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ORIGINAL ARTICLE

Determination of physicochemical properties and degradation kinetics of triamcinolone acetonide palmitate in vitro

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Abstract

Purpose: Triamcinolone acetonide palmitate (TAP) is a lipophilic prodrug of triamcinolone acetonide (TAA) to improve the insoluble TAA physicochemical properties for the preparation of emulsions. *Methods*: This investigation has focused on the preformulation study of TAP, including its physicochemical properties and hydrolysis kinetics in vitro. *Results*: The solubility of TAP in medium-chain triglyceride is about twice greater than that in soybean oil (long-chain triglyceride) (19.17 versus 9.55 mg/g) at 25°C, and in all investigated cases, lecithin (80, 160, and 240 mg/g) as solubilizer provided increased solubility of drugs in medium-chain triglyceride and long-chain triglyceride, whereas the maximum water solubility of TAP was 0.10 μg/mL. The partition coefficient (log *P*) of TAP was 5.79 irrespective of the pH conditions. The hydrolysis of TAP followed pseudo-first-order kinetics in aqueous solutions, and the stable pH range was from pH 5.0 to 9.0. The in vitro enzymolysis kinetics of TAP in rat plasma and liver homogenate was evaluated by measuring the decrease of TAP as well as the increase of TAA at 37°C for 96 hours. The results demonstrated that the TAP may be hydrolyzed mainly by rat plasma esterase and, to a minor extent, by liver esterase, and the hydrolysis half-life of TAP in 100% rat plasma was 17.53 ± 6.85 hours at pH 7.4. *Conclusions*: All these results indicated that TAP had successfully obtained higher lipid-soluble property for the preparation of intravenous emulsion and may be an effective prodrug for sustained release of TAA in vivo.

Key words: *Enzymolysis; hydrolysis; in vitro; partition coefficient; solubility; triamcinolone acetonide palmitate*

Introduction

Triamcinolone acetonide (TAA), a glucocorticoid (Figure 1b) used for its anti-inflammatory effects in disorders of many organ systems and skin, is a more potent intermediate-acting derivative of triamcinolone and is 8 times more potent than prednisone[1]. TAA can quickly relieve the symptoms by blocking the production of a variety of substances in the body that cause inflammatory disorders[2]. Nowadays, the widely used injection formulation of TAA is a sterile crystalline suspension with a solid-particle size of 5–10 μm[3], failing to be administrated intravenously. Kenalog®-40 injection (TAA suspensions) has an extended duration of effect, which could last for several weeks. However, studies have indicated that following a single intramuscular dose of 60–100 mg of TAA, the hypothalamic–pituitary–adrenal axis suppression occurs within 24–48 hours and then gradually returns to normal, usually in 30–40 days[4], and also suggested that this extended inhibition correlates closely with the depot formulation of TAA. In addition, local muscular atrophy at the injection site and damages to joint tissue have been reported and these are mainly because of the insoluble particles[1].

To overcome this barrier, increasing interest has focused on TAA prodrug to improve its aqueous or oil solubility by a chemical modification at the site of carbon 21. Volon® A soluble, as the water-soluble prodrug of TAA 21-phosphate dikalium salt, is available for intravenous administration and reaches its maximal plasma concentration within 5 minutes, but its short biological half-life of 88 minutes means that frequent

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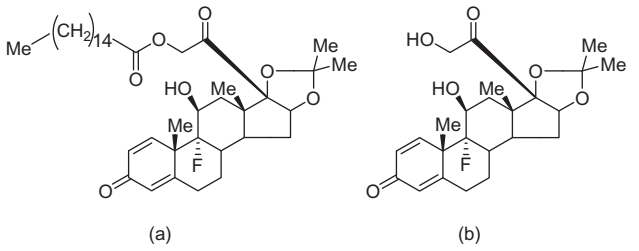

Figure 1. Structure of (a) triamcinolone acetonide palmitate and (b) triamcinolone acetonide.

