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In Vivo Interaction of Morphine and Diclofenac

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Abstract

The number of studies on possible pharmacokinetic interactions between opioid analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs), which are commonly used in combination for the treatment of chronic pain, is limited. In rats, the major metabolic pathway of morphine is glucuronidation to morphine-3-glucuronide (M3G) by UDP-glucuronosyltransferase. In this study, we investigated the influence of diclofenac (NSAID) on the formation of M3G *in vitro* using rat liver tissue homogenates. Competitive inhibition of M3G formation by diclofenac was observed with an average Ki of 19.9 μM. Because these *in vitro* findings suggested that a pharmacokinetic interaction occurs *in vivo*, we investigated whether diclofenac inhibits the glucuronidation of morphine in rats. A single dose of diclofenac increased serum concentrations of both morphine and M3G and showed a higher analgesic efficacy in the Von Frey test. Furthermore, diclofenac caused a net decrease in morphine urine concentrations, but the excretion of M3G through biliary and urinary routes was unchanged. These results demonstrated that in contrast to *in vitro* data a single dose of diclofenac did not alter the glucuronidation of morphine *in vivo*.

Keywords

Morphine, Diclofenac, Udp-Glucuronosyltransferase, Von Frey Test

1. Introduction

Various studies have demonstrated a synergistic analgesic effect of opioid-NSAID combinations [1] [2] [3]. This synergistic effect is considered to be because of known different pharmacodynamic mechanisms of the two groups—opioids act via opioid receptors in the central nervous system and NSAIDs affect the synthesis of prostaglandins by inhibiting cyclooxygenase.

In rats, morphine is metabolized abundantly to morphine-3-glucuronide (M3G) by

UDG-glucuronosyltransferase (UGT) [4]. Diclofenac has previously been demonstrated to induce a marked inhibition of morphine glucuronidation in human liver tissue homogenate [5] [6]. Because morphine clearance is dependent on UGTs, its inhibition by diclofenac may lead to decreased M3G formation, modifying the total effect of opioid. Because *in vitro* findings may not necessarily be of clinical relevance, we aimed to investigate *in vivo* whether diclofenac inhibits morphine glucuronidation in rats with regard to pharmacokinetics and analgesic efficacy.

2. Materials and Methods

2.1. Chemicals and Reagents

Morphine hydrochloride was purchased from the Takeda Chemical Industries (Osaka, Japan). M3G was a generous gift from Prof. Hideyuki Yamada, Kyushu University (Fukuoka, Japan). Diclofenac, naloxone, uridine 5'-diphosphoglucronic acid (UDPGA), and alamethicin were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Animals

Male Fisher rats (7 - 10-week-old; 170 - 200 g) were purchased from Sankyo Laboratory Animal Co., Ltd. (Hamamatsu, Japan). Rats were housed under a 12-h light/dark cycle with free access to food and water. All animal procedures were carried out in accordance with the standards set forth in the Guidelines for the Care and Use of Laboratory Animals at the Takara-machi campus of Kanazawa University, and the experimental protocol was approved by the Institutional Animal Care and Use Committee of Kanazawa University, Japan.

2.3. Morphine Glucuronosyltransferase Activity

Morphine glucuronosyltransferase activity was determined as described by Hara *et al.* [5]. A typical incubation mixture (0.2 mL total volume) contained 50 mM Tris-HCl buffer (pH 7.4), 5 mM $MgCl_2$, 5 mM UDPGA, 25 μg/mL alamethicin, 0.25 mg/mL microsomal protein, and 25 - 200 μM morphine. The reaction was initiated by addition of UDPGA. After incubation at 37˚C for 30 min, the reaction was terminated by the addition of 0.1-mL ice-cold perchloric acid. After removal of protein by centrifugation at 10,000 g for 5 min, at 4˚C, a 100-mL portion of the supernatant was subjected to HPLC (HPLC method is described below). All data were analyzed using the mean of triplicate determinations. Dixon plots were used for determining the type of inhibition. Kinetic parameters were determined by a nonlinear regression analysis using SigmaPlot 13 (Hulinks, Tokyo).

2.4. Prediction of *In Vivo* Drug-Drug Interactions Using *In Vitro* Data

Change in intrinsic clearance (CLint) is expressed using the following equation [7]: CLint (+inhibitor)/CLint (−inhibitor) = 1/(1 + I/Ki), where I is the concentration of the inhibitor and Ki is the inhibition constant. Because data on liver concentrations and protein binding of diclofenac in tissues are not available, maximum plasma concentra-

tions were used.

