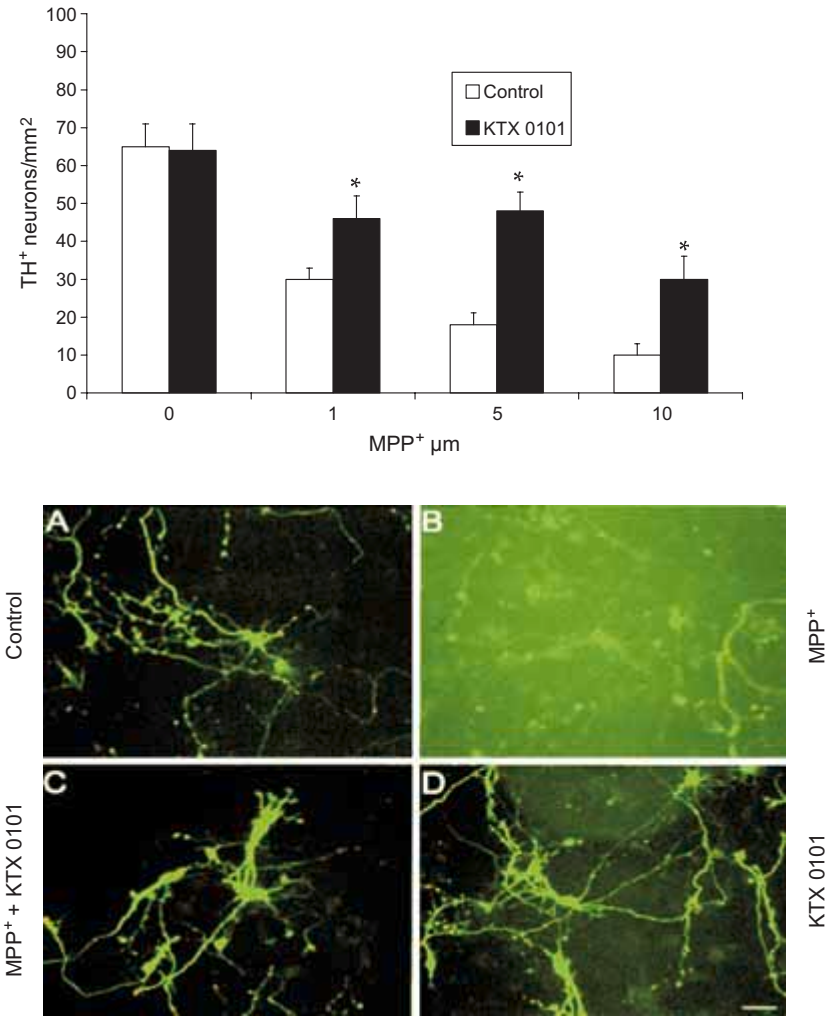

Fig. 11. KTX 0101 protects mesencephalic neurons from MPP^+-induced damage. Mesencephalic cells from 14-day-old embryonic rat brains were cultured for 48 h with 4 mM KTX 0101 before addition of 5 µm MPP^+ for a further 48 h. Cell viability was determined by anti-TH antibody staining on day 7. $*P < 0.05$ compared with corresponding Control group ($n = 20$). Scale bar is 20 µM. Reproduced with permission from: Kashiwaya Y, Takeshima N, Mori N, Nakashima K, Clarke K, Veech RL. D-β-hydroxybutyrate protects neurons in models of Alzheimer's desease. *Proc Natl Acad Sci* 2000;97:5440–5444. Copyright: National Academy of Sciences, 2000.

deficit (77). HPLC measurements showed that sodium βOHB also preserved midbrain and striatal levels of dopamine in the MPTP treated animals when they were killed immediately after the behavioral test (77). Consistent with the hypothesis that βOHB is a metabolic fuel that is able to maintain the viability of neuronal cells in situations of oxidative stress, these investigators have demonstrated that the neuroprotective mechanism of sodium βOHB is almost certainly mediated via improved efficiency of mitochondrial respiration. The rationale for this hypothesis is that MPTP evokes dopaminergic cell loss by

TABLE 2. Sodium D-β-hydroxybutyrate (βOHB) increases the number of tyrosine hydroxylase (TH)-positive cells in the substantia nigra and striatal TH density after MPTP injection in mice

Treatment	Substantia nigra (TH + ve cells)	Striatal TH density (OD × 100)
Vehicle + Vehicle	9700 ± 694	21.8 ± 1.9
NaβOHB + Vehicle	9293 ± 590	23.8 ± 2.1
Vehicle + MPTP	3223 ± 280	1.6 ± 0.2
NaβOHB + MPTP	6300 ± 506**	3.7 ± 0.1*

Note. Sodium βOHB was infused at 1.6 mmol/kg/day (s.c.) for 8 days, starting 24 h before MPTP (18 mg/kg × 4 i.p.). TH was measured immunohistochemically in the brain sub-regions at the end of the treatment period. *$P < 0.05$, **$P < 0.01$ compared with the vehicle + MPTP-treated group (n = 6–9). Data from ref. 77.

blocking Complex I (NADH ubiquinone oxidoreductase) of the mitochondrial electron transport chain (60).

Clinical evidence of therapeutic utility

Recent clinical evidence to substantiate the value of βOHB in the treatment of Parkinson's disease has come from Van Itallie and co-workers (78). These researchers placed 5 patients with Parkinson's disease onto a very strict ketogenic diet to raise their plasma ketone levels to 1–7 mM. After one month, their Unified Parkinson's Disease Rating Scale (UPDRS) disability scores showed a mean 43% reduction and the patients claimed a "moderate" to "very good" improvement in their disorder. These authors ascribed the beneficial effects to elevated blood βOHB concentrations.