dose is needed to maintain the therapeutic efficacy for an extended time, despite the light inhibition of the hypothalamic–pituitary–adrenal axis and little side effects compared with the suspension formulation[1,5]. To enhance oil solubility, saturated palmitic acid was commonly used to confer lipid anchoring to the parent drug, and the palmitoyl chain may act as 'hydrophobic anchor' holding the carboxyl group on the surface of lipid bilayer in liposomes, emulsions, solid lipid nanoparticles, and so on[6]. Except for the enhanced encapsulation, the palmitoylation may also offer extended release and increase the parent drug's activity because of the attachment of the palmitate chain to the lipo layers or biological membranes. For example, it is reported that the anti-inflammatory activity of dexamethasone palmitate (DM-Pal) emulsions was 5.6 times as potent as an equivalent amount of free dexamethasone (DM), chloramphenicol palmitate as an implantable slow release form, azidothymidine palmitate encapsulated in solid–lipid nanoparticle for prolonged release, and so on[6–8]. Therefore, TAA 21-palmitate (TAP, Figure 1a), as a potential lipophilic prodrug, could also enhance the oil solubility of TAA and obtain prolonged release. Goundalkar and Mezei have reported that TAP liposome showed 85% entrapment efficiency compared to that of TAA (5%), and the drug loading was up to 0.1%[9]. Additionally, it is also reported by López et al. that TAP incorporated into liposome lipid was retained in the articular cavity for a much longer period than free TAA and displayed increased anti-inflammatory effect[10].

Compared with the available TAA formulation, the oil-in-water (O/W) emulsions could load more drug than the liposome because of the lipo-core composed of long-chain triglyceride (LCT) or medium-chain triglyceride (MCT) as well as the interface layer constituted mainly by phospholipids. Besides, the O/W emulsions of TAP could also obtain more extended release and longer retention effect in vivo than that of TAA 21-phosphate dikalium salt. However, there were no reports about the physicochemical properties of TAP as well as TAP emulsion formulation. So, in this study, we investigated the partition coefficient, the rate of hydrolysis of TAP in the plasma and in the liver, as well as the physical and chemical stability. All the results were utilized to predict the possibilities of TAP being formulated as O/W emulsions and as a potential lipophilic prodrug with prolonged release in vivo, to facilitate the process of formulations.

Materials and methods

Materials

TAP was a kind gift from China Pharmaceutical University (Nanjing, China). TAA was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Egg lecithin (EPIKURON170, PC72%) was purchased from Degussa Food Ingredients (Shanghai, China). Soybean oil was obtained from Tieling Beiya Foods Ltd. (Liaoning, China) and MCTs from Shanghai Dongshang Shiye Company (Lipoid Co., Germany). All other chemicals and reagents were of analytical or chromatographic grade.

Physicochemical properties

Water and oil solubility

Excess amounts of TAP were separately added to the phosphate buffer solution (PBS) with different pH and MCTs or LCTs, in which egg lecithin with concentrations of 0, 80, 160, and 240 mg/g had been predissolved uniformly under magnetic stirring (DF-101S, YUHUA, YingXiaYuHua Instrument Co., Gongyi, China) at 25°C. Then, the oversaturated solutions were placed in a shaking air bath (HZQ-C, Dongming Medical Instrument Co., Harbin, China) at 25°C and 100 rpm for 72 hours to ensure solubility equilibrium[11]. Three days later, the oil solutions were centrifuged at $1066 \times g$ for 15 minutes, and about 0.5 g of the supernatant was diluted with diethyl ether and methanol in a ratio of 2 : 8 in a 100-mL volumetric flask. The supernatants as well as the water-oversaturated solutions were passed through a 0.45-μm filter membrane, and 10 μL of the filtrate was analyzed by high-performance liquid chromatography (HPLC).

The solubility of TAP in PBS (0.01 mol/L) at pH 4.80, 6.30, 7.07, 8.07, and 9.78 and in distilled water was 0.05, 0.04, 0.06, 0.10, 0.08, and 0.07 μg/mL, respectively; these results showed that TAP is completely insoluble in water. The solubility of TAP in MCTs and LCTs will be discussed in detail later.