2.5. Blood, Bile, and Urine Sampling

Blood samples were taken before drug administration as well as at 0.25, 0.5, 0.45, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 35, and 36 h after subcutaneous (s.c.) administration of morphine and intraperitoneal injection (i.p.) of either saline (vehicle) or diclofenac from the jugular vein under light ether anesthesia. Blood samples were centrifuged after 30 min and serum was stored at −20˚C until analysis. Bile samples were collected via bile duct cannulation at 0 - 1.5, 1.5 - 3, 3 - 4.5, and 4.5 - 6 h after dose administration. Bile pools for each time interval were created by proportional mixing of samples from each rat. Urine was collected before drug administration and at 0 - 3, 3 - 6, and 6 - 12 h after drug administration. The volume was measured and an aliquot of each collection was stored at −20˚C until analysis.

2.6. Determination of Morphine in Rat Blood, Bile, and Urine Samples

Morphine and M3G concentrations were extracted by solid-phase extraction and measured by HPLC as described by Hasegawa *et al.* [8]. Solid-phase extraction was performed using Inertsep C18-C FF cartridges (GL Science Inc., Tokyo, Japan.). 200 µl Samples were added to 20 µl naloxone (internal standard) and loaded onto the cartridges. The plasma samples were loaded undiluted; urine samples were loaded after dilution in saline. The eluent was evaporated at 50˚C under reduced pressure. The residue was dissolved in 300 µl mobile phase, and an aliquot was injected onto the HPLC system. The reverse-phase column (TSKgel ODS-80TM., Tokyo, Japan) was maintained at 50˚C. The mobile phase was [0.1 M phosphate buffer (pH 2.5), acetonitrile, and methanol (72:24:2 v/v)] delivered at a constant flow of 1 ml/min for a total run time of 70 min. Morphine was detected using an electrochemical detector (Coulochem II; Esa Inc., Chelmsford, MA, USA). M3G was quantitated by a sensitive and specific HPLC method using fluorescence detection (excitation: 210 nm; emission: 350 nm).

2.7. Pharmacokinetic Evaluation

Pharmacokinetic parameters were estimated using model-independent moment analysis as described by Yamaoka *et al.* [9]. Data were analyzed using Student's *t*-test to compare the unpaired mean values of two datasets. The number of determinations is noted in each table and figure. p value <0.05 was considered to be statistically significant.

2.8. Determination of Analgesic Effect

Analgesic effects of morphine with and without simultaneous administration of diclofenac were assessed using the Von Frey test as described by Shinoda *et al.* [10]. Briefly, the mechanical sensitivity of the plantar surface of the hind paw was assessed using Von Frey hairs [11]. To assess changes in mechanical nociceptive thresholds, rats were placed in cages with a mesh floor covered with transparent plastic boxes and were al-

lowed to acclimate to their surroundings for a minimum of 15 min before testing in a temperature-controlled room (24˚C). The von Frey hairs were pressed against the plantar surface of the hind paw and withdrawal response frequency was measured from 5 trials. For each trial, the filament was applied at 1 min intervals. Paw-withdrawal threshold was defined as the minimum pressure needed to evoke a response in at least 60% of the trials. Subsequently, the changes from baseline were determined and for each study day the area under the pain threshold change versus time curve was calculated.

The anti-nociceptive effect was expressed as a percentage of the maximum possible effect (MPE): %MPE = (post-drug threshold − pre-drug threshold)/(maximum threshold − pre-drug threshold) × 100, where pre-drug threshold is the mean of the paw-withdrawal threshold in the saline group, post-drug threshold is the paw-withdrawal threshold of each animal treated with drug, and maximum threshold is the mean of the paw-withdrawal threshold in the sham-saline group. The area under the pain threshold versus time curves were presented as mean ± SD and were compared using Wilcoxon matched pairs test. p value <0.05 was considered significant.

3. Results

3.1. Effect of Diclofenac on Morphine Glucuronosyltransferase

Inhibitory effects of drugs on morphine glucuronosyltransferase activities in rat liver microsomes are shown in Figure 1. Our results indicated that morphine glucuronosyltransferase activities were strongly inhibited by diclofenac. The IC_{50} value of diclofenac was 19.9 ± 3.80 μM, and diclofenac exhibited competitive inhibition for morphine glucuronosyltransferase activities.

