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Effects of intravenous infusion of glycerol on blood parameters and urinary glycerol concentrations

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ABSTRACT

In sports, the oral intake and intravenous administration of glycerol as a potential masking agent have been prohibited. The effect of glycerol on blood parameters was investigated by comparing the intravenous administration of glycerol (20 g/200 mL) with that of an electrolyte (8 g glucose/200 mL) as a comparator (n = 7, fixed-dose-rate i.v. infusion, 200 mL in 1 h). This study was also designed to evaluate whether the urinary concentrations reached the positivity threshold after the intravenous infusion of glycerol. Significant decreases of the haemoglobin (HGB, g/dL), haematocrit (HCT, %) and OFF-h Score (OFF-score) values were observed after the infusion of glycerol ($P < 0.05$ at 1–6 h). The differences in the HGB, HCT and OFF-score between pre- and post-administration were -0.49 ± 0.23 g/dL (2 h), -1.54 ± 0.73% (2 h) and -3.89 ± 3.66 (2 h), respectively. Glycerol infusion significantly increased the plasma volume by 12.1% (1 h), 6.3% (2 h) and 5.7% (3 h) compared with the initial values. The infusion of the comparator also increased the plasma volume by 9.6% (1 h), 5.8% (2 h) and 4.9% (3 h) compared with the values before infusion. There were no significant differences in the change of the plasma volume between the intravenous infusions of glycerol and the glucose-based electrolyte (as the comparator) ($P \geq 0.05$). This finding might indicate that glycerol itself only exhibited limited effects on the expansion of plasma. After administration of glycerol, the urinary glycerol concentrations increased from 0.0013 ± 0.0004 mg/mL to 6.86 ± 2.86 mg/mL at 1 h and 6.45 ± 3.08 mg/mL at 2 h. The intravenous infusion of glycerol can most likely be detected using the current urine analysis; however, the dependence of the concentration of urinary glycerol on the urine volume should be considered.

1. Introduction

The abuse of drugs is a serious public issue that can damage human health and cause social problems such as dangerous driving or violence. The misuse of substances or methods that enhance an athlete's physical or mental abilities is prohibited by the World Anti-Doping Agency (WADA) [1]. Drug testing to detect illegal drug use in these situations can play a key role for legal purposes. Blood doping, the use of erythropoiesis-stimulating agents (ESAs) and blood transfusions enhance oxygen-transport capacities and have recently posed a serious problem in sports [2]. Providing statistical analysis of the variation of haematological data, the athlete biological passport (ABP) enables the indirect long-term detection of blood doping and can lead to additional testing for detection of the use of ESAs [3]. A crafty abuser attempts to falsify test results using sample substitution, adulteration, tampering, manipulation or the use of masking agents [4–6]. The administration of diuretics, plasma volume expanders and intravenous transfusions can theoretically alter the elevated concentration of targeted substances and the relevant parameters in urine and blood. To address such illicit behaviour, the misuses of plasma volume expanders and intravenous infusions and/or injections of more than 50 mL per 6 h period are prohibited by the WADA [7].

Since 2010, the oral intake and intravenous administration of glycerol as a potential masking agent have been prohibited [7]. Glycerol, triatomic alcohol (1,2,3-propanetriol), is widely used in products such as liquid soaps, cosmetics, lubricants and antifreeze liquid. For clinical purposes, the addition of glycerol to human blood enables its long-term storage at freezing temperature for long periods; the blood can subsequently be used for blood transfusions [8,9]. Glycerol has also been therapeutically used for the treatment of increased intracranial pressure and acute stroke [10,11]. In sports, the ingestion of glycerol in combination with excess fluid can lead to increased plasma osmolality, reduced urine volume and expanded plasma volume [12]. The hyperhydration and fluid retention resulting from glycerol intake has been

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demonstrated to enhance performance during exercise under hot conditions [13,14]; however, another study demonstrated no significant benefit of glycerol ingestion [15]. The effect of hyperhydration achieved by glycerol ingestion on doping-related haematological blood parameters has been investigated in various situations [16–18]. These studies revealed that the effects of the oral administration of glycerol on blood parameters were limited, which indicated that successful blood manipulation would be difficult to achieve [19].

Glycerol is normally present in the form of its esters (triglycerides) in the human body and is produced in circulation when lipolysis is induced [20]; it is recognised that glycerol metabolism is affected by exercise or fasting [20]. The glycerol concentrations in plasma were reported to be 0.047–0.125 mmol/L-plasma (4.3–11.5 μg/mL) in Beijing residents [21] and 0.04–0.33 mmol/L-serum (3.7–30.4 μg/mL) in 468 outpatients [22].

In sports drug testing, it is important to differentiate exogenous glycerol from naturally occurring glycerol. For urine levels, Thevis et al. reported that the natural occurrence of glycerol in urine does not exceed 0.2 mg/mL based on a population study in 1039 athletes [23]. In 2012, the urinary threshold for glycerol was set as 1.0 mg/mL as evidence of glycerol doping [24]; however, some impaired athletes have been observed to have elevated urinary glycerol caused by self-catheterisation with glycerol for urination [25]. Furthermore, a population study of urinary glycerol in 959 North American athletes was conducted [26]; consequently, the WADA redefined the threshold at 4.3 mg/mL based on statistical data [27]. When the urinary concentration exceeds the decision limit (DL), which is calculated as the sum of the threshold values and the guard band at a 95% confidence interval, it is considered an adverse analytical finding. Since September 2014, the DL of glycerol has been set as 5.3 mg/mL [27].

