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ORIGINAL COMMUNICATION

A randomized controlled trial on the efficacy, safety, and pharmacokinetics of metformin in severe traumatic brain injury

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Abstract

Objective Traumatic brain injury (TBI) is a leading cause of morbidity and mortality worldwide. Metformin is reported to have pleiotropic neuroprotective effects through anti-inflammatory, antioxidative, and anti-ischemic activity, and improvements in vascular hemodynamics and endothelial function. The aim of this study is to examine the efficacy and safety of metformin therapy in severe TBI patients.

Methods This single-blind, parallel-group, randomized controlled trial enrolled adult TBI patients. Of 158 trauma patients assessed, 30 met the eligibility criteria and were randomly allocated in a one-to-one ratio to receive 1 g metformin every 12 h for five consecutive days (intervention group) or to usual management only (control group). For efficacy analysis, temporal profiles of serum levels of S100b, neutrophil to lymphocyte ratio (NLR), and glial fibrillary acidic protein (GFAP) were assessed. For pharmacokinetic analysis, serum concentrations of metformin were evaluated in the intervention group.

Results The two study groups were similar in terms of demographics, baseline clinical characteristics, and on-admission biomarkers' serum levels. Longitudinal analysis of S100b and NLR levels showed statistically significant declines in values toward normal levels in the intervention group (p values of <0.001 and 0.030, respectively), different from the profiles of the control group (p values of 0.074 and 0.645, respectively). Pharmacokinetic analysis demonstrated that metformin absorption is delayed in TBI patients. No events of hypoglycemia and lactic acidosis occurred.

Conclusions Metformin could potentially be an effective and safe therapeutic intervention in patients with severe TBI. Large-scale, multicentre studies are needed to confirm our encouraging results.

Keywords Metformin · Traumatic brain injury · Head trauma · Biomarker · S100b · Pharmacokinetics

Introduction

Traumatic brain injury (TBI) is a significant cause of morbidity and mortality with an overall incidence rate of 939 per 100,000 person-years worldwide. TBI is categorized by a neurological scale, named Glasgow Coma Scale (GCS), into mild, moderate, and severe severity levels. Severe TBI is defined as a GCS score of 8 or less and accounts for 7.95% of head injuries, affecting more than 5.5 million people each year, around the world [1]. Injuries following a TBI are classified into primary injuries, occurring at the moment of trauma, and secondary injuries, delaying from the moment of impact. Primary injuries are induced by mechanical force, and are associated with immediate irreversible neuronal damage. On the other hand, secondary injuries are series of devastating pathophysiological pathways leading to further neuronal death by means of impairing the blood–brain

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barrier (BBB), releasing inflammatory cytokines and neuroinflammation, oxidative stress, ischemia, cerebrovascular injury, and dysfunction of mitochondria within the first few days post-insult [2].

First days following the injury are critical in the management of severe TBI patients. Beyond the crucial supportive care and adequate resuscitation, it is hypothesized that early suppression of the deleterious secondary pathways should be able to minimize poor long-term clinical outcomes [2]. Based on the logic of prevention of neuronal damage by the limitation of the aforementioned secondary processes, several agents are tested in clinical trials for severe TBI. Determination of the worthy agents to be tested in clinical trials hinges on their known biochemical and physiologic effects. The rationale behind the design of severe TBI clinical trials is achieving an improved clinical outcome by means of pharmacologic inhibition of one or more secondary insult(s). Despite meaningful strategies, all pharmacological interventions were heretofore failed to prove efficacy in clinical trials. This impels researchers to seek a medication to be neuroprotective, anti-neuroinflammatory, antioxidant, protective against cerebral ischemia, able to attenuates the BBB breakdown, effective in improving vascular hemodynamics, and beneficial to mitochondrial dysfunction. The complexity of TBI pathophysiology results in the need for a multi-pathway regulative therapy [3–6].

Metformin hydrochloride, the most widely used oral medication in patients with diabetes, is a biguanide molecule being used as an anti-hyperglycemic agent for virtually 60 years. Throughout these years of clinical use and continuous research, multiple physiologic actions and multiple molecular mechanisms are revealed related to its anti-diabetic therapeutic effect [7]. Moreover, metformin is regularly used in the treatment regimens of women with polycystic ovary syndrome and is demonstrated to be beneficial in the prevention and treatment of various forms of cancer and to decrease the risk of cardiovascular diseases [8–10]. Metformin is also found to reduce cognitive impairments, dementia, Alzheimer's disease, Parkinson's disease and many other age-related disorders [11–15].

