Mini Review

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Stroke: a real danger of the use of "Crystal Meth" and "MMA": the unrecognized triggering of hypomagnesemia and release of ceramides, nitric oxide and platelet-activating factor as well as upregulation of NF-kB and proto-oncogenes and the potential role of epigenetics

Abstract

Now days, there is a major drug abuse problem worldwide. In the USA, opioids and other abused drugs like amphetamine derivatives (e.g., "crystal meth" and methyl-methamphetamine [MMA]) are causing approximately 75,000 deaths each year with a growing number of strokes among the youth (16-40 years of age). The mechanisms for these strokes and their prevention and treatment have remained elusive. Our laboratories have been investigating the causes and potential treatments for substance abuse-induced strokes in animals and human subjects since the mid-1970's. Below we review our studies with both "crystal meth" and MMA using a variety of "state-of-the-art" biophysical, biochemical and molecular techniques. Our results, so far, indicate that both "crystal meth" and MMA cause severe vasospasms of microcirculatory blood vessels in the brain cerebral and medullary circulations as evidenced with direct *in-vivo* quantitative TV microscopic observations (at magnifications >6,000x). Concomitant with these vasospasms, we noted adhesion of leukocytes, monocytes and platelets to the endothelial cell walls followed by rupture of post capillary venules with emigration of red blood cells, monocytes, leukocytes and macrophages into the perivascular tissue spaces with increased dose of the amphetamine derivatives. Use of ^{31}P-nuclear magnetic resonance spectroscopy and near-infrared spectroscopy indicated reductions in brain intracellular ATP, ADP, PCR, and pH with concomitant elevation in intracellular P_i and reduction in blood oxyhemoglobin and mitochondrial cytochrome oxidase aa_3. Chronic "crystal meth" or MMA resulted in upregulation of both NF-kB and the proto-oncogenes c-fos and c-jun. Chronic administration of either "crystal meth" or MMA resulted in several epigenetic modifications: 1, acetylation of histone H3, 2. methylation of DNA, 3. upregulation of certain micro-RNAs, and 4. downregulation of telomerases.

Keywords: crystal meth, leukocytes, monocytes, platelets, methamphetamine

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Abbreviations: MMA, methyl methamphetamine; PCR, polymerase chain; DNA, deoxyribonucleic acid; ICH, intracerebral hemorrhage; IHD, ischemic heart disease; NIRS, near-infrared optical spectroscopy; VSMC, vascular smooth muscle cells; PA, prinzmetal angina; SDIHD, sudden-death ischemic heart disease; IHD, ischemic heart disease; PAF, platelet-activating factor; NMRS, nuclear magnetic resonance spectroscopy; NO, nitric oxide; MDMA, methylenedioxymethamphetamine

Introduction

Methamphetamine (Meth), known as "ice", "speed", or "crystal meth" can cause, heart attacks, cardiac arrhythmias, cardiomyopathy, elevated arterial blood pressure, vasculitis, pulmonary hypertension, irreversible lung and kidney damage and failure, liver failure, thromboses, inflamed and broken blood vessels in the brain, psychoses, and strokes (both hemorrhagic and ischemic).[1–7] "Crystal meth" poses extreme dangers for strokes in young people (ages 16-30), even in first-time users.[4–7] Ingestion of methyl-methamphetamine (MMA) is often said to be even more dangerous for induction of strokes than "crystal meth".[4,5] The majority of these strokes appear to be hemorrhagic in nature. One of the quirks, here, is and unlike in older people (i.e., individuals >40 years of age), young people show non-traumatic intracerebral hemorrhage (ICH) that is very seldom associated with hypertension. During the past decade, "crystal meth" and MMA have become increasingly available on "the streets", at very reduced costs, to our youth and is often found in bloods of victims

in association with polydrug use, including opioids. It is important to note, here, that methamphetamine, "crystal meth" and MMA are all strongly associated with development of ischemic heart disease (IHD), atherosclerosis, and accelerated coronary artery and cerebral vascular diseases.[1–5] Both drugs have a high potential for abuse and addiction.