Partition coefficient

n-Octanol was added to PBS with a pH of 4.80, 6.30, 7.07, 8.07, and 9.78 and to distilled water. The two-phase solution was mixed well and then placed in a shaking air bath for 24 hours to make sure that saturation equilibrium had been achieved. After that, the

saturated solutions were left to stand in separator funnels for 2 hours until the two phases had separated completely. Following this, about 10 mg of TAP was dissolved in 1 mL *n*-octanol saturated with different PBS in a 10-mL conical flask and diluted with the corresponding PBS saturated with *n*-octanol[12,13]. Then, the TAP was partitioned between PBS and *n*-octanol for 72 hours at 37 ± 1°C using the shake-flask method. The TAP concentrations in the two phases were separately measured by HPLC, and the partition coefficients at different pH values were calculated from Equation (1)[14]:

$$P = \frac{C_O}{C_W}, \quad (1)$$

where C_O and C_W represent the concentrations of TAP in *n*-octanol and water, respectively.

Hydrolysis in aqueous

Hydrolysis of TAP was studied at various temperatures (80°C, 60°C, and 40°C) in PBS (0.01 mol/L) at pH 2.4, 3.7, 4.4, 5.4, 6.2, 7.4, 8.9, 10.2, and 11.2. Experiments involved adding 3 mL of TAP stock solution (200 μg/mL) to 27 mL of PBS. In all experiments, to evaluate the palmitate bond stability, an aliquot of solution was kept in a water bath (HH.SY11-Ni, Hangfeng Apparatus and Instrument Co., Beijing, China) at 80 ± 1°C. At determined time intervals (0, 0.5, 1, 2, 4, 8, 12, and 24 hours), samples (4 mL) were withdrawn and cooled to room temperature immediately. The solutions were passed through a 0.45-μm filter membrane, and 10 μL of the filtered solution was analyzed without dilution by HPLC. This test was also carried out at 60 ± 1°C and 40 ± 1°C.

Enzymolysis kinetics in vitro

TAP solution enzymolysis in rat plasma

Fresh rat plasma was obtained by centrifugation of whole blood at 4000 rpm for 10 minutes in heparinized tubes and diluted to concentrations of 20%, 50%, 80%, and 100% with physiological saline to give a volume of 2.5 mL. After that, about 0.25 mg of propylparaben (0.01%, m/v), used as a bacteriostatic agent, was spiked into each tube[15]. The enzymatic hydrolysis of TAP in vitro was determined as described below. Each plasma solution was preincubated in an shaking air bath (100 rpm) at 37°C for 5 minutes[16], and the reactions were initiated by adding 100 μL of TAP stock solutions (5 mg/mL). Then, 200 μL samples were withdrawn at 0, 1, 2, 4, 8, 12, 24, 48, and 72 hours, and the reactions were terminated by adding 800 μL of acetonitrile, vortex-mixed for 10 minutes, and centrifuged at 17,050 × *g* for 10 minutes. The upper clear layer was collected and subjected to HPLC analysis.

TAP solution enzymolysis in the rat liver homogenate

In this experiment, 0.5 g of fresh rat liver was homogenized in 1 mL physiological saline, and then the homogenate was centrifuged at 1066 × *g* for 10 minutes[17]. The supernatant was redissolved in physiological saline to prepare 20%, 50%, and 80% liver homogenate solutions. The subsequent treatment was the same as that for rat plasma described under the section **TAP solution enzymolysis in rat plasma**.

HPLC method for determination of TAA and TAP in rat plasma and rat liver homogenate

An HPLC system (HITACHID-7000) fitted with a Kramasil C_{18} column (5 mm, 4.6 × 250 mm) (Dalian, China) consisted of an autosampler (L-7200), a pump (L-7100), and a UV detector (L-7420), all interfaced with D-7000 HSM software. Gradient elution was used to separate TAP and TAA. The initial mobile-phase gradient ratio was 70 : 30 for solvents A and B, respectively, where A is methanol and B is distilled water. The elution was isocratic for the first 8 minutes and then changed gradually to solvent A (100%) over 13 minutes and maintained for 30 minutes, and then the initial mobile-phase composition was restored over 5 minutes and maintained for an additional 10 minutes. The flow rate was set at 1.0 mL/min and the UV detector was set at 254 nm; the column temperature was set at 25 ± 1°C and the injection volume was 10 μL. The HPLC method for TAP determination in physicochemical properties and hydrolysis in aqueous was the same as the single phase of gradient elution method (13–30 minutes).