Metformin exerted anti-inflammatory therapeutic effects in several in vitro experiments, animal studies and clinical trials. Reduced release of pro-inflammatory cytokines and pro-inflammatory prostaglandins is reported following metformin therapy. Inhibition of nuclear factor κB via activation of AMP-activated protein kinase (AMPK) is suggested to be the main mechanism of its anti-inflammatory action [16, 17].

AMPK-dependent activation of nitric oxide synthase following metformin therapy results in improved vascular endothelial cell function and vascular blood flow by enhancing the release of nitric oxide and decreasing the nitric oxide stress [18]. Metformin reduced BBB permeability by diminishing the neutrophil infiltration to the brain in a mice model of cerebral ischemia. Likewise, in an in vitro study on rat brain microvascular endothelial cells, metformin upregulated BBB functions by activating the AMPK pathway [19, 20]. Chronic and acute metformin therapies have been proven to be neuroprotective through AMPK-mediated mechanisms in several animal models of focal and global ischemia [21, 22].

Metformin decreased levels of reactive oxygen species (ROS) and increased glutathione, catalase, and heme oxygenase-1 (HO-1) in an in vitro study on human neural stem cells. Metformin was also capable of reducing the elevated activities of glutathione peroxidase and superoxide dismutase and levels of malondialdehyde in the cerebrum of rats exposed to global cerebral ischemia. The results of such studies revealed the neuroprotective effect of AMPK-dependent metformin signaling against oxidative stress by means of both reduction of ROS production and increase of antioxidant defenses [22, 23].

Pleiotropic neuroprotective effects of metformin, its efficacious concentration profile in the brain and multiplicity of pathologic mechanisms of TBI prompt hypothesis that acute and chronic metformin therapies should be beneficial in head injury patients.

Blood concentrations of S100B and glial fibrillary acidic protein (GFAP) are strongly approved clinical outcome predictors in TBI patients. Early post-traumatic serum levels of S100b and GFAP are highly directly correlated with long-term unfavorable outcomes, morbidity, and mortality [24]. S100b is a calcium-binding protein produced and released mainly by astrocytes of the CNS and its increased post-trauma serum concentration is a result of either glial damage or astrocytic reactions to neural injury. GFAP is a glial intermediate filament protein in astrocytes of the central nervous system (CNS) and its increased post-trauma serum concentration is a result of astrocyte damage [25]. Neutrophil to lymphocyte ratio (NLR) is also shown to have prognostic value in severe TBI patients and is directly associated with poor clinical outcomes [26].

This trial examines the efficacy and safety of metformin therapy in severe TBI patients with serial evaluation of serum concentrations of S100b, GFAP and NLR during 5 days post-admission. Several studies evidenced alterations in the pharmacokinetic behavior of various drugs in critically ill patients [27]. Therefore, we also evaluated metformin pharmacokinetic parameters in severe TBI patients.

Methods

Study design

This was a single-center, single-blind, parallel-group, randomized controlled trial assessing the efficacy and safety of adding metformin to the standard practice of

severe TBI. The study took place in the intensive care unit (ICU) of the Sina University Teaching Hospital in Tehran, Iran, from May 2016 to June 2018. The trial protocol was registered at the Iranian Registry of Clinical Trials (IRCT20180803040681N1).

Study population

We assessed the eligibility of all head injury patients admitted to Sina Hospital during the study period. Inclusion criteria were a definite diagnosis of head trauma, GCS score of 8 or less, an age of 18–65 years, patients on mechanical ventilator, and written informed consent signed by patient's legal guardian. The exclusion criteria were time of admission to ICU of more than 48 h, Nil Per Os (NPO) status on admission day, diabetes, previous treatment with metformin or any other biguanide medication, blood glucose level of ≥ 150 mg/dL or ≤ 70 mg/dL, simultaneous participation in another clinical trial, serum creatinine level of 1.5 mg/dL or more, serum lactate level of 2.5 mmol/L or more, arterial pH of less than 7.25, serum bicarbonate of 13 mEq/L or less, moderate to severe renal impairment [estimated glomerular filtration rate (eGFR) of below 45 mL/min/1.73m^2], cirrhosis, and acute liver failure.

Study interventions

Included severe TBI patients were equally randomly assigned to receive 1 g metformin two times a day at 12-h intervals for five consecutive days through a nasogastric tube (intervention group) or to usual management only (control group). Patients in the intervention and control groups received usual management at the discretion of the critical care team guided by Advanced Trauma Life Support (ATLS) recommendations. We decided not to use a placebo to not perform unnecessary blood sampling in the control group for pharmacokinetic analysis.