Despite the fact that "crystal meth" and MMA have been known to cause strokes and cerebral vascular disease for more than 25 years, the specific mechanisms for induction of these strokes have remained nebulous.[6] Below, we present and review recent human and animal investigations, undertaken by our group, which we believe "shed some light" on the probable molecular mechanisms/pathways underlying the causes of these strokes and biophysical-biochemical methods to diagnose, manage , prevent and treat young people who have ingested, smoked, snorted or injected "crystal meth" or MMA. It has been suggested by numerous investigators that since the heightened awareness effects, hallucinations, and euphoria observed after taking "crystal meth " or MMA are linked to release of catecholamines, serotonin, and dopamine, these substances must be responsible for the high blood pressure, heart attacks and strokes induced by these amphetamine-derivatives.[7–9] However, using experimental animals, and continuous arterial blood pressure measurement, direct, quantitative microscopic in-vivo observations (at magnifications approaching 6,000x) of the brain microvessels and near-infrared optical spectroscopy (NIRS) in the cerebral and medullary areas, together with specific receptor blockers for the aforementioned amines, we could not prevent the hemorrhagic or ischemic strokes induced by either "crystal meth" or MMA.[10]

Use of direct in-situ microscopic observations on both the cerebral and medullary micro vessels at high magnifications (up to 6,000x normal) and NIRS suggest probable causes of ischemic and hemorrhagic strokes

Very recently, two different groups of investigators working with brain mouse arterioles[11,12] and two-photon imaging of mouse brain cerebral blood vessels[13] reported that methamphetamine caused constriction of the blood vessels. However, to our knowledge, no such studies up until our investigations have demonstrated any such effects for 'crystal meth" or MMA.

Working with anesthetized rats, our laboratories found that in-vivo microscopic observations the brains clearly indicated that animals pretreated with specific receptor antagonists to eliminate any contribution of catecholamines, serotonin, dopamine or histamine, both "crystal meth' and MMA induced vasoconstriction of metarterioles, precapillary sphincters, and muscular venules, often followed by rupture of postcapillary venules along with adhesion to the endothelial cell walls (and transudation) of macrophages , monocytes and leukocytes into the perivascular tissue spaces; the higher the dose of either "crystal meth" or MMA, the more likely these postcapillary venules would rupture, thus producing a hemorrhagic stroke.[10] Use of NIRS demonstrated that there was a 50-90% increase in deoxyhemoglobin concomitant with 45-85% increases in reduced mitochondrial cytochrome oxidase aa_3.[10] Therefore, with the potential elimination of the release of catecholamines, serotonin, dopamine, or histamine, we have to conclude that these drugs of abuse probably cause strokes by direct effects on the microvascular smooth muscle and endothelial cells. It also becomes obvious from our data that both "crystal meth" and MMA cause severe inflammatory reactions in the brain (i.e., a vasculitis).

Probable contributions of crystal meth-and MMA-induced cellular depletion of ionized magnesium, phosphocreatine and ATP

Approximately 55 years ago, two us were the very first to report that when isolated vascular smooth muscle cells (VSMC), intact microvessels or primary cultured single VSMC were exposed to low concentrations of extracellular ionized magnesium ($[Mg^{2+}]_0$), the VSMC went into vasospasm and demonstrated increased constrictor activities in the presence of all known circulating neurohumoral, amine, and vasopeptide agonists;[14–25] no known amine or peptide antagonist could prevent these vasospastic actions of low Mg^{2+}. We suggested that these vasospastic actions of low Mg^{2+} could, in large measure, be causal in Prinzmetal angina (PA), ischemic heart disease (IHD), sudden-death ischemic heart disease (SDIHD), refractory hypertension, different forms of vasculitis, preeclampsia-eclampsia, mental retardation, and strokes.[19–27] Further in-vitro and in-vivo studies revealed that low Mg environments resulted in the entry and intracellular release of free Ca^{2+} ions which, in large measure, triggered the vasospasms.[15–17,28–32] We also noted a change in phenotype of primary cultured vascular smooth muscle cells when cultured in low $[Mg^{2+}]_o$.[32] This has led us to believe that reduced dietary Mg intake can be "genotoxic" and probably play an important role in epigenetic phenomena in the cardiovascular system and development of cardiovascular diseases.[33–35] After our original findings and hypotheses were published, numerous labs confirmed our data and reasoning and went-on to confirm our suggestions in patients presenting with PA, IHD, SDIHD, refractory hypertension and strokes.[36–42]