3.2. Predicted Change of *In Vivo* Morphine Clearance by Various Drugs from *In Vitro* Data

We examined the possibility of drug-drug interactions via a metabolic process between morphine and diclofenac. The 1 + I/Ki values calculated for diclofenac were 2.65 for morphine glucuronosyltransferase activities, indicating that the change of plasma

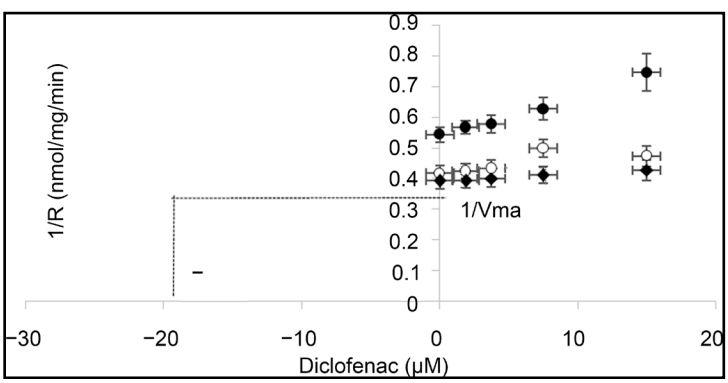


Figure 1. Effect of morphine glucuronosyltransferase activities in rat liver microsomes, ●, 50 μM morphine; ○, 100 μM morphine; ◆, 200 μM morphine. Lines were drawn by linear regression analysis (n = 4).

concentration would be because of the inhibition of morphine glucuronidation by diclofenac.

3.3. Effect of Diclofenac on Morphine and M3G Disposition

Plasma concentrations of morphine after s.c. administration in rats pretreated with vehicle or diclofenac are shown in Figure 2. The plasma concentration of morphine in vehicle-pretreated rats reached a maximum (3.90 μg/ml) of 15 min after administration. (Figure 2(a)) In diclofenac-pretreated rats, the plasma concentration was 7.2 μg/ml at 15 min. The plasma concentration was markedly higher in diclofenac-pretreated rats than in vehicle-treated controls for all time points. AUC was also larger (2.14 fold) in diclofenac-pretreated rats than that in vehicle-pretreated rats. Diclofenac treatment significantly decreased the CL_{total} (by 54.6%) and volume of distribution of morphine (by 51.9%) (Table 1). The plasma concentration time profiles of M3G are shown in Figure 2(b). Concentration of M3G in the plasma in diclofenac-pretreated rats was increased by 1.38 fold at 30 min and 3.34 fold at 90 min compared to that in vehicle-pretreated rats (Figure 2).

The urinary and biliary excretion ratios of morphine and M3G are shown in Figure 3. The biliary excretion ratios of morphine and M3G and the urinary excretion of M3G were similar in the two groups. However, urinary excretion of morphine was significantly lower in diclofenac-pretreated rats [53.7% (0 - 3 h) of vehicle] ($p < 0.05$).

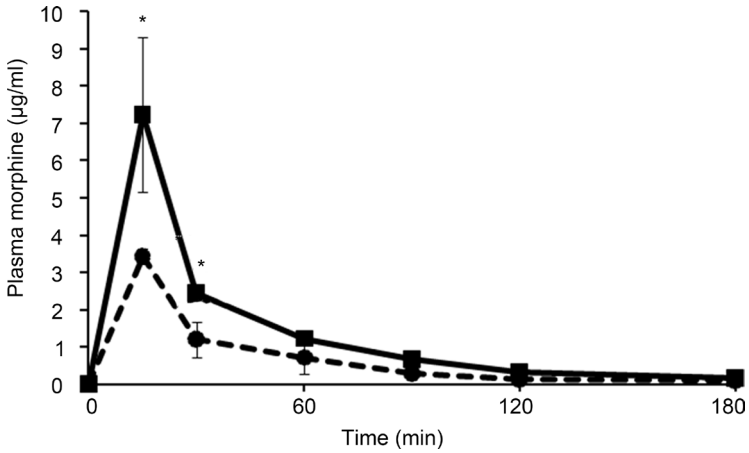
3.4. Effect of Diclofenac on the Anti-Nociceptive Effect of Morphine

The anti-nociceptive effect of morphine was determined by the Von Frey test in vehicle- and diclofenac-pretreated rats as shown in Figure 4. The anti-nociceptive effect of morphine was 18.0% - 68.8% of the MPE in vehicle-pretreated rats. Administration of diclofenac increased the anti-nociceptive effect of morphine to 40.9% - 98.4 % MPE. The area under the anti-nociceptive effect time curve was 1.57-fold greater in diclofenac-pretreated rats than that in vehicle-pretreated rats (16800 ± 790 vs. 9700 ± 855 %MPE·min; mean ± SE; n = 6).