Study outcomes

The primary endpoint in the trial was the 5-day post-trauma serum concentration profile of S100B to evaluate the prognosis of study groups. As secondary endpoints, we analyzed the longitudinal changes of serum GFAP and NLR. With respect to safety considerations, we collected serum levels of blood glucose, creatinine, and lactate and followed patients throughout their hospital stay. To reveal the possible on-admission differences among the study groups, preliminary Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) II score and GCS score, independently of other variables of APACHE II, were compared. Additional secondary endpoint was the plasma concentration of metformin to assess any alterations in pharmacokinetic parameters in study patients.

Randomization

Randomization sequence was created using R-3.2.3 statistical software. Eligible patients were randomly assigned to study groups in a 1:1 ratio using random block sizes of 2 and 4.

Blood sampling

Serial blood sampling for the measurement of serum biomarkers was performed through peripheral veins on admission and at 24 h, 48 h, 72 h and 120 h post-allocation.

Additional blood samples were obtained at 0.5 h, 1 h, 2 h, 4 h, 8 h and 12 h post-intervention in the intervention group for pharmacokinetic analysis.

Serum samples were centrifuged at 3000 rpm for 7 min, aliquoted, and stored at −80 °C until analysis.

Biomarker determination

Serum S100b and GFAP levels were measured using commercially available ELISA kits (S100b ELISA kit, DRG International, Inc., United States and GFAP ELISA kit, Merck, Germany, respectively).

Serum neutrophil, lymphocyte, blood glucose, creatinine, bicarbonate, and lactate levels were extracted from the Hospital Information System (HIS).

Pharmacokinetic analysis

Hydrophilic interaction liquid chromatography (HILIC)-based ultrahigh-performance liquid chromatography (UHPLC) coupled to mass spectrometry was used to separate and detect metformin for pharmacokinetic analysis.

Liquid chromatography was performed with a Flexar UHPLC AS system (Perkin-Elmer, USA) equipped with a degasser, Flexar FX-15 pump, auto-sampler, and PE 200 column oven. Separation column was ZIC-HILIC (100*2.1 mm i.d., 5 µm d) (Merck, Germany) with pre-column ZIC-HILIC 20*2.1 mm, 5 µm d) (Merck, Germany) kept at 35 °C.

For sample extraction, 150 µL of plasma samples were added to 400 µL of 500 ppb melamine in acetonitrile. The mixtures were vortexed for 10 s and centrifuged at 4000 rpm for 20 min. The final supernatants were collected.

Five microliters of each sample was injected into the column and the run time was 3 min. The mobile phase was a 70:30 mixture of solvent A (acetonitrile + 0.1% formic acid) and solvent B (water + 175 mM ammonium formate) with a flow rate of 400 µL/min.

Mass analysis was performed using an ABSciex (Foster City, CA, USA) API3200 instrument, a fully-integrated triple quadrupole mass spectrometer.

The data acquisition and sample quantification were operated using Analyst 1.6.2 software (Foster City, CA, USA).

Calculation of pharmacokinetic parameters

Pharmacokinetic parameters of metformin were calculated using non-compartmental analysis. The elimination rate constant (K_e) was determined as the slope of a least-square linear regression fit of the terminal phase of the logarithmic plasma concentration–time curve. The plasma elimination half-life ($t_{1/2}$) was calculated as $0.693/K_e$. The area under the curves (AUC) from time zero to the last observed time point, $AUC_{(0–12)}$, was calculated using trapezoidal rule. The AUC from last time point to infinity was calculated by dividing the serum concentration at last time point with elimination rate constant (C_{12h}/K_e). The total AUC or $AUC_{0–\infty}$ is calculated using following equation: $AUC_{0–\infty} = AUC_{(0–12)} + AUC_{(12–\infty)}$. Total clearance and apparent volume of distribution are calculated by dividing the administered dose by $AUC_{0–\infty}$ and $AUC_{0–\infty}.K_e$, respectively.

Statistical analysis

Efficacy analyses were performed using a per-protocol approach; therefore, patients who completed the 5-day study period were finally analyzed. Safety analyses were performed using an intention-to-treat (ITT) approach and included all participants who underwent randomization.

For the longitudinal analysis of biomarkers' levels, we used Friedman test within groups, and further Mann–Whitney *U* tests compared levels at each time point. Other continuous variables were compared using Student's *t* tests and Fisher's exact test was applied for the comparison of categorical data.

Data were expressed as mean ± SD and a *p* value of less than 0.05 was considered as statistically significant. All statistical analyses were performed in the GraphPad Prism 6 software.