Use of ^{31}P-nuclear magnetic resonance spectroscopy (^{31}P-NMRS) on the brains of anesthetized animals allowed us to measure and quantify alterations in the brain intracellular concentrations of ATP, ADP, phosphocreatine (PCR), pH, $[Mg^{2+}]_I$, and inorganic phosphate (P_i).[43] As expected, we found that as the doses of "crystal meth" or MMA were increased, there was a greater and greater loss of intracellular free Mg, PCR, ATP and ADP with concomitant rises in intracellular hydrogen ion, Ca^{2+} and P_i concentrations.[43] Thus, in view of these data, we believe that both "crystal meth" and MMA, in large measure, promote strokes by first causing intense vasoconstriction of brain micro vessels followed by sticking of leukocytes, monocytes and macrophages to the endothelial cell walls, and then rupture of postcapillary venules if the doses are high enough (or chronically-administered) or the subject demonstrates a special sensitivity to the drugs. It is, thus, clear to us, that both "crystal meth" and MMA are inducers of inflammatory reactions (e.g., vasculitis) in the brain. Further investigation on bloods removed from jugular vein catheters indicated elevated levels of IL-1a and TNF-alpha after intracarotid arterial administration of "crystal meth" and MMA, thus solidifying an inflammatory reaction.[43] However, this does not appear to be the full explanation because when we placed VSMC in low Mg^{2+} for even short periods of time (i.e., 15-30 min), we found using ^{1}H-NMRS that the cells synthesized and released ceramides, platelet-activating factor (PAF) and several PAF-lipid-like compounds.[44,45]

Use of 1H-NMRS reveals production of ceramides, PAF and PAF-like lipids in microvascular smooth muscle and endothelial cells which exert vasospastic actions/vasoconstriction on brain micro vessels, inflammatory reactions and rupture of post capillary venules: relation to effects of "Crystal Meth" and MMA

Recently, our laboratories reported that short-term dietary Mg deficiency in rats (for 21 days) caused an upregulation of the enzymes that regulate the synthesis of ceramides (i.e., acid, neutral, and alkaline sphingomyelinases; ceramide synthase; sphingomyelinase synthase; ceramide synthase) in cardiac, cerebral, coronary and somatic VSMC and endothelial cells.[46–50] The end result of the upregulation of these enzymes was found to cause increased constriction(and vasospasm) of coronary, cerebral , and some somatic micro- and macro-blood vessels , decreased capillary blood flows and distribution, inflammation of microvessels, adhesion of leukocytes, monocytes and macrophages to the endothelial walls of the postcapillary venules, and rupture of cerebral (and medullary) postcapillary venules, exactly like that seen after administration of both "crystal meth" and MMA (see above).

As indicated above, interestingly we found that short-term Mg deficiency (MGD) in rats (21 days) or MGD in primary cerebral and coronary VSMC caused an increased synthesis and release of PAF and PAF-like lipids,[44,45] all of which are known to cause vasospasm of blood vessels, decreased cardiac output , inflammatory responses, and rupture of some postcapillary venules , responses similar to that seen with administration of "crystal meth" and MMA (see above).

Potential role of nitric oxide in the inflammatory actions of "Crystal Meth" and MMA actions in brain microcirculation