Alzheimer's Disease

Alzheimer's disease is a progressive and ultimately fatal degenerative brain disorder that primarily affects the elderly. It is the most common cause of dementia and loss of cognitive function in this population. Alzheimer's disease is characterized by β-amyloid neuritic plaques and neurofibrillary tangles in the brain, associated with progressive neuronal loss and atrophy (69). More precisely, a proteolytic fragment ($A\beta_{1-42}$) of β-amyloid is implicated in producing these plaques (32). Factors that have been proposed to cause β-amyloid accumulation include abnormalities of $A\beta_{1-42}$ metabolism (68), brain trauma (29), ischemia (42), insulin resistance (48) or impaired brain energy metabolism (27,52). Symptoms found in patients with Alzheimer's disease include gradual memory loss, diminished ability to perform routine tasks, disorientation regarding time and place, learning difficulties, loss of language skills, impaired judgement, rapid mood swings and personality changes. Ultimately, most patients become bedridden, incontinent, totally helpless and unresponsive to the outside world, requiring total care either in a nursing home or the home setting. Alzheimer's disease invariably results in the death of the patient, frequently from pneumonia (82). The time from onset of symptoms to death ranges from 3 to 10 years (11,86). It is estimated that there are 15 million adults with Alzheimer's disease worldwide, including 5 million in the USA alone. The incidence of Alzheimer's disease is

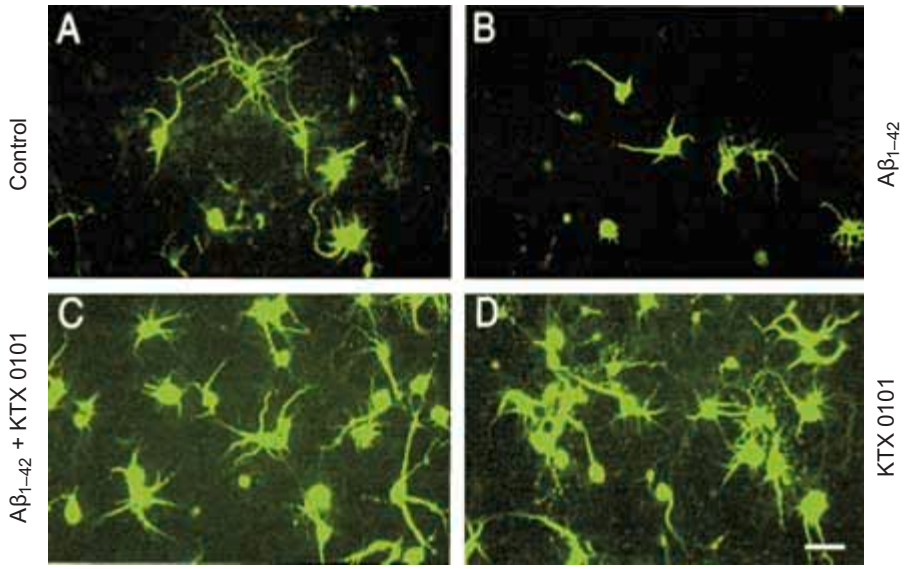

Fig. 12. KTX 0101 prevents β-amyloid ($A\beta_{1-42}$)-induced cell death in hippocampal rat neurons. Day-6 cultures of 18-day-old embryonic rat hippocampal tissue after exposure to 5 μM $A\beta_{1-42}$ + 4 mM KTX 0101 for 14 h. Reproduced with permission from: Kashiwaya Y, Takeshima N, Mori N, Nakashima K, Clarke K, Veech RL. D-β-hydroxybutyrate protects neurons in models of Alzheimer's desease. *Proc Natl Acad Sci* 2000;97: 5440–5444. Copyright: National Academy of Sciences, 2000.

predicted to rise as the population ages: the prevalence increases from 2.5% in those aged 65 years, to 47% in those over 85 years (23).

The observation that cholinergic markers and the number of cholinergic neurons were markedly reduced in the brains of patients with Alzheimer's Disease (10,59,70) led to the hypothesis that a deficiency in central cholinergic neuronal function was responsible for this disorder (16,18). Neurotransmitter replacement therapy (i.e., inhibition of acetylcholine breakdown) has been the main approach to the treatment of Alzheimer's disease for the past 20 years. This therapeutic approach is, however, inadequate for three reasons. First, current drugs provide only a very moderate level of symptomatic relief; second, these drugs are not effective in the later stages of the disease; and third, no current therapy halts or even delays the progression of Alzheimer's disease.

In vitro evidence of neuroprotection

Kashiwaya et al. (43) determined whether KTX 0101 (sodium βOHB) would protect hippocampal neurons *in vitro* against the toxic effects of the proteolytic fragment of β-amyloid, $A\beta_{1-42}$. Hippocampal cells from 18 day-old rat embryos were incubated with 4 mM KTX 0101 for 12 h before addition of 5 μM $A\beta_{1-42}$ and the cells were then cultured for a further 14 h. At the end of the experiment, immunohistochemistry was performed with anti-MAP-2 antibody to determine preservation of neuronal integrity. Exposure of the cells to the toxin, $A\beta_{1-42}$, increased cell mortality, and decreased neurite number and length compared with those in the control culture medium (Fig. 12). The presence of KTX 0101 doubled the surviving cell number and, in addition, increased cell size and

