How to obtain severe hypoglycemia without causing brain or cardiac damage

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A B S T R A C T

Cancer is the second cause of death worldwide, but current therapies are often insufficient or linked with toxicity. Initial evidence in scientific literature seems to support the role of non-pharmacological strategies, including hypoglycemia, in cancer treatment. The biological rationale for hypoglycemia-based treatment of cancer resides in the evidence that cancer cells predominantly utilize glucose as an energy source; notably, cancer cells seem to have damaged glycolysis regulation and few, defective mitochondria showing impaired oxidative phosphorylation. Preliminary data arising from both preclinical and human studies support the role of hypoglycemia in inducing apoptosis on cancer cells. In this paper, we describe how to induce and maintain severe hypoglycemia without causing damage to either the brain or the heart. Our hypothesis is that ExtraCorporeal Membrane Oxygenation (ECMO) and selective glucose perfusion of the carotid vessels are able to induce and maintain severe hypoglycemia in inducing apoptosis on cancer cells. This will allow physicians to study the effect of severe hypoglycemia on cancer cell apoptosis in vivo.

Introduction

With 18.1 million new cases and 9.6 million deaths in 2018 alone [1], cancer is the second cause of death worldwide, and its incidence is expected to rise by around 70% over the next two decades. While there is extensive research concentrating on the identification of new cancer treatments [2], a great number of novel drugs are discarded due to safety issues for possible organ harm. The breadth of chemotherapeutic agents is vast, and some have potent anti-neoplastic effects which are limited by severe toxicity and adverse effects, often requiring cessation of exposure and therapy interruption [3,4]. Tumor lysis syndrome is another issue leading to the limitation of therapies, the incidence of which has increased with newer and more aggressive cytotoxic agents. Together with existing chemotherapeutic agents, older drugs were effective in cancer treatment but showed severe adverse effects and never reached the human experimental phase because of their in vitro and in vivo toxicity. Furthermore, several drugs that have already been commercialized for the treatment of non-cancer diseases may require screening and investigation for potential high dose antineoplastic activity [5–12]. Non-pharmacological strategies and techniques might also have a role in cancer treatment; indeed, initial evidence in literature supports a potential anti-tumoral effect of hypoglycemia [13,14], severe pH changes [15,16], and hyperthermia [17]. There are, however, obvious risks of severe organ damage relating to the application of these techniques. As intensive care physicians, we have vast knowledge of human physiopathology and unique technical skills in monitoring and supporting vital functions. ExtraCorporeal Membrane Oxygenation (ECMO) is an established technique which supports and replaces cardio-pulmonary function with reduced complication rates and has been used successfully in the treatment of acute life-threatening cardiac and pulmonary toxicity. Technology is continuously improving thanks to increased circuit biocompatibility and reductions in size [18]. The use of ECMO in treating respiratory or circulatory failure in acute drug toxicity for patients who are unresponsive to conventional therapy is

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well-established [19]. New technologies and materials developed in the last decade allow for the use of ECMO for several weeks with relatively low risks [20]. Moreover, in high-volume centers providing mechanical circulatory support, ECMO is associated with relatively high success rates and few complications [21]. At the same time, technological improvement has enabled a broadening of the fields of application and to use this technique not only in emergency settings but also in elective conditions and outside the intensive care unit (ICU) [22].

**The hypothesis**

Our hypothesis is that the maintenance of severe hypoglycemia is possible and may induce selective apoptosis on cancer cells, while the cardiac and neurological side effects of hypoglycemia could be prevented with ECMO and selective glucose perfusion of the central nervous system. Furthermore, our aim is to make ECMO available in other settings, such as oncology, where it could provide a safe base for overcoming acute cardiac and pulmonary toxicity of potentially eradicating chemotherapy drugs and techniques, as well as supporting the vital functions.

