BIOLOGICAL SCIENCES

ASSESSMENT OF METFORMIN LEVELS IN RAT LIVER TISSUE AFTER ADMINISTRATION BY DIFFERENT ROUTES

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Abstract

Metformin, a widely used antidiabetic drug, exhibits pleiotropic neuroprotective effects, but the role of hepatic accumulation in mediating these actions remains unclear. This study aimed to compare metformin concentrations in rat liver tissue following oral, intranasal, and intraperitoneal administration of 100 mg/kg/day over 7 and 14 days.

Adult male Wistar rats (n=8 per group) received metformin daily via the specified routes. Liver samples were analyzed using high-performance liquid chromatography with UV detection (HPLC-UV) after protein precipitation and extraction. Data were background-corrected due to limited method selectivity and analyzed via two-way ANOVA with Tukey's post-hoc test.

After 7 days, hepatic metformin levels were highest with oral administration (approximately 20 ng/mg tissue), intermediate with intraperitoneal (10 ng/mg), and lowest with intranasal (5 ng/mg). Prolonging treatment to 14 days significantly reduced concentrations across all routes ($p < 0.05$), with no inter-route differences (approximately 3 ng/mg tissue).

These findings indicate route-dependent initial hepatic uptake, with oral administration maximizing liver exposure short-term, and a general decline over time suggesting potential saturation or enhanced elimination. This provides a pharmacokinetic framework for future studies on metformin's CNS redistribution and neuroprotective potential, highlighting intranasal delivery as a means to bypass hepatic first-pass metabolism.

Keywords: Metformin, HPLC-UV, liver.

1. Introduction

Metformin is one of the most widely used drugs for the treatment of type 2 diabetes mellitus; however, in recent years it has attracted increasing interest as a compound with pronounced pleiotropic effects beyond its classical hypoglycemic action. Experimental and clinical studies have demonstrated the neuroprotective potential of metformin, including its anti-inflammatory, antioxidant [1], and mitochondria-protective effects [2], as well as its ability to modulate autophagy, energy metabolism, and AMPK-related signaling pathways. These effects make metformin a promising candidate for the prevention and treatment of neurodegenerative and cognitive disorders.

Despite intensive research into the neuroprotective properties of metformin, the mechanisms underlying its effects on the central nervous system remain a subject of debate. In particular, it is still unclear to what extent the systemic and peripheral effects of the drug—primarily at the liver level—may mediate its neurotropic action. The liver is a key target organ for metformin and a central regulator of energy and metabolic homeostasis, the disruption of which is closely linked to the development of neurodegenerative processes via the “liver–brain” axis.

The pharmacokinetic characteristics of metformin, including its active transport into hepatocytes via organic cation transporters and pronounced hepatic accumulation, determine both its metabolic and potential systemic effects [3]. At the same time, the route of administration can significantly influence the distribution of the drug between peripheral organs and the central nervous system, as well as the degree of hepatic uptake, which in turn may modulate the neuroprotective effects of metformin.

In recent years, there has been growing interest in alternative routes of metformin administration, including intranasal delivery, which is considered a potential method for targeting the drug to central nervous system structures while partially bypassing systemic circulation and first-pass hepatic metabolism. The intraperitoneal route, widely used in preclinical studies, provides rapid systemic distribution and can serve as a convenient model for comparative pharmacokinetic analyses. However, the impact of different administration routes on hepatic accumulation of metformin and its potential

role in mediating neuroprotective effects remains insufficiently studied.

In this context, the present study aims to comparatively investigate metformin accumulation in liver tissue following oral, intranasal, and intraperitoneal administration as an important step in the comprehensive evaluation of its pleiotropic and neuroprotective properties. The resulting data will help clarify the contribution of hepatic drug distribution to its systemic and central effects and provide a pharmacokinetic basis for further research on metformin as a neuroprotective agent.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (body weight 320 ± 20 g) were used in the present study. The animals were obtained from the Federal State Unitary Enterprise "Laboratory Animal Nursery RAPPOLOVO" (Leningrad Region, Russian Federation). Rats were housed in standard laboratory cages (4–5 animals per cage) under controlled environmental conditions, including a temperature of 24 ± 1 °C, relative humidity of 45–65%, and a 12 h light/12 h dark cycle. Standard laboratory chow and tap water were provided *ad libitum* throughout the experimental period.