4. Discussion

The possible pharmacokinetic interaction between morphine and diclofenac could have clinical implications. Considering that diclofenac inhibited morphine glucuronidation in rat liver microsomes, it was important to verify whether morphine glucuronidation was also inhibited by diclofenac *in vivo* after administration of a commonly used dose. While the inhibition of morphine glucuronidation may result in elevated morphine serum levels and a greater analgesic effect, it could also lead to potentially increased adverse effects.

The Ki of diclofenac for M3G formation by rat microsomes was calculated to be 19.9 ± 3.80 μM. The exposure (AUCi (+inhibitor)/AUC (−inhibitor)) ratios were predicted using the equation for drug clearance mediated by metabolism catalysed by a single enzyme. The predicated AUCi/AUC ratio is 2.65 which suggests a significant *in vivo*



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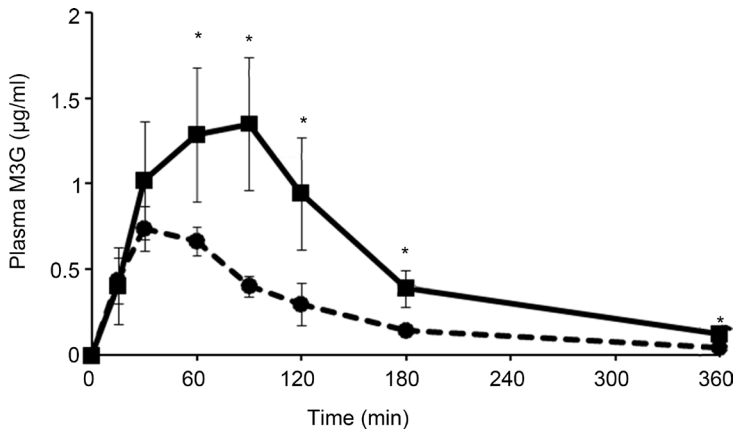


(b)

Figure 2. Plasma morphine (a) and morphine-3-glucuronide (b) concentration following subcutaneous administration in vehicle- and diclofenac-pretreated rats. ●, Morphine; ▪, Morphine + Diclofenac. Each symbol and bar represents the mean ± SD of the three rats. *, Significantly different from control rats ($p < 0.05$).

Table 1. Pharmacokinetic parameters calculated from plasma concentrations of morphine after subcutaneous administration (5 mg/kg) in rats pretreated with vehicle or diclofenac (5 mg/kg, intraperitoneal).

(a) Morphine		
Parameter	**Vehicle**	**Diclofenac**
Tmax (min)	15.0 ± 0002	15.0 ± 0.002
Cmax (μg/ml)	3.90 ± 0.180	7.2 ± 0.189*
AUC (mg/ml·min)	0.132 ± 0.0368	0.282 ± 0.0247*
t1/2 (min)	22.0 ± 0.472	21.0 ± 0.389
Vd (L/kg)	2.70 ± 0.970	1.40 ± 0.337*
CLtot (L/hr/kg)	0.033 ± 0.00953	0.018 ± 0.00152*

Continued

	(b) M3G	
Parameter	**Vehicle**	**Diclofenac**
Tmax (min)	30.0 ± 15.5	90.0 ± 15.4
Cmax (μg/ml)	0.74 ± 0.0091	1.35 ± 0.00322*
AUC (mg/ml·min)	0.098 ± 0.00923	0.232 ± 0.00442*
t1/2 (min)	21.7 ± 0.211	20.3 ± 0.213

$T_{1/2\alpha}$; distribution half-life, $T_{1/2\beta}$; elimination half-life, AUC; area under the blood concentration-time curve, CL_{tot}; total body clearance, Vdss; steady-state volume of distribution, V_1; distribution volume in central compartment, V_2; distribution volume in peripheral compartment, k_{10}; k_{12}; k_{21}; kinetic constants. Values are means ± SD. *$P < 0.05$ vs. control group. *; Significantly different from control rats ($p < 0.05$; n = 3).

pharmacokinetic interaction (AUCi/AUC >2) [12]. After a single dose administration of morphine and diclofenac in rats, the serum concentrations of morphine and, moreover, its glucuronide (M3G) were increased. M3G/morphine AUC ratio, which is gross indictor of hepatic metabolism of morphine, [13] were similar in the two groups (vehicle- and diclofenac-pretreated rats; 0.742 vs 0.823). This results indicated that morphine glucuronidation was not inhibited by diclofenac *in vivo*.

