Influence of small doses of various drug vehicles on acetaminophen-induced liver injury

Tomislav Kelava, Ivan Čavar, and Filip Ćulo

Abstract: The biological effects of drug vehicles are often overlooked, often leading to artifacts in acetaminophen-induced liver injury assessment. Therefore, we decided to investigate the effect of dimethylsulfoxide, dimethylformamide, propylene glycol, ethanol, and Tween 20 on acetaminophen-induced liver injury. C57BL/6 male mice received a particular drug vehicle (0.6 or 0.2 mL/kg, i.p.) 30 min before acetaminophen administration (300 mg/kg, i.p.). Control mice received vehicle alone. Liver injury was assessed by measuring the concentration of alanine aminotransferase in plasma and observing histopathological changes. The level of reduced glutathione (GSH) was assessed by measuring total nonprotein hepatic sulfhydrls. Dimethylsulfoxide and dimethylformamide (at both doses) almost completely abolished acetaminophen toxicity. The higher dose of propylene glycol (0.6 mL/kg) was markedly protective, but the lower dose (0.2 mL/kg) was only slightly protective. These solvents also reduced acetaminophen-induced GSH depletion. Dimethylformamide was protective when given 2 h before or 1 h after acetaminophen administration, but was ineffective if given 2.5 h after acetaminophen. Ethanol at the higher dose (0.6 mL/kg) was partially protective, whereas ethanol at the lower dose (0.2 mL/kg) as well as Tween 20 at any dose had no influence. None of the vehicles (0.6 mL/kg) was hepatotoxic per se, and none of them was protective in a model of liver injury caused by D-galactosamine and lipopolysaccharide.

Key words: acetaminophen, dimethylsulfoxide, dimethylformamide, propylene glycol, ethanol, Tween 20, liver injury.

Introduction

Acetaminophen (APAP) is a widely used analgesic and antipyretic drug that is considered safe at therapeutic doses. Overdose of APAP causes liver damage and for the last 2 decades is the leading cause of acute hepatic failure in the Western world (Lee 2004; Norris et al. 2008). The toxicity of APAP is initiated by formation of a reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), by liver microsomal enzymes; CYP1A2 and CYP2E1 seem to be the most important isoforms in mice (in humans, CYP2E1 and CYP3A4 appear to be the most important isoforms). NAPQI first depletes glutathione (GSH) and then covalently binds to cellular proteins (Mitchell et al. 1973; Nelson 1982; Zaher et al. 1998; Laine et al. 2009). APAP-induced liver injury (AILI) in mice is the most extensively used model for study-
ing mechanisms of drug-induced liver injury (Gunawan and Kaplowitz 2007; Masson et al. 2008).

Some of the major biases in AILI investigations have occurred because the drug vehicles had biological effects per se, particularly dimethylsulphoxide (DMSO). For example, El-Hassan et al. (2003) reported a protective effect of the pancaspase inhibitor Z-VAD-fmk against APAP toxicity in mice. For controls, they used the cathepsin inhibitor benzylloxy-carbonyl-Phe-Ala-fluoromethylketone (Z-FA-fmk) and achieved no protection with this compound. However, they did not add DMSO to Z-FA-fmk, which was used to dilute Z-VA-fmk (El-Hassan et al. 2003). Subsequently, it was shown that DMSO and not Z-VA-fmk protected mice from APAP (Jaeschke et al. 2006). Likewise, it was reported that natural killer cells have a pathogenic role in AILI (Liu et al. 2004), but recently it was shown that this happens only when DMSO is used to help dissolve APAP (Masson et al. 2008).