In 1987, two of us reported, for the first time, that coronary and cerebral arteries required synthesis/release of nitric oxide (NO) in order for Mg^{2+} ions to cause these blood vessels to relax (i.e., undergo vasodilation).[51] Subsequently, our laboratory found that intact micro vessels, in the intestinal, cerebral, and skeletal muscle micro vasculatures, also required synthesis/release of NO in order to promote vasodilatation in response to free Mg ions.[52] Release of NO in the microvasculature has been reported by numerous workers to be critical in inflammatory reactions.[53–55] In the early 1980's, our laboratories and that of Furchgott's first found that that the endothelium of all blood vessels, including micro vessels, released NO in response to most vasodilator vasoactive agents (i.e., acetylcholine, ATP, kinins, histamine, etc.).[56–58] The NO was found, in these studies, to be the actual agent that promoted vasodilatation and, in large measure, the inflammatory responses. Several years ago, a number of investigators found, in animals, that amphetamines, meth-amphetamine, and methylenedioxymethamphetamine (MDMA) ingestion, snorting or "main-lining" were associated with synthesis/release of NO in several areas of the brain promoting neurotoxicity.[12,13,59,60] Administration of two different inhibitors of NO were found in these studies to inhibit/ attenuate the depletion of both dopamine and serotonin in frontal cortex and parietal cortex n response to these analogs of amphetamine.[59,60] We, thus, believe, rather strongly, that "crystal meth"- and MMA-induced strokes owe their inflammatory mechanisms of actions, in part, in the brain, to synthesis/release of NO (reviewed above). Using MGD (21days) rats, we have reported that loss of cellular levels of free Mg ions activates all three major enzymes required for synthesis of NO both *in-vitro* and *in-situ.*[61]

Our findings, observed with MGD animals and primary VSMC in culture, thus, lead us to implicate depletion of Mg, and synthesis/ release of ceramides, NO, PAF and PAF-like lipids in the mechanisms of action for "crystal meth"- and MMA-induced strokes, at least in experimental animals, VSMC and endothelial cells. Very preliminary experimental studies with rats suggest that a combination of systemic administration of inhibitors of ceramide, PAF and NO synthesis attenuates the stroke-inducing actions of "crystal meth" and MMA.[62] Whether this will turn-out to be correct in youth and adult subjects who present with hemorrhagic/ischemic strokes after administration of either "crystal meth" or MMA will remain a hypothesis until clinical studies are undertaken with non-invasive biophysical techniques such as NIRS and both ^{1}P-NMRS and ^{31}P-NMRS. If our findings are confirmed, they would suggest potential avenues for the prevention, management and treatment of victims intoxicated/ addicted to 'crystal meth' and MMA. That is, the use of specific receptor blockers/ inhibitors for ceramides, NO and PAF should be critical in unraveling these molecular pathways. Our use of biophysical and biochemical tools to explore various molecular pathways involved in "crystal meth"- and MMA-induced strokes should prove very useful in the precise identification of these molecular pathways.

Expression of proto-oncogenes and NF-kB in cardiovascular tissues and cerebral cells from animals chronically-treated with "Crystal Meth" or MMA: relation to ceramide levels

Several years ago, our group found a stark relationship the finding of an increased expression of both NF-kB and proto-oncogenes (c-fos and c-jun) in cardiovascular tissues and cells excised from rats exposed to short-term Mg deficiency (i.e., 21 days) and cerebral and coronary arterial VSMC exposed to low Mg^{2+} in primary cell cultures.[48,49,63–66] NF-kB and the proto-oncogene families (e.g., c-fos and c-jun) are known to be principal players in regulation of growth, cellular differentiation, vascular remodeling, migration, inflammatory signals, cognitive awareness, immune events, and cell death.[67–71] It is not clear, however, exactly what triggers these important cellular signals. We have suggested that Mg deficiency is most likely one of the main activators.

Since we have demonstrated, at least in rats, that "crystal meth" and MMA reduce brain cellular levels of free intracellular Mg^{2+} (see above), we decided to determine whether chronic treatment of animals (i.e., 21 days) with either "crystal meth" or MMA would increase (activate) either or both NF-KB and the proto-oncogenes. The answers, not surprisingly, demonstrated that both chronic "crystal meth" and MMA turned-on both NF-kB and the proto-oncogenes [unpublished findings]. Although these studies are yet preliminary, we are tempted to speculate that low-induced brain cellular levels of Mg^{2+} some- how trigger some of the molecular mutations that may be stimulated by these amphetamine derivatives.