Results

Flow diagram of the progress of participants through the study is shown in Fig. 1. From May 2016 to June 2018, 158 potential patients were screened to participate in the study in the ICU of Sina Hospital. A total of 128 patients were excluded due to the age of > 65 years ($n = 5$), mild–moderate severity of head injuries and GCS levels of > 8 ($n = 48$), refusal of consent by their guardians ($n = 7$), NPO status at the time of allocation which impedes oral drug administration ($n = 16$), on-admission lactate level of > 2.5 mmol/L ($n = 11$), admission time to the ICU of > 48 h post-insult ($n = 35$), and other reasons ($n = 6$) including history of diabetes and metformin intake ($n = 2$), blood glucose level of ≥ 150 mg/dL ($n = 2$), and serum creatinine level of ≥ 2.5 mg/dL ($n = 2$).

The two study groups were similar in terms of demographic and baseline clinical characteristics (Table 1). There were no significant imbalances between groups concerning age, sex, etiology of the injury and incidence of polytrauma (*p* value > 0.05). Initial APACHE II scores were 14.13 ± 3.52 and 13.88 ± 3.23 in control and intervention groups, respectively (*p* value = 0.88). On-admission GCS score was 6.91 ± 1.14 and 6.5 ± 1.22 in control and intervention groups, respectively (*p* value = 0.48).

Serum lactate levels

No case of severe increase in blood lactate concentration (> 4 mmol/L) was seen either within the 5-day study period or in the following in-hospital stay in study groups.

On-admission lactate levels were 16.09 ± 4.21 mg/dL and 14.64 ± 4.15 mg/dL in control and intervention groups, respectively. Primary mild–moderate elevated lactate levels were cleared within 5 days of sampling for all included patients. End-of-study levels of lactate were 10.91 ± 4.43 mg/dL and 10.18 ± 3.6 mg/dL in control and intervention groups, respectively. There was no statistically significant between-group difference in initial and final serum lactate levels (*p* values of 0.42 and 0.68, respectively).

Blood glucose levels

None of the participants in either group experienced a hypoglycemic condition (glucose level of 3.9 mmol/L (70 mg/dL) or less) during the study period and following in-hospital stay.

Serum creatinine levels

No patient had serum creatinine rise to > 1.5 mg/dL among groups during the study period. On-admission and end-of-study creatinine levels were 1.13 ± 0.13 mg/dL and 1.00 ± 0.15 mg/dL in control group, and 1.02 ± 0.19 mg/dL and 0.95 ± 0.16 mg/dL in intervention group, respectively. There was no statistically significant between-group difference in initial and final serum creatinine levels (*p* values of 0.17 and 0.42, respectively).

Longitudinal serum concentrations of S100b

Dynamics of serum S100b levels in two study groups are shown in Fig. 2. Initial S100b values were not