The gradient elution method was validated by analyzing the sample solutions obtained from rat plasma. The selectivity of the method showed that no interference from endogenous substances was observed in the chromatograms of drug-free rat plasma at the retention times of the analytes. For TAP and TAA, the linearity ranges were from 0.02–10 μg/mL (r = 0.9984) to 10–80 μg/mL (r = 0.9972) and from 0.01–10 μg/mL (r = 0.9982) to 2–80 μg/mL (r = 0.9979), and the limit of quantitation was 0.02 and 0.01 μg/mL, respectively. The precision was <15% with an accuracy of 100 ± 15% at three concentrations (2, 20, and 50 μg/mL for TAP and 5, 20, and 50 μg/mL for TAA; n = 6), whereas the mean extraction recoveries were 90.64 ± 2.28% and 89.4 ± 4.69% at the three concentration levels.

Results and discussion

Physicochemical properties

Oil solubility

The solubilities in oil are shown in Figure 2. The TAP solubility in MCT was higher than that in LCT. It is

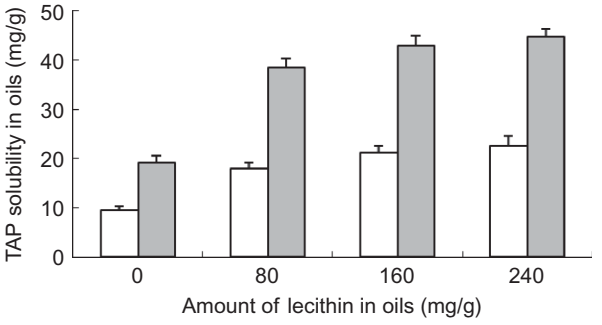

Figure 2. TAP solubility in different oils at 25°C; the blank columns represent the solubility of TAP in LCTs, and the solid columns represent that in MCTs ($n = 3$).

obvious that the solubility of TAP in MCT is about twice greater than that in LCT (19.17 versus 9.55 mg/g) without added lecithin, and in all cases investigated lecithin used as a solubilizer increased the solubility of TAP. The optimal solubilizing effect was obtained when the lecithin concentration was about 80 mg/g, and a further increase in lecithin had no marked effect.

In this article, LCT and MCT were utilized to determine the TAP solubility in oil because they are known for their long-term commercial acceptability when used in parenteral emulsions[18]. In addition, lecithin is a surfactant generally recognized as a safe solubilizer and an emulsifier very frequently used in injectable emulsion formulations[19]. The solubility of TAP in MCT is higher than that in LCT; the reason is that the presence of a saturated fatty acid with 6–12 carbons per head group in the MCT chain leads to a smaller molecular size and stereo-specific blockade compared with that of 12–18 carbons per head group in LCT, which facilitates co-absorption with the saturated fatty acid chain of palmitate of TAP molecules based on the theory of 'similarity and intermiscibility'[20].

After dissolving in the oil, lecithin as a solubilizer can increase the solubility of TAP compared with the pure oil phase. Because lecithin can interact with TAP in a special noncovalent manner that lowers the chemical potential of the TAP molecules in oil, a higher solution concentration (i.e., solubility) is required to reach a solution free energy that matches the solid free energy[21]. However, the tendency of lecithin to form lamellar and liquid-crystal phases in nonaqueous solvents results in limited solubilization ability, because it is hard for lamellae to swell in the interior volume of the micelles[22]. Moreover, the increased incorporation of TAP into the micelle phase could be offset by the long chain of MCT or LCT[23]. Therefore, lecithin can only increase the solubility of TAP in oil to a certain degree.