After oral administration of glycerol (combination with 1.0 g/kg and 25 mL fluid/kg) [16,18], the glycerol content in urine increased to approximately 50 mg/mL after 2 h and exceeded the WADA DL. Thus, the oral use of glycerol can be effectively detected using the current urine analysis approved by the WADA. However, to date, the effects of the intravenous administration of glycerol on the doping-relevant blood parameters and urinary glycerol levels have rarely been investigated. The effect of glycerol on the blood parameters was investigated by comparing the intravenous administration of glycerol with that of an electrolyte as a comparator. This study was also designed to evaluate whether the urinary concentrations reached the WADA DL after intravenous administration of glycerol.

2. Experimental

2.1. Chemicals

N-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA, >98.5%, Fluka 69479) was obtained from Sigma (St. Louis, MO, USA). Ultra-purified water was prepared using a Milli-Q Ultra purified system (Millipore, Bedford, MA, USA). Human whole blood (female with type O blood, citrate phosphate dextrose adenine-1: CPDA-1) was purchased from Kojin Bio Co., Ltd. (Saitama, Japan). Glycerol (86%) was purchased from Carl Roth GmbH (Karlsruhe, Germany). [1,1,2,3,3-$^2H_5$]glycerol (D_5-glycerol, 99%) was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Because small amounts of glycerol are detected in normal urine and blood, a matrix of calibration solutions was used using 0.9% (w/v) NaCl (saline). The stock solution of glycerol was prepared in saline with a concentration of 10 mg/mL. The calibration solutions were prepared at 0.001, 0.003, 0.006, 0.012, 0.024 and 0.048 mg/mL by diluting with saline. The internal standard (D_5-glycerol) solution was prepared in ultra-purified water; its concentration was 1.32 mg/mL.

For the excretion studies, GLYCEOL® was purchased from Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). The contents of 200 mL GLYCEOL® were 20 g glycerol, 10 g fructose and 1.8 g NaCl. The electrolyte KN No. 4 Injection (as comparator) was purchased from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). The contents of 200 mL of KN No. 4 Injection were 8 g glucose, 0.2 g sodium L-lactate and 0.2 g NaCl.

2.2. Blood parameters

The blood samples were delivered to the laboratory within 36 h of blood collection, where the blood markers haematocrit (HCT, %), haemoglobin (HGB, g/dL) and reticulocytes percentage (RET%) were measured using a Sysmex XT-2000i (Hyogo, Japan) within 12 h after their arrival. All the procedures were performed in accordance with the WADA ABP operating guidelines [3]. The OFF-h Score (OFF-score) [28] was calculated according to Equation (1):

$$\text{OFF-score} = \text{HGB} - 60 \times \sqrt{\text{RET\%}} \quad (1)$$

The differences in HCT, HGB and OFF-score between pre- and post-administration were expressed as ΔHCT, ΔHGB and ΔOFF-score, respectively. The change in plasma volume (PV%) was calculated according to Equation (2) reported by Dill and Costill [29].

$$\text{PV\%} = (((100 - \text{HCT}_{\text{post}})/\text{HGB}_{\text{post}})/((100 - \text{HCT}_{\text{pre}})/\text{HGB}_{\text{pre}}) - 1) \times 100. \quad (2)$$

Note that the whole blood samples were diluted using an 'isotonic' reagent in the measurement of HCT using the most commercially available automated cell counters, such as Sysmex XT-2000i. Thus, these HCT values may not reflect the true circulating red cell volume when the plasma osmolality is different from that of the 'isotonic' diluent [30]. Therefore, PV% calculated using the HCT values might include the effects induced by the change of plasma osmolality after administration. Nonetheless, the HCT values measured by the Sysmex analyser were used for calculating the plasma volume change in this study because the purpose of this study was to evaluate the effect of the administration of glycerol on the ABP programme using a Sysmex analyser.

2.3. Determination of glycerol concentration

The glycerol concentrations were determined using gas chromatography/mass spectrometry (GC–MS) based on the stable isotope dilution method developed by Thevis *et al.* [23]. When the concentrations of glycerol were outside the calibration range, the samples were diluted using saline to fit the range calibrated.

2.3.1. Urine analysis

The determination of urinary glycerol was validated and conducted according to the previous study [25]. In brief, the urine samples (20 μL) were fortified with D_5-glycerol solution (20 μL), and the mixtures were subsequently evaporated for 30 min at 80 °C. The dried residue was dissolved in MSTFA (60 μL) and heated for 20 min at 60 °C. The reaction mixture (1 μL) was injected into the GC–MS system (split 47:1, 300 °C). The GC–MS system was an Agilent 6890 N GC-5973MSD (Agilent Technologies, Palo Alto, CA, USA). The column separation was conducted using a DB-5 (Agilent, 15 m × 0.25 mm, 0.25 μm, He at 15 psi). The oven temperature was set to 80 °C (hold 5 min) and was increased at a rate of 0.5 °C/min to 83 °C and then at a rate of 40 °C/min to 320 °C (hold 5 min). The interface temperature and source temperature were set to 320 °C and 230 °C, respectively. Electron ionisation was conducted at 70 eV. All the data were acquired using the selected ion-monitoring mode