**Evaluation of the hypothesis**

Severe hypoglycemia has a potential anti-tumoral effect since cancer cells are metabolically dependent on glucose. Glucose deprivation is the most frequently used method to separate cancer from normal cells in the laboratory, and in 1924, a Nobel prize winning research demonstrated that neoplastic cells rely on anaerobic glycolysis for their metabolic needs [23]. Cancer cells show increased glucose consumption even under normal oxygenated conditions, a phenomenon that is known as the Warburg effect [24]. Cancer cells have few and defective mitochondria, their metabolism shows an impaired oxidative phosphorylation [25,26], and damaged glycolysis regulation [27]. The key point of hypoglycemic treatment is that most cancers requires glucose as a predominant energy source [28], and that this high fuel requirement is due to the high metabolic activity and proliferation of these cells. Recently, Elgendy et al. [29], showed that fasting -induced hypoglycemia suppressed tumor growth in mice and that comitant treatment with metformin to inhibit mitochondrial oxidative phosphorylation added a synergic anti-neoplastic effect.

Previous *in vitro* experiments [30] investigated the molecular mechanism underlying glucose deficiency-induced cytotoxicity, which is the desirable effect, demonstrated that blood glucose reduction to 2 mM/l (36 mg/dl) could be an effective cancer treatment on HeLa cells [31] and apoptosis induction mediated by caspase-3 activity was increased for glucose concentration of 1 mM (=18 mg/dl). Furthermore, from a molecular point of view, a glucose level of 25–30 mg triggers a rise in HSP72 plasma concentrations, which may act as a danger signal to the bodily tissues, thus enhancing the state of immune and metabolic surveillance [32].

According to the above cited evidence, a blood glucose target of 20–30 mg/dl has been defined in order to account for variability during *in vivo* blood glucose management and to address safety issues. Although we will perform central nervous system protection through selective glucose perfusion, blood glucose levels below 18 mg/dl could cause irreversible brain damage [33]. Thus, a blood glucose level between 20 and 30 mg/dl could be a realistic target to address efficacy and safety concerns, also taking into account possible glycemic fluctuations.

In terms of safety for all the other organs that are not directly involved in the protection plan, a glycemic threshold of 36 mg/dl has already been evaluated as being safe in healthy volunteers [34]. Indeed, as mentioned in the paper, normal cells benefit from a certain metabolic flexibility, and thus under aerobic conditions, ATP production can be guaranteed by the Kreb’s Cycle throughout the consumption of metabolic substrate other than glucose: multiple anaplerotic reactions replenish the Kreb’s cycle, starting from both fat and proteins. As previously hypothesized by Mathews et al. [35], we believe that blood glucose concentration could be further lowered below 36 mg/dl, under aerobic conditions, and providing special protection to the most sensitive organs. ECMO allows for protecting the heart throughout the reduction of its work under such metabolic stress, as well as ensuring blood oxygenation and blood flow to the other tissues.

The swine and large canine experimental model could be appropriate for this aim, providing sufficient vessel size to allow for Veno-Arterial (VA)-ECMO cannulation. Feasibility is already proven by the cardiac surgery experience, a setting where swine models are routinely used to perform cardiovascular research with extracorporeal support. A small sample size of swine could be used to confirm the safety of the proposed method, while large dogs affected by untreatable neoplasms and identified through veterinary clinics could receive compassionate treatment, which will confirm the efficacy of the method.

Only those animals affected by tumors potentially responsive to hypoglycemia will be treated with severe hypoglycemia under VA-ECMO and selective brain glucose perfusion. Notably, we must acknowledge that not all cancers are exclusively glycolytic, and some may be also oxidative or rely on glutamine [36–44]. Thus, in order to candidate the eligible animals to hypoglycemic treatment, we would suggest performing a Positron Emission Tomography (PET) to assess if the neoplasm is sufficiently glycolytic to be susceptible to the hypoglycemic insult. In fact, the standardized uptake value (SUV) is a semi-quantitative method establishing the uptake of glucose analogue by cancer cells [45], and the mean SUV of solid neoplasm is above 5.0 [42]. Mathews et al. suggested that in order to consider a neoplasm susceptible of hypoglycemic treatment, SUV neoplasm should be above 5.0 [35].