According to the clinical dosage of TAA, intra-articular TAA for joints was 2.5–15 mg (4% or 1%, m/v), and the doses for the intravenous injection for asthma range from 10 to 80 mg, depending on the specific disease being treated. Supposing that TAP was dissolved in whole MCT with 160 mg/g of lecithin added (the solubility is 42.98 mg/g) and the oil phase is 20%, the drug-loading efficiency can be up to 8.60 mg/mL of TAP in emulsion (0.56%, m/v, based on TAA), which was 5.6 times greater than that of TAP liposome, suggesting TAP emulsions may be a more potential drug carrier system and will facilitate the clinical administration for some acute or severe inflammation[9]. The articular cavity could receive 0.5–2.0 mL of injections while there was no limits to the intravenous injection[24], so the results of solubility seem to suggest that the solubility of TAP in oil is high enough so that an O/W emulsion can be formulated in a reasonable volume for clinical use. Additionally, it is reported that the enhanced stability of emulsion was mainly attributed to the probable formation of a complex interfacial film between the poloxamer and the lecithin molecules at the oil–water interface[25]. In this experiment, the optimal solubilizing effect of lecithin (80 mg/g) ensured the varied amount of lecithin, which means the more stable interfacial film could be obtained by increasing the amount of lecithin. Moreover, the oil-phase composition in emulsions also plays an important role in their formulation and influences the physicochemical properties and the stability of parenteral lipid emulsions[26]. As mentioned above, TAP is very soluble in MCT, and the lipid emulsion containing MCT might provide more stable all-in-one admixtures[27]. All these results indicated that the solubility of TAP was great enough in oil such that an O/W emulsion was feasible to formulate.

Partition coefficient

After 3 days of distribution equilibrium, the concentrations of TAP in *n*-octanol and water were measured by HPLC. It is obvious that the partition coefficient of TAP was independent of the pH, for the calculated value of log *P* was from 5.73 ± 0.09 to 5.89 ± 0.07 ($n = 3$) between pH 4.80 and 9.80 and the mean value of log *P* was 5.79.

As TAP is extremely hydrophobic, the volume of the water phase was ninefold higher than *n*-octanol to avoid the concentration in water being too low to be quantified[28]. As expected, TAP had a suitable partition coefficient, because if the value of log *P* is too high (>6) or too low (<3), the drug may present poor transport characteristics[29]. Compared with the parent drug, TAP successfully increased the hydrophobicity of TAA, displaying that its log *P* was enhanced from 2.54[10] to 5.79, which favors the formulation of a stable emulsion, as it has been reported that the calculated values of log *P* for the active substance should preferably exceed 4.0[30].

Hydrolysis dynamics

Order of reaction and route of hydrolysis

Plotting the natural logarithm of the remaining concentration of TAP (ln C) versus time at different temperatures allowed the order of the hydrolysis reaction to be identified. As expected, the hydrolysis of TAP followed first-order kinetics in aqueous solutions, as seen in Figure 3.

When TAP is hydrolyzed in PBS, the esterolysis usually occurs through two parallel routes of hydrolysis and they may be represented as follows:

The reaction can be represented as follows[31]:

$$\frac{dc}{dt} = -\frac{d[TAP]}{dt} = \frac{d[TAA]}{dt} = K[TAP]\left[H^+\right] \quad (2)$$

or

$$\frac{dc}{dt} = -\frac{d[TAP]}{dt} = \frac{d[TAA]}{dt} = K[TAP]\left[OH^-\right], \quad (3)$$

where K is the chemical rate constant for the reaction and [TAP] is the concentration of TAP. As the pH is constant, TAP hydrolysis in aqueous is considered to follow pseudo-first-order kinetics[12], and the equation can be rewritten as

$$\frac{dc}{dt} = K[TAP]. \quad (4)$$

Here, the temperature and concentration of PBS were kept the same, so K can completely represent the reaction characteristics, including the hydrolysis rate, stability, and half-life of degradation.