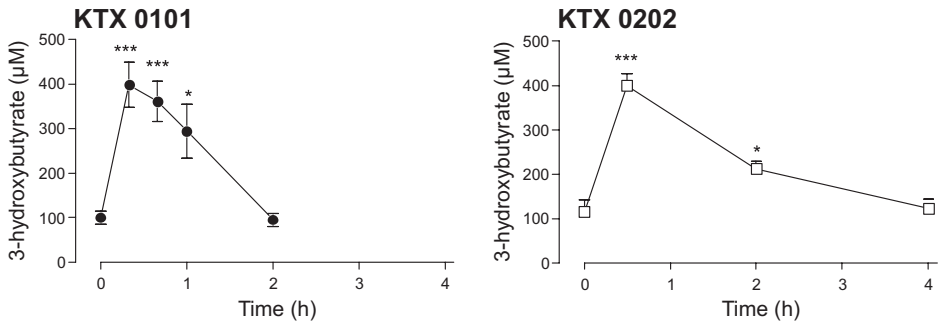

Fig. 13. The improved oral bioavailability of KTX 0202 compared with KTX 0101. Rats were dosed orally with 30 mg/kg KTX 0101 or KTX 0202 and plasma was taken by cardiac puncture. Levels of plasma βOHB were measured spectrophotometrically. *$p < 0.05$, ***$p < 0.001$ compared with baseline control ($n = 4$).

neurite outgrowth in comparison to those exposed to $A\beta_{1-42}$ alone (Fig. 12). Furthermore, exposure of cells to 4 mM βOHB alone for 14 h also increased surviving cells and neurite number compared to the control cells (Fig. 12).

Developing Potential Ketone Body Approaches for Chronic Neurological Disorders

The scientific evidence presented above demonstrates a clear rationale to indicate that KTX 0101 may be therapeutically beneficial in neurological disorders, like Parkinson's disease, Alzheimer's disease and epilepsy. Not only could this agent provide symptomatic relief, but its powerful neuroprotective properties suggest it also has the potential to be a 'disease modifier,' slowing or perhaps even halting the progression of these neurodegenerative disorders. Whilst this evaluation is very positive, there are key features of KTX 0101 that will preclude its use in the treatment of chronic neurological disorders. First, ketone bodies are very rapidly metabolized in man and have to be administered in relatively large quantities to achieve and maintain therapeutically relevant plasma concentrations of 1–3 mM βOHB. Whilst this is readily achievable in the surgical setting with an intravenous infusion, this method of delivery is obviously inappropriate for chronic therapy. Second, KTX 0101 is orally bioavailable, but even setting aside the shortcoming of its rapid utilization as a metabolic fuel with a resulting short biological half-life, KTX 0101 would still not be suitable for long-term use because of the sodium load associated with ingestion of large quantities of this compound. To address the twin issues of sodium load and rapid metabolism of KTX 0101, KetoCytonyx has synthesized a range of oligomers and derivatives of βOHB potentially for use as oral medications for the chronic conditions of epilepsy, Parkinson's and Alzheimer's diseases. Although this research is still at an early stage of development, various orally bioavailable NCE's have already been synthesized. Of these compounds, several have an oral bioavailability in rats that is at least equivalent to KTX 0101 and some, e.g., KTX 0202, have biological half-lives that are 2 to 3-fold longer than that of KTX 0101 (Fig. 13). A nutritionally suitable form of these oligomers would be ingested by patients without the unpalatability and induction of hypercholesterolemia associated with the ketogenic diet. βOHB is not used by the liver and,

therefore, cannot be used as a substrate for lipid synthesis. The only possible effect of long-term ingestion of βOHB oligomers is the competition of βOHB with long-chain fatty acids, mainly in muscle, for peripheral metabolism. However, it is known that the Inuit people of North America eat a diet containing large quantities of oily fish that leads to constantly elevated plasma levels of βOHB, without ill effect. The major problem for Western society is the consumption of ketogenic bars in addition to the required daily caloric intake. The consequence is obesity with associated comorbidities.

TOXICOLOGICAL EVALUATION OF KTX 0101

KTX 0101 (sodium βOHB) is a white or faintly yellow odorless powder. The purity of the various batches of KTX 0101 used in toxicological testing was within the range 97.9–100% with excellent chemical stability for the duration of the study period. The only identified impurity, crotonic acid, was always within the specification of <0.3%. All testing was conducted using intravenous KTX 0101 administration because it is the intended route for clinical administration. KTX 0101 was administered intravenously in a dose volume of 20 mL/kg at an infusion rate of 2 mL/min for rats, 4 mL/min for rabbits and 5 mL/min for dogs. Different dose levels were achieved by varying the concentration of KTX 0101 in the formulation. Since the osmolality of plasma is ~300 mOsm/L, all of the solutions employed were mildly to markedly hypertonic (Table 3).

In single dose tolerability studies, KTX 0101 was tested at doses ≤3800 mg/kg in rats and ≤4000 mg/kg in dogs. KTX 0101 produced no overt signs in rats at doses ≤2000 mg/kg in females and ≤3000 mg/kg in males. One of five females in each group died at 2400 and 3000 mg/kg and 2/5 at 3800 mg/kg; in males, 2/5 also died at this top dose. The females that died shortly after dosing were lying prone and manifesting tachypnea, whilst the males survived longer having previously displayed decreases in motor activity and respiratory rate, clonic convulsions, prostrate posture and some self-soiling. In the surviving animals at these doses, all symptoms rapidly reversed and had normalized within 6 h. In 2/2 females and 1/2 males that died, cerebral hemorrhage was noted at *post mortem* and, at the highest doses of KTX 0101, lesions of the kidneys were also observed in some animals. These pathological findings were considered to be a direct consequence of the volume and nature of the solution used to inject KTX 0101, rather than toxic effects from KTX 0101 itself. No other significant findings were reported. In dogs, no deaths oc-

TABLE 3. Osmolality of the KTX 0101 formulations

KTX 0101 dose (mg/kg i.v.)	Concentration (%)	Osmotic pressure (mOsm/L)
500	2.5	365
1000	5	765
2000	10	1510
3000	15	2295
4000	20	3120

curred at any dose. At 2000 mg/kg, increased micturation and drinking were observed; at 3000 mg/kg, these effects were accompanied by dryness of the nasal speculum, attenuated pupillary reflex and salivation. The highest dose of KTX 0101 produced staggering, tachypnea, tachycardia, hyperthermia and hindlimb extension. In all animals, the symptoms disappeared within 24 h. No abnormalities were observed at autopsy, except reduced spleen weight at the highest dose that may not have been treatment related. From these studies, the maximum tolerated intravenous dose for repeated administration was 2000 mg/kg/day to rats or dogs.