**Brain protection**

The hypothetical target blood glucose level (30–20 mg/dl) will be reached through continuous intravenous (i.v.) insulin infusion. Severe hypoglycemia has never been attempted in humans because of the frailty of noble organs such as the brain and the heart. Thus, in order to avoid an irreversible hypoglycemic encephalopathy, a sufficient glucose provision must be ensured to the central nervous system. To reach this aim, the brain could be selectively perfused with glucose solution using small cannulas inserted through percutaneous arterial access.

**Heart protection**

To protect the heart from hypoglycemia and unconventional metabolic condition, a low-consume rest status and temporary bypass will be achieved with Veno-Arterial ECMO (VA-ECMO).

ECMO is used daily in humans in order to prevent or treat organ failure [22] and it is reasonable to perform feasibility studies in animals to verify both the therapeutic efficacy of hypoglycemia on tumors, and the safety of severe hypoglycemia under VA-ECMO and selective brain glucose perfusion conditions.

Femoro-femoral VA-ECMO will be electively positioned with fluoroscopy and trans-esophageal echocardiography (TEE) guidance. The ECMO flow will be set according to the animal’s body surface area (BSA). Low dose heparin or bivalirudine will be used as a anticoagulant to achieve the target activated clotting time (ACT) of 50 s. Intravenous esmolol will be administered to prevent tachycardia and further reduce cardiac oxygen consumption, if required. Carotid and collaterals of spinal cord artery cannulation will be performed to selectively deliver glucose solution.

The administration of 50% glucose solution will be started at a dose of 1 ml/min and will then be adjusted according to neurologic electroencephalographic (EEG) monitoring and to the glycemic concentration in the mixed venous blood.

This amount has been calculated as follows: according to a
hypothetical blood flow of 5 l/min: the cerebral blood flow is approximately 15% of the total blood flow (approximately 750 ml/min of 5 l/min). The accepted normoglycemia in an awake patient is approximately 60 mg/dl. A50% glucose solution contains 0.5 g of glucose for each ml, thus 7.5 dl/min * 60 mg/dl = 450 mg of glucose per minute = 900 mg of 50% glucose infusion per min = 0.9 g per min = approximately 1 ml/min = 20 drops. Thus, the administration of 5% glucose can be started at a dose of 10 ml/min if fluid overload is not an issue.

In order to address the safety issue, intraprocedural monitoring will be performed as follows: electrocardiogram (ECG), pulse oximeter, invasive arterial blood pressure, mixed venous oxygen saturation through a central venous 4 lm catheter, jugular venous oximetry, mixed venous glyceremia and jugular venous glyceremia, electroencephalography (EEG) near infrared spectroscopy (NIRS), bispectral index (BIS), trans-thoracic echography (TTE), trans esophageal echocardiography (TEE), and peripheral somatosensory evoked potentials.

Paraplegia is an extremely rare complication in survivors of cardiac arrest, confirming that the spinal cord is more resistant than the brain cells to the (hypoxic) insults. Nonetheless, we will select only those animals with an identifiable Adamkiewicz artery (or a similar artery that is big enough to be cannulated). The spinal cord will be further protected through contact cooling (e.g. mattress). Cooling and pharmacological protection will also be performed through an intrathecal/epidural catheter.

Kidney function will be carefully monitored during the first few cases and selective renal perfusion considered in the following cases if permanent dialysis or permanent chronic renal failure ensues.

Sensitive organs (e.g. pancreas) monitoring will be performed twice a day in the first few cases to evaluate if selective organ perfusion is necessary.

