Effect of Succinic Acid and Tween-80 on Glucuronidation of 2-Ethyl-6-Methyl-3-Hydroxypyridine

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 7, pp. 62-64, July, 2008
Original article submitted October 1, 2007.

We studied the effect of succinic acid on the process of glucuronidation of 2-ethyl-6-methyl-3-hydroxypyridine after peroral and intraperitoneal administration in the form of succinate or a base. Since the basic form of 2-ethyl-6-methyl-3-hydroxypyridine is insoluble in water, it was administered in 5% Tween-80. It was necessary to evaluate also the effect of Tween-80 on glucuronidation of 2-ethyl-6-methyl-3-hydroxypyridine in different administration routes. Quantitative assay of glucuronidated fractions was performed by the method of reversed-phase HPLC with fluorometrical detection. The detection limit for this method was 10 ng/ml. We confirmed that the major excretion pathway for 2-ethyl-6-methyl-3-hydroxypyridine is conjugation with glucuronic acid. It was found that succinic acid increased excretion of glucuronidated metabolite after both peroral and intraperitoneal administration of 2-ethyl-6-methyl-3-hydroxypyridine in the form of succinate and base in 5% Tween-80. The effect of Tween-80 was detected only after peroral administration, which was probably related to its effect on absorption of this compound. Tween-80 increased excretion of glucuronate after peroral administration of 2-ethyl-6-methyl-3-hydroxypyridine in the form of succinate and in 5% Tween solution.

Key Words: *succinic acid; Tween-80; 2-ethyl-6-methyl-3-hydroxypyridine; glucuronidation*

MATERIALS AND METHODS

Experiments were carried out on 83 outbred male mice weighing 18-22 g (Stolbovaya nursery, Russian Academy of Medical Sciences) maintained under standard vivarium regimen in V. V. Zakusov Institute of Pharmacology). The animals were maintained on water diet for 12 h before the experiments. The animals were randomly divided into 4 groups (including the control group).

Aqueous solution of 2-ethyl-6-methyl-3-hydroxypyridine succinate (HPS) and solutions of 2-ethyl-6-methyl-3-hydroxypyridine (HP)-Tween and HPS-Tween-80 (5% Tween) were prepared *ex tempore* and were administered to fasting animals *per os* with a metal probe or intraperitoneally with an insulin syringe. The dose of HPS and HPS-Tween administered to mice was 100 mg/kg, and the dose HP-Tween was corrected with consideration for the molecular weight of succinic acid (118 g/mol) and was 53.7 mg/kg.

The animals were placed into individual metabolic cages with free access to water for 24 h. Diurnal urine was collected into glass tubes and adjusted to a volume of 5 ml with water: 2.5 ml was taken for measurement of initial substance and 2.5 ml was used for measurement of glucuronidated metabolite (all dilutions were taken into account in further calculations).

Isolation of HP and its glucuronate was performed as follows: 3 ml 1 M borate buffer (pH 9.0) was added to 1 ml urine and extracted with 6 ml

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0007-4888/08/1461-0056

ethylacetate (Reakhim) for 15 min on an electrical shaker; the procedure was repeated twice. The extracts were pooled and evaporated on a water bath at 77°C. The dry residue was dissolved in 5 ml mobile phase.

Glucuronidated fractions were assayed in the urine after its incubation with 3000 U/ml β-glucuronidase (from cattle liver, DiaM) at 23°C for 24 h.

Quantitative assay was performed by the method of reversed-phase HPLC in isocratic regimen (Beckman Coulter System Gold 127) with fluorometrical detection (Shimadzu RF-10A XL). Chromatography conditions were as follows: stationary phase: Luna C18(2) Phenomenex column (250× 4.60 mm, 5 μ); mobile phase: methanol (Merck): citrate-phosphate buffer (pH 5.0) (2.5:6.0 v/v); mobile phase flow rate 1.0 ml/min; detection at 310 nm (excitation) and 405 nm (emission); chromatograph loop volume 20 μl. Under these conditions the retention time for HP was 4.60 min. The detection limit for this method was 10 ng/ml.

RESULTS

It was found that after both peroral and intraperitoneal administration of all three HP formulas (HPS, HP-Tween, and HPS-Tween), the urinary excretion of glucuronidated metabolite surpassed that of the unchanged compound ($p<0.05$, Table 1). For instance, after peroral and intraperitoneal administration, elimination of glucuronate surpassed that of HP by 7 and 10 times, respectively (Fig. 1, 2).

Detailed analysis of excretion showed that the maximum amount of unchanged compound is excreted with the urine after peroral and intraperitoneal administration of HPS-Tween (2.89±2.40% and 3.00±2.69% from administered dose, Fig. 1, Table 1). The maximum amount of glucuronate is excreted after peroral and intraperitoneal administration of both HPS-Tween and HPS (Fig. 2, Table 1). Thus, succinate considerably modulates conjugation of HP with glucuronic acid, which is clearly seen from the data on excretion of glucuronidated

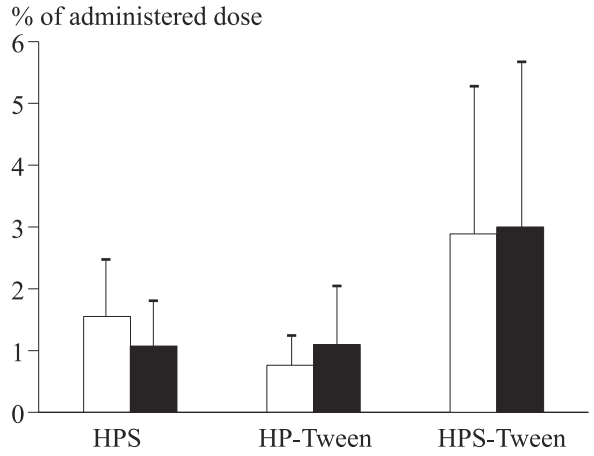

Fig. 1. Excretion of unchanged substance after peroral (light bars) and intraperitoneal (dark bars) administration of HP forms.