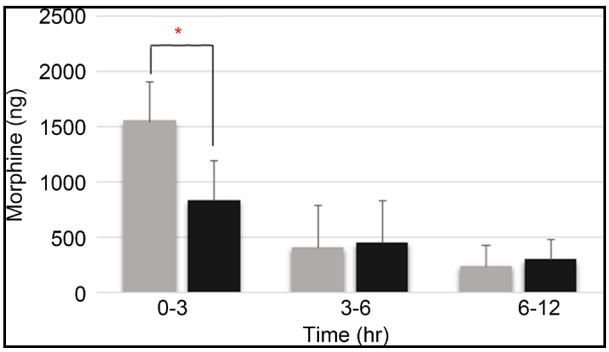
Ammon *et al.* showed that a single dose of diclofenac did not alter the formation of codeine-6-glucuronide in healthy volunteers. They speculated that diclofenac does not achieve serum levels fast enough and high enough to inhibit codeine glucuronidation *in vivo*. In this research the time to achieve peak serum concentrations of M3G (0.5 hr) was faster than that of diclofenac (1.73 hr) [14]. Therefore, it is likely that diclofenac, at least after administration of a common single dose, does not achieve adequately high and fast serum levels to inhibit morphine glucuronidation *in vivo*.

A pharmacokinetic interaction between morphine and diclofenac lead to higher serum levels of morphine and an increase in pain threshold where the area under the pain threshold versus time curve did differ significantly after diclofenac treatment. The increase of morphine concentrations in serum was a result of inhibition of morphine renal excretion by diclofenac. Accordingly, the formation of M3G was increased and the bile and renal excretion of M3G was not unchanged by diclofenac. As a result, M3G concentration in serum was increased.

There are several studies demonstrating the benefit of NSAIDs-opioids combination in comparison to opioids alone in the treatment of postsurgical pain, pain induced by arthrosis, and chronic pain in cancer patients [15] [16] [17]. Thus, our findings that diclofenac influences the pharmacokinetics/pharmacodynamics of morphine may be of clinical relevance.

5. Conclusion

In conclusion, our results indicate that a single dose of diclofenac did not alter the glucuronidation of morphine *in vivo*, which is in contrast to *in vitro* data. However,



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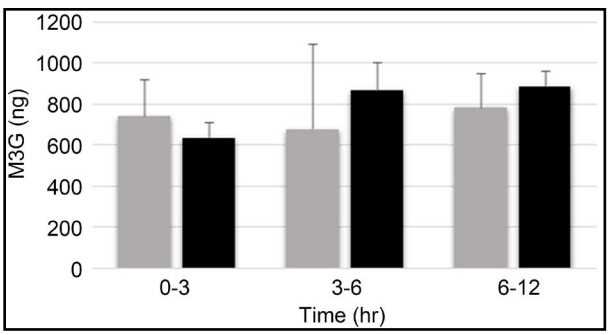

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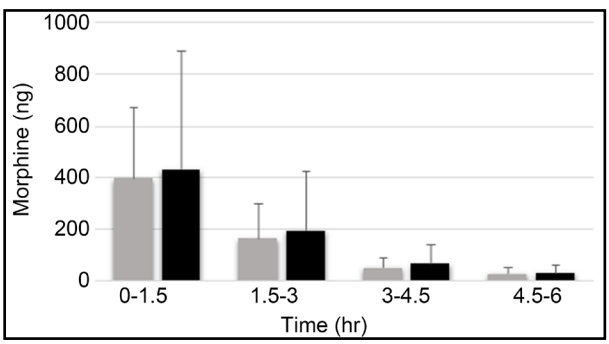