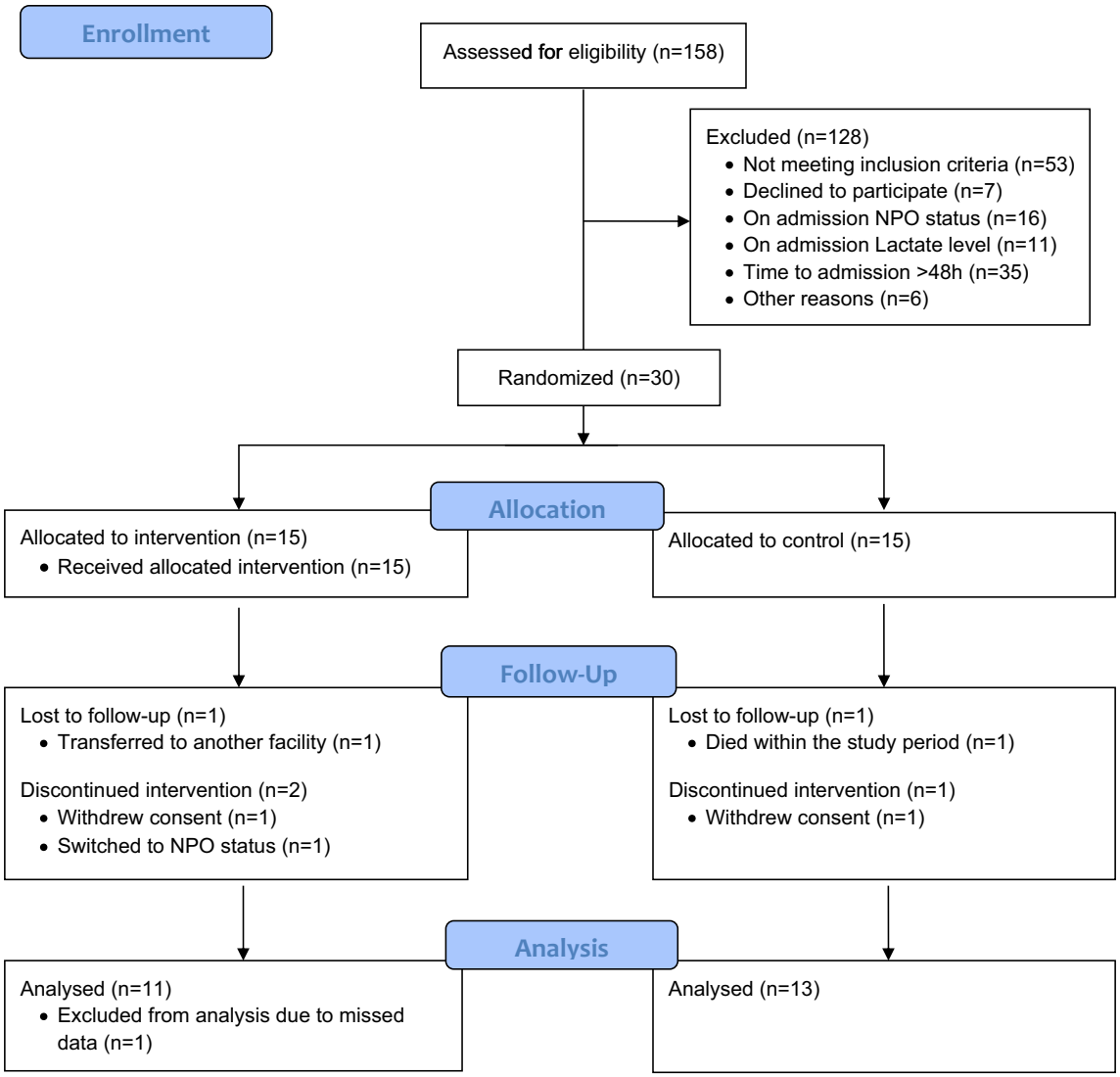

Fig. 1 Flow diagram of the progress of participants through the study

statistically different between groups (174.8 ± 94.2 pg/mL and 220.3 ± 56.7 pg/mL in control and intervention groups, respectively, p value = 0.074); however, there were significant differences among groups at 48 h, 72 h, and 120 h time points (p values of 0.025, 0.001, and 0.028, respectively).

Friedman test revealed statistically significant changes within the intervention group with time (p value < 0.001); meanwhile, there were no significant differences between repeated measures in the control group (p value = 0.066). There were no significant differences between individuals in either group (p value > 0.05).

Longitudinal serum concentrations of GFAP

Dynamics of serum GFAP levels in two study groups are shown in Fig. 3. GFAP values were not statistically different between groups at all study time points (p value > 0.05).

Longitudinal values of NLR

Dynamics of NLR ratios in two study groups are shown in Fig. 4. Initial NLR ratios were not statistically different between groups (7.31 ± 5.48 and 9.06 ± 5.00 in control and

Table 1 Demographic and baseline clinical characteristics of patients included in the trial

Characteristics	Intervention group ($n=15$)	Control group ($n=15$)
Age, median (range; years)	30 (21–48)	31 (19–61)
Sex: men, *n* (%)	15 (100)	15 (100)
Cause of injury, *n*		
Traffic accidents	7	9
Falls	3	2
Struck by an object	3	1
Assault	1	0
Sport-related injuries	0	1
Unknown	1	2
Polytrauma, *n* (%)	4 (27)	5 (33)
On-admission APACHE II score, mean ± SD	13.88 ± 3.23	14.13 ± 3.52
On-admission GCS score, median (range)	7 (5–8)	7 (5–8)
GCS 5–6, *n* (%)	7 (47)	6 (40)
GCS 7–8, *n* (%)	8 (53)	9 (60)

APACHE Acute Physiologic Assessment and Chronic Health Evaluation, *GCS* Glasgow Coma Scale