All experimental procedures were conducted in accordance with institutional animal care guidelines, the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and applicable national regulations (Ministry of Health of the Russian Federation Order No. 267, June 19, 2003; Guide for the Use of Laboratory Animals, Moscow, 2005). The study protocol was reviewed and approved by the Local Ethics Committee of the Federal State Budgetary Scientific Institution "Institute of Experimental Medicine".

2.2. Experimental design

The animals were randomly assigned to seven experimental groups, with eight rats in each group. Metformin was administered via different routes for either 7 or 14 consecutive days. Group 1 received metformin orally for 7 days, Group 2 received metformin intranasally for 7 days, and Group 3 received metformin intraperitoneally for 7 days. Group 4 received metformin orally for 14 days, Group 5 received metformin intranasally for 14 days, and Group 6 received metformin intraperitoneally for 14 days. Metformin was administered at a dose of 100 mg/kg. An additional control group consisted of intact animals that did not receive any treatment. At the end of the experiment, the rats were deeply anesthetized via intraperitoneal injection of Zoletil (15 mg/kg) and euthanized by decapitation using a guillotine (Open-Science AE1601, RPC Open-Science Ltd, Russia). All collected tissues were immediately frozen and stored at −80°C until further analysis.

2.3. High performance liquid chromatography for determination of metformin

Quantitative determination of metformin was performed using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. Chromatographic separation was carried out on a reversed-phase analytical column Hypersil BDS C18 (250 × 4.6 mm) under isocratic conditions. The mobile phase consisted of 10 mM ammonium acetate, acetonitrile (15%, v/v), and octanesulfonic acid (3 mM), adjusted to pH 6.8. The mobile phase was freshly prepared, filtered through a 0.22 μm membrane filter, and degassed prior to use.

Elution was performed at a constant flow rate under ambient column temperature. The injection volume was set according to the analytical protocol. Metformin was detected using a UV detector at a wavelength of 233 nm. Chromatographic data acquisition and processing were carried out using the chromatography system software.

Quantification of metformin was based on peak area measurements using an external calibration curve constructed from reference standard solutions. The method demonstrated adequate selectivity, with no interfering peaks observed at the retention time of metformin, as well as acceptable linearity and reproducibility within the concentration range relevant to the study [4].

For the quantitative determination of metformin concentration in liver samples using HPLC-UV, a calibration curve was constructed using the external standard method (Fig.1). Initially, a series of standard solutions of metformin hydrochloride in the mobile phase were prepared across a concentration range of 0.1 to 50 μg/mL. Each standard solution was analyzed under the same chromatographic conditions as the experimental samples, and the peak area at the retention time corresponding to metformin was recorded. Based on the obtained data, a calibration curve was plotted as peak area versus concentration and fitted by linear regression. The concentration of metformin in each sample was automatically calculated from the regression equation based on the measured peak area.