Effect of pH

Plotting the natural logarithm of the chemical rate constant (ln K) versus the corresponding pH value gives the most stable pH of TAP in aqueous solution (Figure 4). In this figure, it is apparent that TAP hydrolysis is an acid–base reaction, the decomposition of TAP through hydrolysis was minimal at pH 5.4, and the relatively stable pH range was about 5.0–9.0. Below pH 5.0 or above pH 9.0, the decomposition increased rapidly. Besides, the behavior of TAP hydrolysis dynamics was very similar with that of DM palmitate (DMP) as depicted in the determination of the physicochemical property of DMP[6], suggesting that the same mechanism of hydrolysis reaction mainly occurs at the sites of palmitoylation. Compared with the hydrolysis behavior of free TAA[32], the long palmitoyl chain may protect the original hydrolysis site through the stereoscopic shadowing, enhancing the stability of the parent steroids.

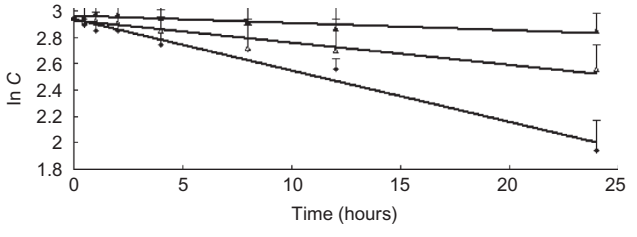

Figure 3. TAP hydrolysis curve in aqueous solutions at the same pH but different temperature: ♦, r = 0.9849 at 80°C and pH 7.4; Δ, r = 0.9685 at 60°C and pH 7.4; and ▲, r = 0.9311 at 40°C and pH 7.4 (n = 3). Solid lines were obtained by linear regression.

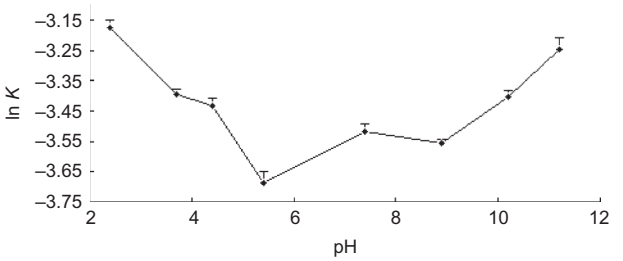

Figure 4. The pH–rate profile curve from ln K versus pH values at 80°C in different PBS solutions (n = 3).

Moreover, the relatively stable pH value could not only ensure the slowest degradation of TAP but also minimize the rate of hydrolysis of egg lecithin and triglycerides[33–36], which could be utilized as an effective protection for the long-term stability of TAP emulsion. In general, the pH of injection is preferably about 4–8, and a pH above 9 has a probability of causing hemolysis and local irritation[37], so the relatively stable pH range for TAP solution was very suitable for the preparation of intravenous injection.

Effect of temperature

In this study, TAP hydrolysis experiment was carried out in parallel at 40°C, 60°C, and 80°C. According to the Arrhenius equation, the temperature (T) is closely related to the chemical rate constant (K):

$$\ln K = -\frac{Ea}{RT} + \ln A. \quad (5)$$

Assuming ln K is linear with $1/T$, the effect of temperature on the hydrolysis of TAP was observed in Figure 5.

As expected, significant positive correlation was observed between the reciprocal of temperature and the natural logarithm of the chemical rate constant (ln K). Moreover, according to the Arrhenius equation, the half-life ($t_{1/2}$) of TAP hydrolysis at 25°C and 37°C can be calculated using the ln 2/K. For example, the $t_{1/2}$ of TAP hydrolysis at pH 7.4 was 341.88 and 175.41 hours at 25°C and 37°C, respectively.

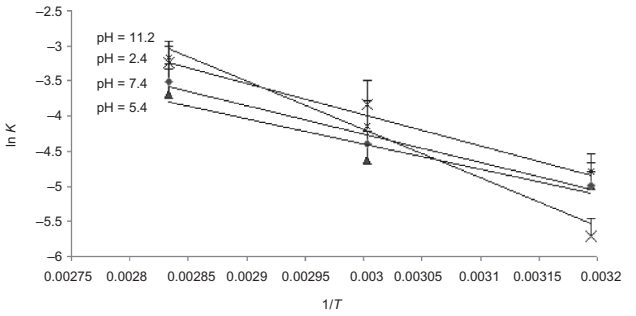

Figure 5. The effect of temperature on the hydrolysis rate of TAP at pH 2.4 (r = 0.9898), pH 5.4 (r = 0.9601), pH 7.4 (r = 0.9901), and pH 11.2 (r = 0.9667) (n = 3).