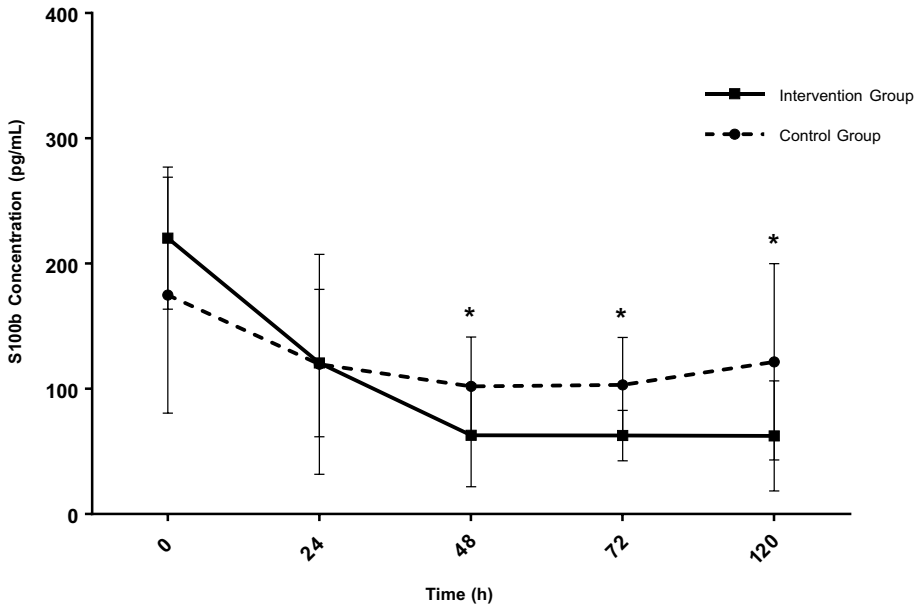

Fig. 2 Mean ± SD serum concentrations of S-100b over time for patients in control and intervention groups; *p value <0.05 between patients in study groups

intervention groups, respectively, p value = 0.574). Friedman test revealed statistically significant changes within the intervention group with time (p value = 0.017); however, there were no significant differences between repeated measures in the control group (p value = 0.085). There were no significant differences between individuals in either group (p value > 0.05).

Pharmacokinetic profile

Metformin pharmacokinetic parameters in study participants are presented in Table 2. Averaged $AUC_{0-\infty}$ and Cl/F are within normal range, C_{max} and K_e are lower than normal values and Vd/F, t_{max}, and $t_{1/2}$ are greater than normal amounts [28].

Discussion

Utilizing serum biomarkers as predictors of outcome in TBI patients has been gaining importance in recent years and application of a combination of potential biomarkers is proposed to have a role in assessing the efficacy of treatment. This trial assessed the effect of metformin oral administration on dynamic levels of serum S100b, GFAP and NLR at five discrete time points over 5 days from admission. The

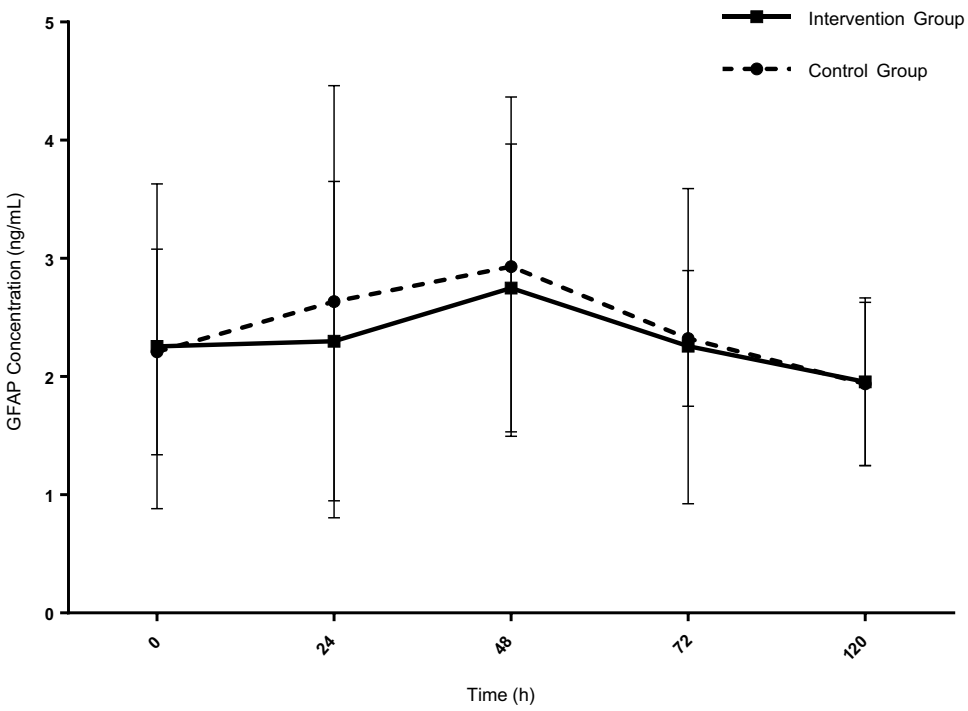

Fig. 3 Mean ± SD serum concentrations of GFAP over time for patients in control and intervention groups