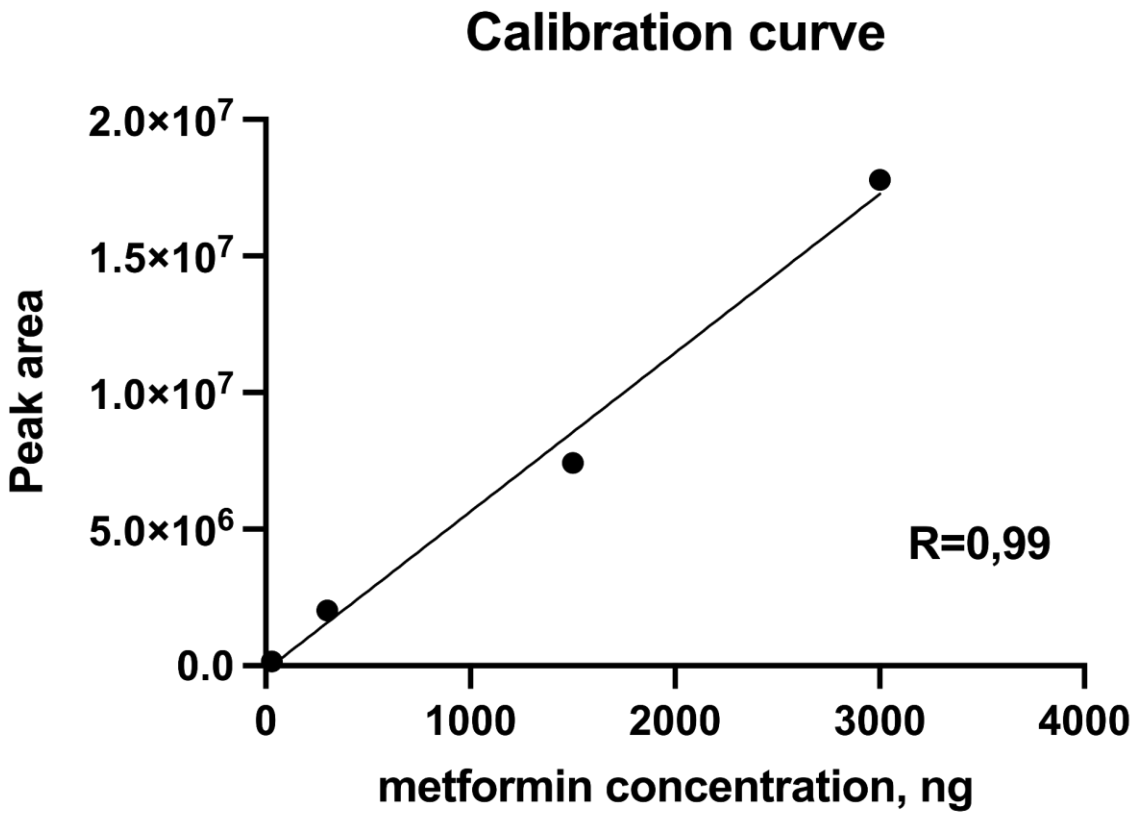

Fig. 1 *Calibration curve for metformin*

2.4.Sample preparation

Liver tissue samples were homogenized in a mixture of acetonitrile and methanol (5:4, v/v) to precipitate proteins and extract metformin. The resulting homogenates were centrifuged, and the supernatant was carefully collected. The supernatant was then evaporated to dryness by heating at 60 °C for 2 h.

The dried residue was reconstituted in the mobile phase, followed by centrifugation to remove any insoluble material. The final supernatant was transferred to autosampler vials and injected into the HPLC system for chromatographic analysis.

2.5.Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0.1 (GraphPad Software, USA). Normality of data distribution was assessed using the Shapiro-Wilk test. All data are presented as mean ± standard error of the mean (SEM) and individual data points. A two-way ANOVA was applied to evaluate the effects of two independent factors: (1) type of administration, and (2) time of administration. Post-hoc comparison procedures were conducted using Tukey's multiple comparison test. Statistical significance was set at $p < 0.05$ for all analyses.

3. Results

The HPLC–UV method used in the present study demonstrated limited selectivity for metformin. A detectable peak at the retention time corresponding to metformin was also observed in liver samples obtained from intact control animals. This background signal was quantified, averaged across control samples, and subsequently subtracted from the values obtained in metformin-treated groups. All data presented therefore represent background-corrected metformin concentrations in liver tissue.

Despite this methodological limitation, the corrected results consistently demonstrate that oral administration is associated with greater hepatic exposure to metformin during short-term treatment, whereas prolonged administration leads to a general decrease in detectable liver levels irrespective of the route of administration.