Four-week, daily intravenous administration studies were performed in rats and dogs at 500, 1000, and 2000 mg/kg/day. In rats, no effects were observed at doses ≤1000 mg/kg/day. At the highest dose, decreases in red cell count, hemoglobin and hematocrit values were found. Spleen weight was increased and histopathological examination revealed exacerbated, extramedullary, erythrocytic hematopoiesis; these changes reversed on withdrawal. Similarly, at doses ≤1000 mg/kg/day KTX 0101 produced no effects in dogs. However, the highest dose induced vomiting, salivation, decreased motor activity and reddish urine; these effects were transiently observed during and immediately subsequent to dosing. There were no changes of body weight, food consumption, hematology, or blood chemistry, and at autopsy, there were no alterations in organ weights or histopathological changes. The data from these chronic studies indicate that KTX 0101 is safe and well tolerated when given in repeated doses ≤1000 mg/kg.

In genotoxicity testing, KTX 0101 showed no evidence of mutagenic potential in the bacterial mutation and mammalian chromosomal aberration assays *in vitro*, or in the mouse micronucleus assay *in vivo*.

A complete program of reproductive toxicology experiments was performed in female rabbits and male and female rats using intravenous doses of 500, 1000, and 2000 mg/kg/day. Two of 6 rabbits died at the highest dose of KTX 0101 indicating that an intravenous dose of 2000 mg/kg is close to the lethal limit in this species. No effects on reproductive function or fetal development were detected at any dose. In rats, KTX 0101 had no effect on male or female fertility and no effect on reproductive function or fetal development. Prolonged diestrus occurred in 3 female rats at the dose of 2000 mg/kg/day, but the incidence was too low to demonstrate unequivocally that KTX 0101 influences the estrus cycle.

Overall, these data demonstrate that KTX 0101 is safe. The osmotic load, sodium levels, volume load and tonicity of the solution are all considered to have contributed to the minor findings in the toxicological assessments. Theoretical risks of KTX 0101 administration to patients include (dose-dependently) acidosis, sodium and osmotic overloads with respective fluid shifts. This may be avoided by the close monitoring of blood pH and bicarbonate levels, sodium and potassium levels, arterial and jugular venous pressure, pulse, ECG, and respiration rate.

PHARMACOKINETICS OF KTX 0101

The pharmacokinetics and distribution of KTX 0101 (sodium β-hydroxybutyrate) have been investigated after single and multiple intravenous administration to rats, rabbits, and

dogs (Table 4). Endogenous levels of βOHB were approximately 1 mg/dL in rats, 1–2 mg/dL in rabbits, and usually below the detection limit of the assay in dogs. βOHB was rapidly cleared from the plasma in all three species. In rats, after intravenous administration of KTX 0101 at 30, 500, 1000, or 2000 mg/kg, plasma levels of βOHB returned to endogenous levels within 30 to 60 min. At a dose of 2000 mg/kg, βOHB was still detectable after 1 h, but not at the next sampling point of 24 h. The same dose given to dogs was not detectable after 3 h.

There was no evidence of any gender differences in the pharmacokinetics of KTX0101. There was also no evidence of accumulation or depletion of βOHB after repeated administration of KTX0101 for ≤1 month in rats or dogs or ≤13 days in female rabbits. The $C_{5\ min}$ and $AUC_{5\ min–24\ h}$ were approximately dose-proportional in all three species, except at the highest doses in dogs where the values were greater than dose-proportional.

The tissue distribution of βOHB was studied using $CH_3[^{14}C]H(OH)CH_2COONa$ ([^{14}C]KTX 0101) with a radiochemical purity of >99%. In male rats, plasma levels of radioactivity declined rapidly in a biphasic manner with a half-life of 32 min for <2 h, and 37 h from 2–120 h after administration of single doses of 10, 30, or 100 mg/kg or an infusion of 30 mg/kg/h for 1 h. βOHB-related material was widely distributed in the tissues of rats after intravenous administration of 30 mg/kg [^{14}C]KTX 0101. Five min after dosing, high levels of radioactivity were present in blood, skeletal muscle, skin, urine, liver, Harderian gland, parotid salivary gland, tongue, and kidneys. Low radioactivity was found in the brain, eyes, thyroid gland, epididymis and prostate gland. Concentrations of [^{14}C]KTX 0101 decreased over a period of 24–120 h when radioactivity was still present in the nasal cavity, Harderian gland, adrenal gland, spinal cord, skeletal muscle and skin. The level in the fat increased during this time, probably due to *de novo* synthetic incorporation of the radiolabel into fat constituents. There was no evidence of [^{14}C]KTX 0101 binding to plasma proteins with rat, dog, or human plasma *in vitro*.