Enzymolysis kinetics

Order of reaction

Plotting the natural logarithm of the residual concentration of TAP (ln C) versus time shows the order and relative rate of enzymic hydrolysis in vitro in rat plasma and liver homogenate, as seen in Figure 6.

As we know, there is a carboxylate group in the TAP structure. When absorbed or injected intravenously into the circulatory system, this prodrug will be hydrolyzed to the active drug TAA by the nonspecial carboxylesterase lipase (CEL). In this article, the individual rat plasma and liver homogenate incubated at 37°C were used to simulate the metabolism of TAP in vivo for their high levels of carboxylesterase[38]. As shown in Figure 6, the enzyme kinetics followed a first-order process and resulted in a quantitative reversion to parent drug evidenced by HPLC analysis. Moreover, the character of TAP enzymolysis reaction was comparable with that of other prodrugs such as DM-Pal in 80% human plasma and steroids with amino acids in human serum[6,39], inferring that TAP could release the parent drug in vivo at a reasonable rate. The hydrolysis rate constant (K) in 50% and 80% rat plasma was 0.033 and 0.020 per hour, respectively, whereas the values were 0.004 and 0.012 per hour in the same concentration of liver homogenate, showing that the hydrolysis rate in rat plasma was about 4.6- and 2.6-fold than that of liver homogenate at a concentration of 50% and 80%, respectively. Furthermore, only 0.5% (0.404 μg/mL) and 0.2% (0.183 μg/mL) of the original TAP in 50% and 80% plasma solutions could be detected after 72 hours, whereas 64.44% (15.939 μg/mL) and 53.37% (12.379 μg/mL) remained in the corresponding concentration of liver homogenate, respectively. These results show that TAP is hydrolyzed mainly by rat plasma esterase and only to a minor extent by liver esterase. CEL in plasma is generally considered to be the first quantitatively important enzyme that hydrolyzes xenobiotics with an ester linkage, especially for drugs[40].

Moreover, the accuracy of the result can be validated by the changed molar concentration of TAP and TAA, showing that the decreasing molar concentration of TAP was equal to the generated molar concentration of TAA at each time point of the same concentration, and their molar sum was constant at around 50×10^{-9} mol/mL. However, the molar equilibrium was disrupted after 48-hour incubation, which may be because of further degradation of TAA[1].

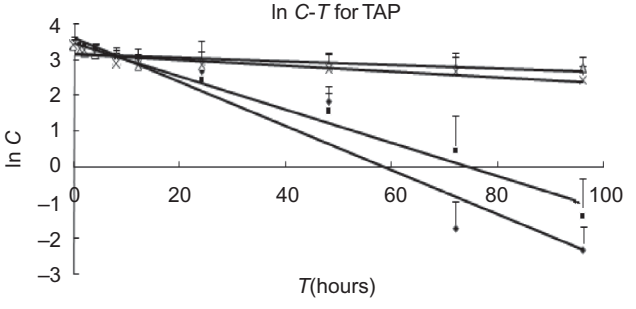

Figure 6. The ln C of TAP versus time for TAP hydrolyzed in rat plasma and liver homogenate solutions with different concentrations for 96 hours at 37°C. (♦)—in 80% rat plasma (r = 0.9987); (■)—in 50% rat plasma (r = 0.9960); (×)—in 80% rat liver homogenate (r = 0.9128); and (□)—in 50% rat liver homogenate (r = 0.9298). C represents the mean concentration of TAP in each reaction at the same time point (n = 4).

Terminal half-life ($t_{1/2}$) in vitro

The terminal half-life ($t_{1/2}$) of TAP in various concentrations of rat plasma and liver homogenate can be calculated by linear regression analysis, according to the hydrolysis theory in aqueous solution (Figure 7).