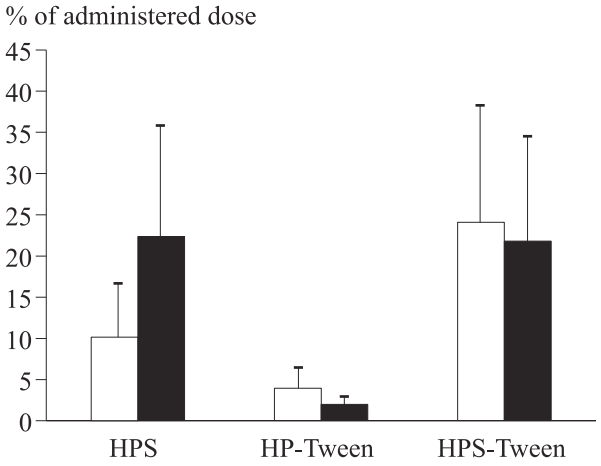

Fig. 2. Excretion of glucuronidated substance after peroral (light bars) and intraperitoneal (dark bars) administration of HP forms.

TABLE 1. Excretion of HP and Its Glucuronate after Peroral and Intraperitoneal Administration of HPS, HP-Tween, and HPS-Tween (%)

Formula	Peroral administration		Intraperitoneal admionistration	
	unchanged compound	glucuronidated metabolite	unchanged compound	glucuronidated metabolite
HPS	1.55±0.95	10.14±6.52	1.07±0.77	22.35±13.64
	$P=0.001$		$P=7.28\times10^{-7}$	
HP-Tween	0.76±0.46	3.92±2.53	1.10±0.95	1.97±0.96
	$P=5.91\times10^{-4}$		$P=0.045$	
HPS-Tween	2.89±2.40	24.07±14.55	3.00±2.69	21.79±12.83
	$P=5.59\times10^{-5}$		$P=1.21\times10^{-4}$	

TABLE 2. Effect of Succinic Acid on Excretion of HP and Its Glucuronate

Formula	Peroral administration		Intraperitoneal admionistration	
	unchanged compound	glucuronate	unchanged compound	glucuronate
HP-Tween	0.76±0.46	3.92±2.53	1.10±0.95	1.97±0.96
	$P=4.63\times10^{-5}$	$P=2.48\times10^{-5}$	$P=0.039$	$P=5.35\times10^{-5}$
HPS-Tween	2.89±2.40	24.07±14.55	3.00±2.69	21.79±12.83

TABLE 3. Effect of Tween-80 on Excretion of HP and Its Glucuronate

Formula	Peroral administration		Intraperitoneal admionistration	
	unchanged compound	glucuronate	unchanged compound	glucuronate
HPS	1.55±0.95	10.14±6.52	1.07±0.77	22.35±13.64
	$P=0.131$	$P=0.015$	$P=0.011$	$P=0.915$
HPS-Tween	2.89±2.40	24.07±14.55	3.00±2.69	21.79±12.83

metabolite after intraperitoneal and peroral administration of HPS-Tween and HP-Tween.

Thus, comparative analysis of the results obtained after administration of HPS and HP in 5% Tween-80 showed that the presence of succinic acid increased ($p<0.05$) excretion of unchanged and glucuronidated HP by on average 3 and 9 times, respectively, both after peroral and intraperitoneal administration (Table 2, Fig. 1, 2).

The effect of Tween-80 was analyzed by comparing the data obtained after administration of aqueous solutions of HPS and HPS-Tween. It was found that the presence of Tween-80 increased excretion of native compound after intraperitoneal injection, and had no effect after peroral administration (Fig. 1, Table 3) and excretion of glucuronidated metabolite after peroral administration (Fig. 2, Table 3).

Thus, we can conclude that the presence of succinic acid definitely affects the process of glucuronidation of HP. The effect of Tween-80 on glucuronidation of HP is less pronounced, but after peroral administration it almost 3-fold increased excretion of glucuronate.

Thus, we studied the effect of succinic acid and Tween-80 on glucuronidation of HP administered via different routes. Similarly to previous experiments [1,2], we confirmed that conjugation with glucuronic acid is the main excretion pathway for HP. We also demonstrated the effect of succinic acid on glucuronidation process. Succinic acid probably increased the degree of ionization of HP compared to the basic form. Increased ionization reduces reabsorption of HP in renal tubules and hence, promotes its excretion [3].

Tween-80 modulates glucuronidation of HP only after peroral treatment. It can be hypothesized that Tween-80 possessing pronounced hydrophilic properties and promoting micelle formation increases absorption of 2-ethyl-6-methyl-3-hydroxypyridine from the gastrointestinal tract to systemic circulation.

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