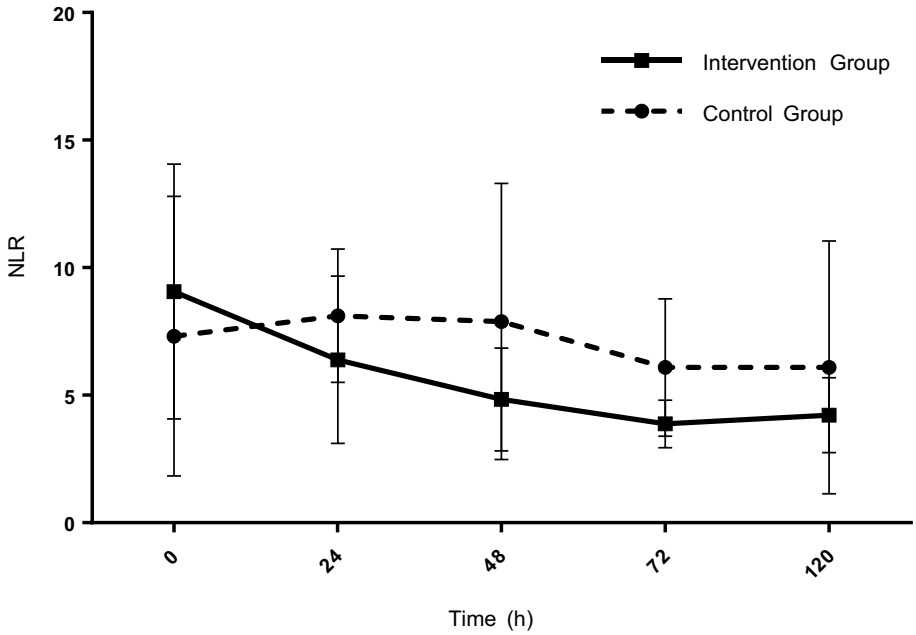

Fig. 4 Mean ± SD serum NLR ratios over time for patients in control and intervention groups

safety and pharmacokinetic profile of metformin in patients with severe TBI was also evaluated in this study.

S100b is the most studied biomarker in the assessment of brain injury and is shown to be a valuable predictor of prognosis in patients with TBI. Several publications have appeared in recent years documenting the significant correlation between serum levels of S100b during early time points post-insult and risk of unfavorable clinical outcomes. Murillo-Cabezas et al. [29] and Berger et al. [30] argue that patients with subsequent serum S100b levels in steady decline have a lower risk of unfavorable outcome. Results of Friedman analysis of our data on S100b levels demonstrated different patterns of change in control and intervention groups. We show through our analysis that metformin administration results in a downward trend in serum S100b levels few days post-trauma. A secondary increase in serum S-100b levels to > 200 pg/mL at the 120 h time point in three participants of the control group reveals ongoing

Table 2 Pharmacokinetic key parameters of metformin in study participants

PK parameter	Value (mean ± SD)
$AUC_{0-\infty}$ (ng/mL h)	12,944.23 ± 3427.28
C_{max} (ng/mL)	1260.43 ± 223.81
t_{max} (h)	3.43 ± 0.98
$t_{1/2}$ (h)	4.45 ± 0.53
K_e (1/h)	0.16 ± 0.02
Cl/*F* (L/h)	81.71 ± 19.84
Vd/*F* (L)	522.76 ± 139.07

AUC area under the curve, C_{max} maximum concentration, *Cl/F* total clearance, K_e elimination rate constant, $t_{1/2}$ plasma elimination half-life, *Vd/F* apparent volume of distribution

destructive processes of neural tissue. The lack of a secondary increase in the intervention group participants suggests that metformin protects the nervous system from further cell destruction.

Much research on the role of neutrophils in the pathogenesis of brain inflammation and BBB breakdown has been done. NLR is known to be a significant indicator for predicting the inflammatory status in patients and its usefulness as a prognosis predictor has been proven in several critical illnesses including nervous system disorders. Chen et al. studied NLR as a predictor of outcome in patients with severe TBI and showed the association of increased NLR with unfavorable 1-year functional outcome and mortality rate in patients [26]. The results obtained by Guo et al. in stroke patients suggest that NLR is a dynamic variable and its temporal variation presents clinical value in identifying risk for unfavorable outcome [31]. In our study, there were no statistically significant between-group differences in the mean levels of NLR at study time points; however, longitudinal analysis of our data showed a more rapid decline in NLR values toward normal levels in the intervention group as compared to the control group. These values correlate favorably with Wang et al. [32], Ibáñez et al. [33], and Soraya et al. [34] and further support the concept of anti-inflammatory benefits of metformin through attenuation of neutrophilia and normalizing the ratio of neutrophils to lymphocytes.