After intravenous administration to male rats of [^{14}C]KTX 0101 (10, 30, or 100 mg/kg bolus doses or 30 mg/kg given as a continuous infusion over 1 h), 168 h after dosing,

TABLE 4. Summary of pharmacokinetic data for KTX 0101

Species/sex	Dose (mg/kg/day i.v.)	$C_{5\ min}$ (mg/dL)	$AUC_{5\ min–24\ h}$ (mg/h/dL)	$t_{1/2}$
Rat (m)/acute	30	2.5*	0.43*	5
Rat (m/f)/1 d and 28 d	500	49–62	24–42	N.D.
	1000	132–158	55–64	N.D.
	2000	306–335	147–225	N.D.
Dog (m)/acute	30	4.8*	0.68*	5
Dog (m/f)/1 d and 28 d	500	39–42	10–17	8–20
	1000	122–138	70–82	16–23
	2000	292–374	291–319	23–28
Rabbit (f)/6 d and 18 d of gestation	500	49–68	19–25**	10–30
	1000	141–174	69–82**	15–23
	2000	314–457	293–356**	20–23

Note. N.D., not determined; *, $C_{2\ min}$, *, $AUC_{-\infty}$; **, $AUC_{-3\ h}$.

2.7–6.0% of the radiolabel had been excreted in the urine, 1.2–1.8% in the feces, but the overwhelming majority of the radioactivity, i.e., 81.0–89.2%, was eliminated as radioactive carbon dioxide. This observation is consistent with the mitochondrial metabolism of βOHB in the TCA cycle to convert this energy source ultimately into carbon dioxide and water. The residual percentage of [^{14}C]KTX 0101 in the body was 6.7–9.0% at this time-point. A very small percentage of 0.7% was excreted into the bile of bile duct cannulated rats over a 48 h period.

The kinetics of βOHB has also been studied in humans. For example, Hetherington et al. (34) monitored sodium [^{13}C]βOHB utilization in normal human brain after intravenous injection during acute hyperketonemia. They employed magnetic resonance spectroscopy to demonstrate that, at a plasma concentration of βOHB of 2.25 ± 0.24 mmol/L, the apparent brain tissue concentration was 0.18 ± 0.06 mmol/L during the final 20 min of the 2-h study. βOHB was oxidized primarily in the neuronal pool, metabolized at a rate of 0.03 ± 0.01 mmol/kg/min and it accounted for 6.4 ± 1.6% of total coenzyme A oxidation. An earlier study by Hall et al. (31) measured the post-absorptive plasma concentrations and the kinetics of βOHB and acetoacetate in 11 subjects after administration of [^{14}C]βOHB and [^{14}C]acetoacetate. The mean plasma ketone levels were 0.07 ± 0.04 mmol/L for βOHB and 0.05 ± 0.02 mmol/L for acetoacetate and the mean rates of ketone release and removal were ~110.7 ± 105.9 μmol/min/m^2. Turnover time in the blood for these molecules was about 2 min (84). These results are, therefore, consistent with rapid turnover of ketones in other tissues in humans (79).

PHASE I CLINICAL EVALUATION OF KTX 0101

Although a final report is not available at this time, KetoCytonyx recently provided a press release announcing the preliminary findings from their Phase I clinical trial with KTX 0101. The compound was tested in 20 volunteers in whom it was well tolerated with no incidence of serious adverse events. The pharmacokinetics of the drug in man was as predicted from the animal studies. EEG was employed as the marker of the central actions of KTX 0101. This compound produced a change in the EEG spectrum that was similar to the one evoked by hypothermia. The latter is widely reported to provide neuroprotection against ischemic damage in man providing additional evidence to support the hypothesis that KTX 0101 has neuroprotective properties.

SUMMARY

There is a dearth of new treatments, not only to prevent cognitive impairments due to multiple, small ischemic foci in the brain caused by major surgery, but also for more chronic disorders including epilepsy, Parkinson's and Alzheimer's diseases. New therapies will need to provide long-term symptomatic relief with a reduced burden of serious side effects and adverse events, but they should also deliver some degree of neuroprotection to delay the progression of these diseases.

As evidenced by the data presented in this review, there is good scientific rationale to support the view that increasing metabolic efficiency may be a viable approach to reduce or prevent deficits in a wide range of neurological disorders. KTX 0101 (or generic sodium βOHB) has been shown to have cytoprotective effects in models of *status epilepticus*, excitotoxicity, hypoxia, cardiac and cerebral ischemia/reperfusion injury, lung hemorrhage, and also in models of chronic neurological disorders, e.g., Parkinson's disease.

Overall, the findings reviewed here indicate that KTX 0101, administered intravenously before and during surgery, has the potential to provide clinically relevant neuroprotection against ischemia caused by CPB and other types of major surgical procedures. Although KTX 0101 is unsuitable for long-term therapy in epilepsy, Parkinson's and Alzheimer's diseases, because it has to be administered in large amounts and is very rapidly metabolized, these limitations are being addressed by KetoCytonyx through the design and synthesis of multimers of βOHB and other βOHB derivatives. These orally bioavailable compounds are being developed to yield therapeutically relevant plasma levels of βOHB over a much longer period than KTX 0101 in order to permit a twice or thrice daily dosing regime.

Acknowledgments. The authors would like to thank Dr. Liz Jagger for performing the *in vitro status epilepticus* experiments. We are also grateful to Ms. Jane Gosden for preparing all of the figures and tables for the manuscript.

Addendum. Abbreviations

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate;
βOHB, D-β-hydroxybutyrate;
CABG, coronary artery bypass graft;
CPB, cardiopulmonary bypass;
GFAP, glial fibrillary acidic protein;
KTX 0101, sodium D-β-hydroxybutyrate;
MAP-2, microtubule-associated protein-2;
MPP^+, 1-methyl-4-phenylpyridinium;
MPTP, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine;
MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide);
NCE, new chemical entities;
PARP, poly (ADP-ribose) polymerase;
PDC, L-trans-pyrrolydine-2,4-dicarboxylate;
SOD-1, copper/zinc dependent superoxide dismutase;
TCA, tricarboxylic acid;
TH, tyrosine hydroxylase;
TTC, triphenyltetrazolium chloride;
UPDRS, Unified Parkinson's Disease Rating Scale.