Potential roles of epigenetics in "Crystal Meth"- and MMA-induced strokes

Although single doses of "crystal meth "or MMA often do not induce strokes, repeated (or chronic) administration will often result in hemorrhagic/ ischemic strokes in susceptible victims. Since

we have shown that Mg-deficient diets can induce oxidation and fragmentation of DNA and several forms of programmed cell death (i.e., apoptosis, necroptosis, ferroptosis).[33–35,72–76] as well as phenotypic alterations in cerebral and coronary VSMC in disease,[32,34,35,75] thus suggesting epigenetic alterations in the cardiovascular system, at least in experimental animals and VSMC, we thought that chronic use of amphetamines, such as "crystal meth" and MMA, might also induce epigenetic changes which could prime in-situ coronary and cerebral VSMC for heart attacks and strokes. Recent studies , in our labs, have shown that cerebral and cardiac VSMC excised from animals subjected to short-term Mg deficiency (21 days), as well as canine cerebral and coronary VSMC in primary cell cultures, demonstrated acetylation-modifications in histone H3, methylations of histones, upregulation of several micro -RNAs, and downregulation of telomerases [unpublished findings]. With a profile of such cellular cardiovascular modifications, at least in experimental tissues and cells, we have to, tentatively, conclude that epigenetic alterations in VSMC most-likely play important roles in priming cardiac and cerebral VSMC for coronary artery disease and strokes in response to chronic use of "crystal meth", MMA and other amphetamine derivatives.

Conclusions and future thoughts

Over the past two decades, there has been a number of reports indicating that administration of amphetamines, "crystal meth" and MMA, all can induce hemorrhagic and ischemic strokes with youths showing a preponderance of hemorrhagic strokes unlike older adults (people >45 years of age) who mainly present with ischemic strokes. The exact mechanisms for both types of stroke have remained elusive although it has been suggested that increases in arterial blood pressure, release of sympathomimetic amines, dopamine, histamine and serotonin along with unknown forms of vasculitis may play important roles. Our investigations, using anesthetized animals and direct, quantitative in-vivo microscopic observations on the cerebral and medullary microcirculations indicate that all of these drugs of abuse cause vasospasm and inflammation (i.e., a vasculitis) of arterioles, metarterioles and precapillary sphincters with concomitant adherence of leukocytes, monocytes and macrophages to the post capillary venular endothelial cell walls. As the doses of both 'crystal meth' and MMA were increased, post capillary venules ruptured with emigration of leukocytes, monocytes, macrophages, and red blood cells into the perivascular tissue spaces, thus producing hemorrhagic strokes.

Further investigation, using isolated cerebral vascular smooth muscle as well as VSMC in primary cell culture revealed that "crystal meth" and MMA reduced the intracellular concentrations of Mg^{2+} followed by phenotypic alterations in cell geometry and changes in membrane transport and intracellular release of Ca^{2+}. Patients admitted to our ERs with intoxication of "crystal meth "or MMA also demonstrated reduction in blood levels of ionized Mg and increased Ca^{2+}/Mg^{2+} ratios. Use of both ^{1}H-NMRS and ^{31}P-NMRS, and near-infrared optical spectroscopy on animals given "crystal meth" or MMA revealed that intact brain cerebral hemispheres, cerebral blood vessels and brain medullary vessels demonstrated increased levels of ceramides, NO, PAF and PAF-like lipids concomitant with reduced intracellular levels of ATP, PCR, ADP, and pH together with increased intracellular levels of P_i. Preliminary observations on human subjects who ingested either "crystal meth" or MMA also showed increased blood levels of ceramides and PAF. Preliminary animal studies revealed that cerebral tissues and VSMC excised or cultured from rats chronically treated with either "crystal meth" or MMA showed elevated cellular levels of NF-kB and proto-oncogenes. All of our new studies, when viewed together, strongly suggest that 'crystal meth 'and MMA produce hemorrhagic strokes in youth by a series of direct actions on brain microvascular and endothelial cells with concomitant synthesis/release of ceramides, NO, PAF and PAF-like lipids. Biomarkers for alterations in histones structure, DNA methylation, upregulation of certain micro-RNAs, and downregulation of telomerases suggest that epigenetics probably play roles in strokes induced by chronic administration of both "crystal meth' and MMA. Use of the non-invasive biophysical techniques, employed in our investigations, should help to prove whether our hypothesis is valid and could be used to better diagnose, manage and treat subjects intoxicated with "crystal meth", MMA and other designer- amphetamine derivatives. Our findings, we believe, are suggestive of the therapeutic use of Mg together with receptor blockers/inhibitors of ceramides, NO and PAF.

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Conflicts of interest

The author declares there are no conflicts of interest.

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