GFAP is a highly specific biomarker of brain tissue damage. Previous research has demonstrated that elevated levels of GFAP following brain trauma are correlated with long-term unfavorable outcomes. The pattern of change in serum GFAP levels of our study participants fits well with Nyle´n et al. [35], Pelinka et al. [36], and Papa et al. [37]. As illustrated in Fig. 3, the value of GFAP serum levels increased initially, reached a peak at 48 h after trauma and declined steadily over the study period. There were no statistically significant differences among study groups in GFAP levels at study time points. Although GFAP is more brain specific as compared to other serum biomarkers, a long biologic half-life of about 24–48 h makes it less suitable for the assessment of treatment efficacy in studies with short-interval sampling [38]. Metformin administration could provide beneficial impacts on the dynamics of GFAP over time if patients were sampled late after the trauma in studies with longer durations.

Statistically distinct longitudinal profiles of serum S100b levels and NLR values in control and intervention groups have further strengthened our conviction that acute metformin administration in severe TBI patients is effective in normalizing the levels of TBI biomarkers, which may favorably affect the risk of secondary injuries of brain trauma.

No events of hypoglycemia and lactic acidosis occurred during either the 120-h study period or the following in-hospital stay period. This finding points to the safety of metformin administration in critically ill patients. As proposed by Lalau et al. [39] metformin-unrelated lactic acidosis is the most common scenario in lactic acidosis reports in diabetic patients and direct metformin-related mortality is almost zero. Metformin improves blood glucose control without increasing insulin levels and thus the potential risk of hypoglycemia is rare. Considering the high incidence rate of severe hypoglycemia and increased risk of death following intensive insulin therapy, metformin would seem to be a highly desirable option for the management of stress-induced hyperglycemia in critically ill patients [40].

In a very recent article attempting to investigate the potential neuroprotective effects of metformin in a rat model of TBI, LiTao et al. stated that metformin significantly ameliorated neurological deficit, cerebral edema, and neuronal apoptosis, decreased the production of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6, reduced neuronal apoptosis and suppressed NF-κB and MAPK activation after TBI [41]. We have obtained satisfactory results indicating that metformin is a safe therapeutic intervention in severe TBI patients which positively influences the serum biomarkers of brain injury. Given that our findings are based on the assays of biomarkers with high prognostic value, the results from such analyses should thus be treated with the utmost caution.

Although our results differ to some extent from those of our previous study on the pharmacokinetics of metformin in critically ill traumatized patients in 2008 [42], it could nevertheless be argued that physiologic alterations in these patients significantly alter the pharmacokinetics of metformin. On average, we found values for the area under the time–metformin concentration curve that are comparable with those of healthy subjects. However, the time to reach the maximal concentration was delayed and the maximal concentration was diminished when compared to normal values. The most likely explanation of the aforementioned results is the motility dysfunction of the gastrointestinal

(GI) tract in critically ill patients. Metformin is absorbed through the small intestine and gastric emptying is likely to be a rate-limiting step in the absorption. Hence, dysmotility of the stomach and small intestine delays drug absorption, prolongs time to reach the maximum concentration and flattens the time–metformin concentration curve. Administration of certain medications, including opioids, catecholamines, and anticholinergics, immobility, shock, being on mechanical ventilation, electrolyte disturbances, and head and spinal injuries are risk factors associated with abnormal GI motility in study patients [43, 44]. As reported by Labuzek et al. [27], orally administered metformin rapidly penetrates the BBB and accumulates in various structures of the CNS. Given that the drug absorption is delayed in study patients, the duration of the lag time to reach the site of action in the CNS will be extended. As was mentioned in "Introduction", earlier suppression of the secondary pathways is critical in the management of the severe TBI patients. Due to the frequent GI abnormalities with potential impacts on the time of drug absorption in critically ill patients, intravenous administration is preferred to achieve a more predictable pharmacokinetic profile.

The current study was limited by the small sample size due to too narrow inclusion/exclusion criteria. However, the strict criteria resulted in the recruitment of patients with a high degree of homogeneity with similar demographic and baseline clinical characteristics, including GCS and APACHE, and on admission biomarkers' levels. Furthermore, individual differences within groups in longitudinal analyses were not significant (p value > 0.05) which diminishes the potential confounders in the study.

Further studies, which take the administration of metformin in TBI patients into account, will need to be undertaken to increase the amount of available data. Despite the small sample size, our work has led us to conclude that metformin could potentially be an effective and safe therapeutic intervention in patients with severe TBI. The strength of our study lies in the widely accepted prognostic value of analyzed biomarkers for long-term clinical outcomes.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical standards The study was performed according to the principles of the declaration of Helsinki and was approved by the Research Ethics Committee of Tehran University of Medical Sciences. Written informed consents were signed by patients' legal guardians prior to their inclusion in the study.

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