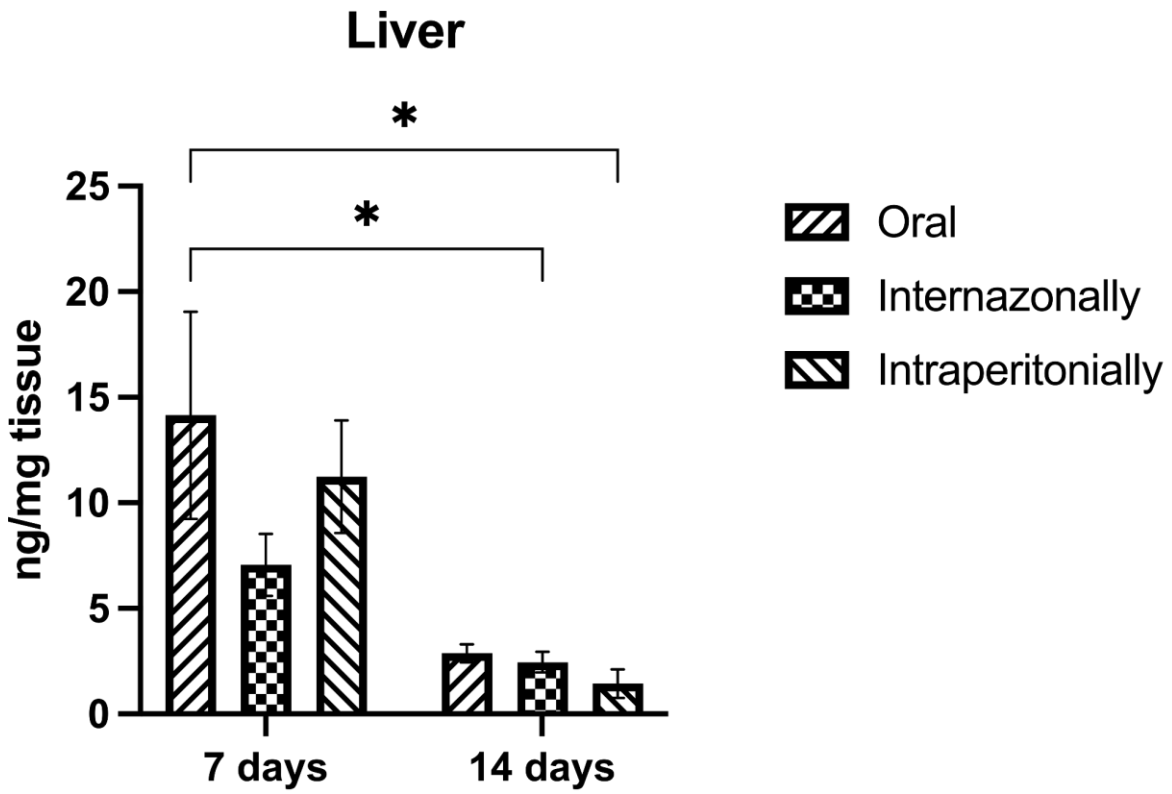

Fig. 2 *Metformin accumulation in liver tissue following oral, intranasal, and intraperitoneal administration for 7 and 14 days. Data are presented as mean ± SEM. Two-way analysis of variance (ANOVA) with Tukey's post-hoc test was used; * – $p < 0.05$.*

Analysis of metformin concentrations in liver tissue revealed significant differences depending on both the route and duration of administration. After 7 days of treatment, hepatic metformin levels were highest following oral administration, intermediate after intraperitoneal administration, and lowest after intranasal administration.

Extension of treatment duration to 14 days resulted in a significant reduction of metformin concentrations in the liver compared with the corresponding 7-day groups ($p < 0.05$). This decrease was observed irrespective of the route of administration. At 14 days, hepatic metformin levels were low and did not differ significantly between the oral, intranasal, and intraperitoneal groups.

Discussion

The present study provides a comparative analysis of metformin accumulation in rat liver tissue following oral, intranasal, and intraperitoneal administration over 7 and 14 days. These findings advance our understanding of how pharmacokinetic distribution between peripheral organs and the central nervous system (CNS) may influence metformin's pleiotropic neuroprotective effects.

The results clearly show that the route of administration markedly affects initial hepatic uptake. Highest liver concentrations after 7 days of oral dosing confirm the liver as a primary depot under standard administration, driven by extensive first-pass extraction and active uptake via organic cation transporters (OCT1) [5]. In contrast, intranasal delivery resulted in the lowest hepatic levels, consistent with partial bypassing of systemic circulation and first-pass metabolism, potentially favoring greater CNS availability [6], [7]. Intraperitoneal administration yielded intermediate accumulation, reflecting rapid systemic absorption without pronounced hepatic first-pass effects.

Most notably, hepatic metformin concentrations significantly decreased after 14 days across all routes. This time-dependent decline likely reflects adaptive processes, such as saturation of hepatic uptake mechanisms or enhanced elimination, allowing greater redistribution to extrahepatic tissues—including the brain—during prolonged treatment.

These dynamics have direct implications for metformin's neuroprotective potential. Early high hepatic exposure (especially oral) may prioritize peripheral metabolic actions, whereas reduced liver accumulation over time, or bypass via intranasal delivery, could enhance direct CNS effects (e.g., AMPK activation, anti-inflammatory actions). Intranasal administration thus emerges as a promising strategy to minimize hepatic sequestration and maximize brain targeting in neurodegenerative models.

Study limitations include the HPLC-UV method's limited selectivity (requiring background correction) and lack of concurrent brain or plasma measurements to confirm redistribution. Future studies should employ more sensitive techniques (e.g., LC-MS/MS) and directly assess CNS accumulation alongside cognitive outcomes to validate the hepatic bypass/redistribution hypothesis.

In summary, route- and duration-dependent hepatic pharmacokinetics provide a rational framework

for optimizing metformin regimens aimed at neuroprotection, with intranasal delivery offering particular promise for enhancing brain exposure.

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