REFERENCES

1. Aitken SP, Brown WM. Treatment of epilepsy: Existing therapies and future developments. *Front Biosci* 2000;5:E124-E152.
2. Alam HB, Austin B, Koustova E, Rhee P. Resuscitation-induced pulmonary apoptosis and intracellular adhesion molecule-1 expression in rats are attenuated by the use of ketone ringer's solution. *J Am Coll Surg* 2001;193:255–263.
3. Al-Mudallal AS, LaManna JC, Lust WD, Harik SI. Diet-induced ketosis does not cause cerebral acidosis. *Epilepsia* 1996;37:258–61.
4. Arrowsmith JE, Harrison MJ, Newman SP, Stygall J, Timberlake N, Pugsley WB. Neuroprotection of the brain during cardiopulmonary bypass: A randomized trial of remacemide during coronary artery bypass in 171 patients. *Stroke* 1998;29:2357–2362.
5. Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF. Synaptic reorganisation by mossy fibers in human epileptic fascia-dentata. *Neuroscience* 1991;42:351–363.
6. Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* 1985;14:375–403.
7. Bernasconi N, Bernasconi A, Caramanos Z, et al. Entorhinal cortex atrophy in epilepsy patients exhibiting normal hippocampal volumes. *Neurology* 2001;56:1335–1339.
8. Bough KJ, Eagles DA. A ketogenic diet increases the resistance to pentylenetetrazole-induced seizures in the rat. *Epilepsia* 1999;40:138–143.
9. Bough KJ, Valiyil R, Han FT, Eagles DA. Seizure resistance is dependent upon age and calorie restriction in rats fed a ketogenic diet. *Epilepsy Res* 1999;35:21–28.
10. Bowen DM, Davison AN. The failing brain. *J Chronic Dis* 1983;36:3–13.
11. Brookmeyer R, Corrada MM, Curriero FC, Kawas C. Survival following a diagnosis of Alzheimer disease. *Arch Neurol* 2002;59:1764–1767.
12. Cahill GF, Aoki TT. Alternate fuel utilization in brain. In: Passonneau JV, Hawkins RA, Lust WD, Welsh FA, Eds. Cerebral Metabolism and Neural Function. Baltimore: Williams and Wilkins, 1980;234–242.
13. Cahill GF (Jr), Veech RL. Ketoacids? Good medicine? *Trans Am Clin Climatol Assoc* 2003;114:149–161; discussion: 162–163.
14. Cheung N, Pascoe CJ, Giardina SF, John CA, Beart, PM. Micromolar L-glutamate induces extensive apoptosis in an apoptotic-necrotic continuum of insult dependent, excitotoxic injury in cultured cortical neurones. *Neuropharmacology* 1998;37:1419–1429.
15. Cohen G. Oxidative stress, mitochondrial respiration, and PD. *Ann NY Acad Sci* 2000;899:112–120.
16. Coyle JT, Price DL, DeLong MR. Alzheimer's disease: A disorder of cortical cholinergic innervation. *Science* 1983;219:1184–1190.
17. Dardzinski BJ, Smith SL, Towfighi J, Williams GD, Vannucci RC, Smith MB. Increased plasma beta-hydroxybutyrate, preserved cerebral energy metabolism and amelioration of brain damage during neonatal hypoxia ischemia with dexamethasone pretreatment. *Pediatric Res* 2000;48:248–255.
18. Davies P. The neurochemistry of Alzheimer's disease and senile dementia. *Med Res Rev* 1983;3:221–236.
19. DeVivo DC, Leckie MP, Ferrendelli JS, McDougal DB, Jr. Chronic ketosis and cerebral metabolism. *Ann Neurol* 1978;3:331–337.
20. Diegeler A, Hirsch R, Schneider F, et al. Neuromonitoring and neurocognitive outcome in off-pump versus conventional coronary bypass operation. *Ann Thorac Surg* 2000;69:1162–1166.
21. Dodson WE, Prensky AL, DeVivo DC, Goldring S, Dodge PR. Management of seizure disorders: Selected aspects. Part I. *J Pediatry* 1976;89:527–40.
22. Dodson WE, Prensky AL, DeVivo DC, Goldring S, Dodge PR. Management of seizure disorders: Selected aspects. Part II. *J Pediatry* 1976;89:695–703.
23. Evans DA, Funkenstein HH, Albert MS, et al. Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. *J Am Med Assoc* 1989;262:2551–2556.
24. Fery F, Balasse EO. Ketone body production and disposal in diabetic ketosis. A comparison with fasting ketosis. *Diabetes* 1985;34:326–332.
25. Freeman JM, Vining EPG, Pyzik PL, Gilbert DL. Beta-hydroxybutyrate levels in blood correlate with seizure control in children on the ketogenic diet. *Epilepsia* 1998;39:167–171.