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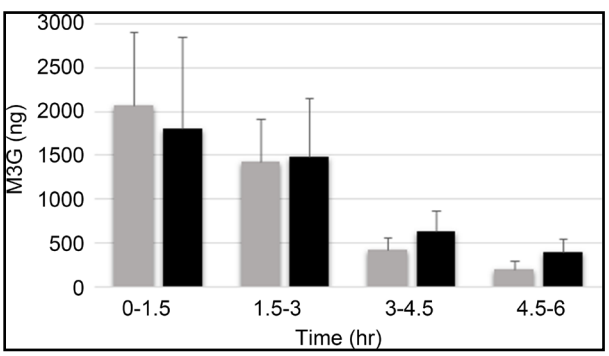
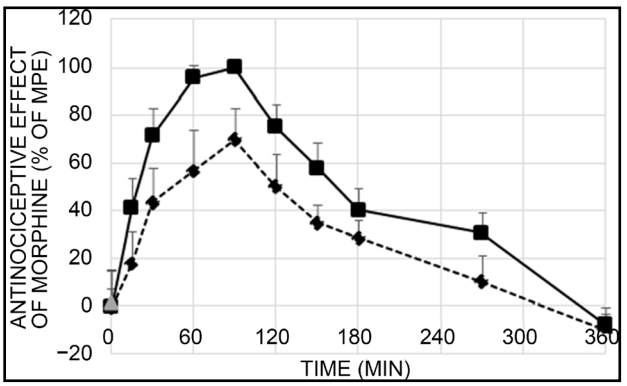


(d)

Figure 3. Urinary (a, b) and biliary (c, d) levels of morphine and morphine-3-glucuronide (M3G). Levels of morphine (a, c) and M3G (b, d) were determined after subcutaneous administration of 5 mg/kg morphine in rats. ▪, Morphine; ▪, Morphine + Diclofenac. Each symbol and bar represents the mean ± SD of the six rats. *, Significantly different from control rats ($p < 0.05$).

diclofenac inhibited the renal excretion of morphine *in vivo*, leading to higher serum levels of morphine and an increase in pain threshold (Figure 5).



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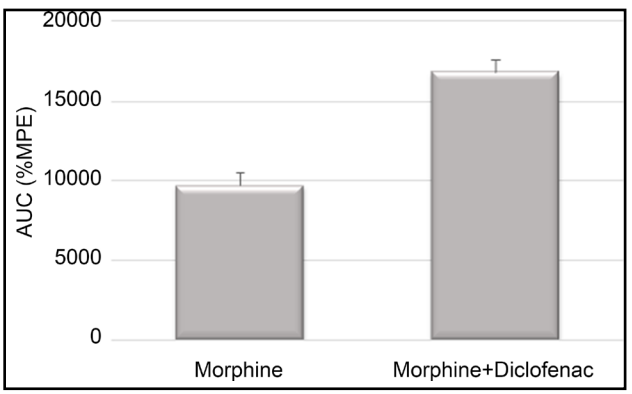

(b)

Figure 4. Effects of diclofenac on the anti-nociceptive effect of morphine in rats as measured by Von Frey threshold. Rats were treated with morphine (5 mg/kg s.c.) after the intraperitoneal administration of vehicle or diclofenac. ◆, Morphine; ▪, Morphine + Diclofenac. Points are mean ± SE (n = 6 rats). $^{*}p < 0.05$ (Morphine vs. Morphine + Diclofenac). MPE; maximum possible effect. Area under the effect curve of only morphine and morphine + diclofenac treated male rats.

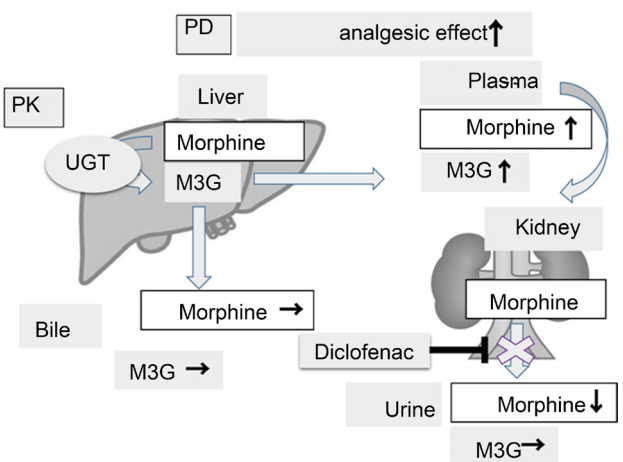

Figure 5. Summary of this research.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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