The half-life in vitro is an important parameter used to predict the enzymolysis behavior of TAP in vivo. Here, the results of the terminal half-life also showed that the TAP hydrolysis was faster in rat plasma than in liver in vitro. For example, the half-life in 50% liver homogenate was 4.6-fold longer than that in the same

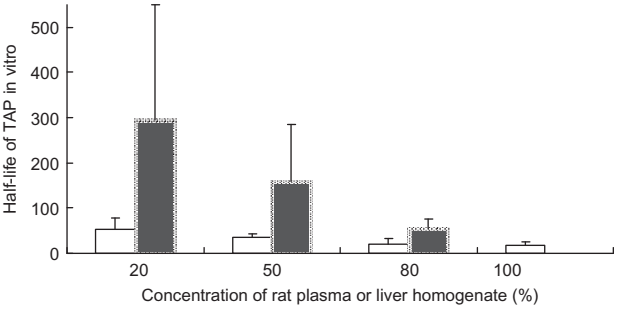

Figure 7. Half-lives ($t_{1/2,}$ hours) of TAP in various concentrations of rat plasma and liver homogenate for 96 hours; the blank columns represent the $t_{1/2}$ of TAP in rat plasma, and the solid columns represent that in liver homogenate. The values are mean ± SD (n = 4).

concentration of rat plasma (159.47 versus 34.58 hours), and the half-life of TAP in whole rat plasma was 17.53 ± 6.85 hours in vitro. In contrast, it is reported that the half-life of TAA phosphate was within 3–4 minutes and was difficult to determine clinically[5], so these results indicated that TAP could obtain the prolonged plasma retention and the enhanced permeability and retention effect in vivo. Moreover, the same results have already been demonstrated by the similar cortical steroids of DM prodrugs, whose phosphate hydrolysis was more rapid than that of the palmitate in vitro and the free DM concentrations were detected for 24 hours in animals administered DM-Pal but not in animals administered the same dose of DMP solution in vivo[6,41]. Additionally, it is noteworthy that this prediction obtained from the enzymic hydrolysis in vitro is comparable with a report of the higher potency of TAP in the inhibition of the arthritis of rabbits in vivo compared with free TAA[10]. Compared with the hydrolysis half-life in PBS at pH 7.4 and 37°C ($t_{1/2}$ = 218.58 hours), CEL present in blood or liver can catalyze enzymolysis reactions to a greater degree, indicating a reasonable rapid cleavage for TAP in plasma and liver. Eventually, the enzymolysis of TAP in vitro showed that TAP was a good lipophilic prodrug for plasma or liver enzymes and TAP emulsion appeared to be suitable candidate as prolonged release system, which could improve the delivery of the parent drugs and relieve the system side effects.

Conclusion

In conclusion, the solubility results suggest that TAP as prodrug effectively enhances the hydrophobicity of TAA and increases the calculated value of log *P* from 2.54 to 5.79. The solubility of TAP in oil was great enough such that an O/W emulsion was feasible to formulate in a reasonable volume for clinical use. When TAP was dissolved in whole MCT with 160 mg/g of lecithin added and the oil phase is 20%, the drug-loading efficiency can be up to 8.60 mg/mL of TAP in emulsion. The hydrolysis kinetics indicated that the relatively stable pH range for TAP solution was from 5.0 to 9.0, and the most stable pH was 5.4, which could minimize the hydrolysis of TAP. Moreover, the hydrolysis kinetics in rat plasma and liver homogenate showed that TAP is hydrolyzed mainly by plasma esterase and, to a minor extent, by liver esterase, and the terminal half-life of TAP in 100% blank plasma in vitro was 17.53 ± 6.85 hours, indicating that TAP can delay the release of the parent drug and offer a long-acting anti-inflammatory effect. All these results from the preformulation study indicate that TAP is lipophilic enough to allow the preparation of an O/W emulsion formulation, which could be administrated intravenously as prolonged release system against acute or severe inflammation.

Declaration of interest

The authors report no conflicts of interest.

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