26. Fujikawa DG, Shinmei SS, Cai BY. Kainic acid-induced seizures produce necrotic, not apoptotic, neurons with internucleosomal DNA cleavage: Implications for programmed cell death mechanisms. *Neuroscience* 2000;98:41–53.
27. Gabuzda D, Busciglio J, Chen LB, Matsudaira P, Yankner BA. Inhibition of energy metabolism alters the processing of amyloid precursor protein and induces a potentially amyloidogenic derivative. *J Biol Chem* 1994;269:13623–13628.
28. Gilman S. Cerebral disorders after open heart surgery. *N Engl J Med* 1965;272:489–498.
29. Graham DI, Gentleman SM, Nicoll JA, et al. Altered beta-APP metabolism after head injury and its relationship to the aetiology of Alzheimer's disease. *Acta Neurochir* 1996;66:96–102.
30. Grocott J, Phillips-Bute B, Newman M. Lidocaine does not prevent cognitive dysfunction after cardiac surgery. *Anesth Analg* 2004;98:SCA1–134.
31. Hall SEH, Wastney ME, Bolton TM, Braaten JT, Berman M. Ketone body kinetics in humans: The effects of insulin-dependent diabetes, obesity and starvation. *J Lipid Res* 1984;25:1184–1194.
32. Hardy JA, Higgins GA. Alzheimer's disease: The amyloid cascade hypothesis. *Science* 1992;256:184–185.
33. Heal DJ, Jagger L, Martin KF. KTX 0101 protects hippocampal neurones in an *in vitro* model of *status epilepticus*. 34th Annual Meeting of the Society for *Neuroscience*, San Diego, 2004; Abstract No. 2635.
34. Hetherington HP, Pan JW, Spencer DD. ^{1}H and ^{31}P spectroscopy and bioenergetics in the lateralization of seizures in temporal lobe epilepsy. *J Magn Reson Imaging* 2002;16:477–483.
35. Ide T, Steinke J, Cahill GF Jr. Metabolic interactions of glucose, lactate and β-hydroxybutyrate in rat brain slices. *Am J Physiol* 1969;217:784–791.
36. Ilic TV, Jovanovic M, Jovicic A, Tomovic M. Oxidative stress indicators are elevated in *de novo* PD patients. *Funct Neurol* 1999;14:141–147.
37. Imamura K, Takeshima T, Kashiwaya Y, Fukuhara Y, Nakaso K, Veech RL, Nakashima K. D-β-hydroxybutyrate (DBHB) protects SH-SY5Y dopaminergic neuroblastoma cells in the rotenone and MPP^{+} models of Parkinson' disease. 34th Annual Meeting of the Society for *Neuroscience*, San Diego, 2004; Abstract No. 1013.15.
38. Jagger L, Cheetham SC, Heal DJ, Mason R. Validation of an *in vitro* model of *status epilepticus*. *Br J Pharmacol* 2005; In press.
39. Jagger L, Parker TL, Starkey S and Mason RM. Kainic acid-induced epileptiform activity and neuronal cell death in hippocampal organotypic slices. *Br J Pharmacol* 2002;135:103P.
40. Janigro D. Blood-brain barrier, ion homeostatis and epilepsy: possible implications towards the understanding of ketogenic diet mechanisms. *Epilepsy Res* 1999;37:223–32.
41. Jenner P. Oxidative stress in PD. *Ann Neurol* 2003;53:S26–S36; discussion: S36–S38.
42. Kalaria RN, Bhatti SU, Lust WD, Perry G. The amyloid precursor protein in ischemic brain injury and chronic hypoperfusion. *Ann NY Acad Sci* 1993;695:190–193.
43. Kashiwaya Y, Takeshima T, Mori N, Nakashima K, Clarke K, Veech RL. D-β-Hydroxybutyrate protects neurons in models of Alzheimer's and PD. *Proc Natl Acad Sci USA* 2000;97:5440–5444.
44. Kondratyev A, Gale K. Temporal and spatial patterns of DNA fragmentation following focally or systemically-evoked *status epilepticus* in rats. *Neurosci Lett* 2001;310:13–16.
45. Kong RS, Butterworth J, Aveling W, et al. Clinical trial of the neuroprotectant clomethiazole in coronary artery bypass graft surgery: A randomized controlled trial. *Anesthesiology* 2002;97:585–591.
46. Koustova E, Rhee P, Hancock T, et al. Ketone and pyruvate Ringer's solutions decrease pulmonary apoptosis in a rat model of severe hemorrhagic shock and resuscitation. *Surgery* 2003;134:267–274.
47. Kunikowska G, Jenner P. Alterations in m-RNA expression for Cu, Zn-superoxide dismutase and glutathione peroxidase in the basal ganglia of MPTP-treated marmosets and patients with PD. *Brain Res* 2003;968: 206–218.
48. Kuusisto J, Koivisto K, Mykkanen L, et al. Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein E4 phenotype: Cross sectional population based study. *Br Med J* 1997;315:1045–1049.
49. Laffel L. Ketone bodies: A review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes Metab Res Rev* 1999;15:412–426.
50. Li H, Dryhurst G. Oxidative metabolites of 5-S-cysteinyldopamine inhibit the pyruvate dehydrogenase complex. *J Neural Transm* 2001;108:1363–1374.
51. Li PA, He QP, Csiszar K, Siesjo BK. Does long-term glucose infusion reduce brain damage after transient cerebral ischemia? *Brain Res* 2001;912:203–205