Animal experimental phase

Experimental treatment will be performed for at least 24 h. All cases will undergo clinical, radiological and laboratory follow-up for 30 days. At the end of the follow-up, all swine models will be sacrificed to check anatomo-pathological changes, investigating neurological, pulmonary and cardiac damage in particular. Furthermore, healthy and tumoral tissues will be examined to verify the effects of hypoglycemia on normal and cancer cells.

All the above described expertise is currently available in large volume cardiovascular hospitals, where ECMO is routinely performed both in emergency and, more recently, also under elective conditions. The rate of vascular complication is very low in urgent cases and almost non-existent in elective cases.

Selective cannulation of middle-sized arteries is performed daily by cardiologists worldwide (coronary cannulation), vascular surgeons, neuro-radiologists, neurosurgeons (carotid cannulation), and interventional radiologists (almost every existing middle-sized artery is cannulated daily in cases of bleeding).

At the end of the experimental phase, data will be collected and analyzed to verify the hypothesis.

Since no literature on the efficacy of hypoglycemic treatment in vivo is available, a precise sample size estimation cannot currently rely on preliminary results.

We recognize and agree that animal welfare is a value of the European Union, and as such all legislative and ethics requirements in terms of housing, feeding, watering, and handling of experimental animals will be respected, and all experiments will be conducted in an environment characterized by expertise in animal handling. Indeed, we are aware that animal welfare may impact the quality of the research data, and veterinary anesthesiologists may assess the health of the animals (clinical conditions, nourishing status, safety, comfort, behavior, distress, and pain assessment) on a daily basis, and indeed eligibility for the procedure.

Any unpleasant and painful procedures will be performed under intravenous or gaseous anesthesia. Analgesia will be provided for possible postoperative pain. The euthanasia procedure will be performed under deep general anesthesia, and any post-mortem procedures will be performed upon ascertaining permanent cessation of cardiac activity. We are aware of the ethical burden of the use of animal models in scientific experiments, however we feel that the possibility of substantial progress in the field of cancer research justify the application of our hypothesis on an animal in vivo model. Any euthanasia procedures will be performed at the end of the in vivo swine experimental procedures, because confirmation that ECMO had indeed provided protection from hypoglycemic neurological and cardiopulmonary toxicity, as well as the safety of the treatment on the other organs, can only be identified through anatomo-pathological examination. The efficacy of the intervention with regard to tumors can then be shown through the clinical, radiological, biopsy and biochemical examinations of tissues in cases in which the animal does survive treatment and pathology; or alternatively, through pathological and biochemical studies on the animal models’ organs post-mortem, in cases where the animal does not survive the illness.

Consequence of the hypothesis and discussion

This is the first study proposal aiming at investigating the safety and efficacy of severe hypoglycemia to induce apoptosis on cancer cells. The cardiac and neurological side effects of hypoglycemia may be prevented using ExtraCorporeal Membrane Oxygenation and selective glucose perfusion of the central nervous system.

This experimental plan has several strengths. A thorough literature review and straight-forward protocol definition will guarantee the best possibilities to the intervention. The study of the effects of severe hypoglycemia into a tumoral animal model has never been attempted before. Our project allows us to evaluate both the effects on the organism of severe hypoglycemia with ECMO support and with selective central nervous system glucose perfusion, and the effects on tumoral tissues. Moreover, this study will help further development of research on non-pharmacological therapeutic strategy, and on extracorporeal circulation in animal models, rare and underdeveloped fields of research. If our hypothesis is confirmed, we will be able to improve the therapeutic chances of cancer patients. If hypoglycemia is demonstrated as safe and effective through preliminary experimental animal studies, it will be possible to investigate the role of hypoglycemia in human subjects. Furthermore, we could investigate the combination of hypoglycemia with other chemotherapies, possibly reducing drugs toxicity, improving the efficacy of currently-used treatment, allowing for lower drug dosages, including patients previously excluded from the chemotherapy protocols due to comorbidities, and finding new strategies for treating cancer.

Declaration of Competing Interest

None of the authors have any conflict of interest to declare. Note: No financial support was provided for this manuscript.

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