52. Lin L, Georgievska B, Mattsson A, Isacson O. Cognitive changes and modified processing of amyloid precursor protein in the cortical and hippocampal system after cholinergic synapse loss and muscarinic receptor activation. *Proc Natl Acad Sci USA* 1999;96:12108–12113.
53. Llinas R, Barbut D, Caplan LR. Neurologic complications of cardiac surgery. *Prog Cardiovasc Dis* 2000;43: 101–112.
54. Longa EZ, Weinstein PR, Carlson SC, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989;20:84–91.
55. Lowenstein DH. Status epilepticus: An overview of the clinical problem. *Epilepsia* 1999;40:S3–S8.
56. Mark DB, Newman MF. Protecting the brain in coronary artery bypass graft surgery. *J Am Med Assoc* 2002; 287:1448–1450.
57. Massieu L, Haces ML, Montiel T, Hernandez-Fonseca K. Acetoacetate protects hippocampal neurons against glutamate-mediated neuronal damage during glycolysis inhibition. *Neuroscience* 2003;120:365–378.
58. Masuda R, Monahan JW, Fukuhara Y, et al. Neuroprotective effect of D-β-hydroxybutyrate against hypoxia in serum free hippocampal primary culture. *J Neurosci Res* 2005; In press.
59. Nakamura S, Koshimura K, Kato T, et al. Neurotransmitters in dementia. *Clin Ther* 1984;7:18–34.
60. Nicklas WJ, Yougster SK, Kindt MV, Heikkila RE. MPTP, MPP^+ and mitochondrial function. *Life Sci* 1987;40:721–729.
61. Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF, Jr. Brain metabolism during fasting. *J Clin Invest* 1967;46:1589–1595.
62. Owen OE, Reichard GA Jr, Patel MS, Boden G. Energy metabolism in feasting and fasting. Adv Exp Med Biol 1979; 111:169–188.
63. Pan JW, Bebin EM, Chu WJ, Hetherington HP. Ketosis and epilepsy: ^{31}P spectroscopic imaging at 4.1 T. *Epilepsia* 1999;40:703–7.
64. Peterman MG. The ketogenic diet in the treatment of epilepsy. A preliminary report. *Am J Dis Child* 1924;28:28–33.
65. Pollard H, Charriaut-Marlangue C, Cantagrel S, et al. Kainate-induced apoptotic cell death in hippocampal neurons. *Neuroscience* 1994;63:7–18.
66. Sato KY, Kashiwaya CA, Keon N, et al. Insulin, ketone bodies, and mitochondrial energy transduction. *FASEB J* 1995;9:651–658.
67. Schwartz RH, Eaton J, Bower BD, Aynsley-Green A. Ketogenic diets in the treatment of epilepsy. Short-term clinical effects. *Dev Med Child Neurol* 1989;31:145–151.
68. Selkoe DJ. Alzheimer's disease: Genotypes, phenotypes, and treatments. *Science* 1997;275:630–631.
69. Selkoe DJ. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. *J Alzheimer's Dis* 2001;3:75–80.
70. Sims NR, Bowen DM, Smith CC, et al. Glucose metabolism and acetylcholine synthesis in relation to neuronal activity in Alzheimer's disease. *Lancet* 1980;1:333–336.
71. Simuni T, Stern MB. Does levodopa accelerate PD. *Drugs Aging* 1999;14:399–408.
72. Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol* 1989;26:321–330.
73. Suzuki M, Suzuki M, Kitamura Y, et al. Beta-hydroxybutyrate, a cerebral function improving agent, protects rat brain against ischemic damage caused by permanent and transient focal cerebral ischemia. *Jpn J Pharmacol* 2002;89:36–43.
74. Suzuki M, Suzuki M, Sato K, et al. Effect of β-hydroxybutyrate, a cerebral function improving agent, on cerebral hypoxia, anoxia and ischemia in mice and rats. *Jpn J Pharmacol* 2001;87:143–150.
75. Swink TD, Vining EP, Freeman JM. The ketogenic diet. *Adv Pediatry* 1997;44:297–329.
76. Tamura A, Graham DI, McCulloch J, Teasdale G. Focal cerebral ischemia in the rat. I. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 1981;1:53–60.
77. Tieu K, Perier C, Caspersen C, et al. D-β-Hydroxybutyrate rescues mitochondrial respiration and mitigates features of PD. *J Clin Invest* 2003;112:892–901.
78. VanItallie TB, Nonas C, Di Rocco A, Boyar K, Hyams K, Heymsfield SB. Treatment of Parkinson disease with diet-induced hyperketonemia: A feasibility study. *Neurology* 2005;64:728–730.
79. VanItallie TB, Nufert TH. Ketones: Metabolism's ugly duckling. *Nutrition Rev* 2003;61:327–341.
80. Veech RL. The therapeutic implications of ketone bodies: The effects of ketone bodies in pathological conditions: Ketosis, ketogenic diet, redox states, insulin resistance, and mitochondria metabolism. *Prost Leuk Essential Fatty Acids* 2004;70:309–319.
81. Vining EPG. Clinical efficacy of the ketogenic diet. *Epilepsy Res* 1999;37:181–190.

82. Volicer L, McKee A, Hewitt S. Dementia. *Neurol Clin* 2001;19:867–885.
83. Wang D, Wu X, Li J, Xiao F, Liu X, Meng M. The effect of lidocaine on early postoperative cognitive dysfunction after coronary artery bypass surgery. *Anesth Analg* 2002;95:1134–1141.
84. Wastney ME, Hall SEH, Berman M. Ketone body kinetics in humans: A mathematical model. *J Lipid Res* 1984;25:160–174.
85. Wilder RM. The effect of ketonemia on the course of epilepsy. *Mayo Clin Bull* 1921;2:307–308.
86. Wolfson C, Wolfson DB, Asgharian M, et al. Clinical Progression of Dementia Study Group. A reevaluation of the duration of survival after the onset of dementia. *N Engl J Med* 2001;344:1111–1116.
87. Woodyatt RT. Objects and method of diet adjustment in diabetics. *Arch Intern Med* 1921;28:125–141.
88. Young RS. Controversies in treatment of neonatal seizures. *Pediatry Neurol* 1993;9:331–332.
89. Zinker BA, Britz K, Brooks GA. Effects of a 36-hour fast on human endurance and substrate utilization. *J Appl Physiol* 1990;69:1849–1855.
90. Zou Z, Sasaguri S, Rajesh KG, Suzuki R. dl-3-Hydroxybutyrate administration prevents myocardial damage after coronary artery occlusion in rat hearts. *Am J Physiol Heart Circ Physiol* 2002;283:H1968–H1974.