Commonly used vehicles in AILI experiments include DMSO (El-Hassan et al. 2003), ethanol (ETOH), corn oil (Donthamsetty et al. 2008), olive oil (Jodynis-Liebert et al. 2005), Tween 20 (Renić et al. 1993), Tween 80 (Kamanaka et al. 2003), propylene glycol (PG) (Oz and Chen 2008), and methylcellulose (Harrill et al. 2009). They are used for 2 purposes: (1) to facilitate solution of some other substance being investigated for its effect on AILI and (or) (2) to help dissolve APAP itself; in the latter case PG is frequently used. Despite its obvious importance, the influence of vehicles on AILI is rarely investigated. So far, it has been shown that DMSO, PG, and ETOH, if injected 15–30 min before or 1–2 h after APAP administration, reduce its toxicity, most probably by inhibition of cytochrome P450, which converts APAP to NAPQI. However, most of these investigations were conducted for doses of vehicles larger than 1 mL/kg (Hughes et al. 1991; Thomsen et al. 1995; Lee et al. 1999), which are 5–10 times higher than doses usually used in AILI experiments. A notable exception is DMSO, for which an effective dose-dependent study was conducted showing a strong protective effect even when DMSO was administered at a dose of 0.2 mL/kg (Yoon et al. 2006). The effects of various vehicles, to our knowledge, have not been compared.

Therefore, we decided to compare the effects of the 3 most frequently used solvents (DMSO, PG, and ETOH) on AILI, as well as to investigate the effects of Tween 20 and dimethylformamide (DMF), whose influence in this model have not been investigated. The main object of our study was to show which vehicles at doses of 0.2 and 0.6 mL/kg change the intensity of AILI and which do not. Besides common parameters of APAP toxicity, such as serum aminotransferase levels and liver pathohistology, the influence of APAP on GSH content in liver and microsomal enzyme activity was also investigated.

Materials and methods

Animals

C57BL/6 mice were raised in an animal colony unit at the Department of Physiology, School of Medicine, University of Zagreb, Zagreb, Croatia. Male mice aged 12–16 weeks and weighing 20–25 g were used in all experiments. The
(0.2 mL/kg, i.p.) or saline was injected 30 min before APAP administration.

In all experiments food was returned 4 h after APAP administration, and after an additional 3 h mice were anesthetized with Avertin; blood was collected by puncture of the medial eye angle with heparinized glass capillary tubes, and the livers were removed for analyses.

It is important to note that the doses of vehicles were adjusted to be equal in millilitres per kilogram (not in mg/kg or mol/kg). This was done deliberately, because the volume of the solvent is what is important when addition of a solvent is being considered.

**Hepatic injury induced by GalN and lipopolysaccharide (LPS)**

Mice were injected simultaneously with GalN (300 mg/kg, i.p.) and LPS (15 μg/kg, i.p.). Saline, DMSO, DMF, ETOH, PG, or Tween 20 was injected (0.6 mL/kg, i.p.) 30 min before GalN plus LPS treatment.

**Evaluation of liver injury**

Alanine aminotransferase (ALT) levels were determined from plasma by standard laboratory techniques in a clinical diagnostic laboratory. A portion of the liver was fixed in 10% neutral formalin, processed by histological techniques, stained with hematoxylin–eosin, and examined for morphological evidence of liver injury.

**Total hepatic nonprotein sulfhydryl content determination**

GSH level was assessed by measuring total hepatic nonprotein sulfhydryls, using the method of Ellman (Ellman 1959). A portion (approximately 100 mg) of liver was homogenized in Tris–HCl buffer (25 mmol/L, pH 7.4), and 0.1 mL of the homogenate was mixed with 0.2 mL of 5% trichloroacetic acid, followed by centrifugation at 860 g for 10 min. Supernatant was mixed with DTNB. The absorbance was determined at 412 nm. The absorbance was compared with a standard curve prepared using 6 different GSH concentrations. From the rest of the homogenate, the protein concentrations were determined using the Bradford method, using bovine serum albumin as the standard (Bradford 1976), and GSH content was calculated in nanomoles per milligram of protein.

**Measurement of phenobarbitone sleeping time**

Influence on duration of phenobarbitone sleeping time was determined as an in vivo measure of liver microsomal enzyme activity (Piel et al. 1969; Chan et al. 2009). Mice (n = 4 per group) were injected with saline, DMSO, DMF, PG, ETOH, or Tween 20 (0.6 mL/kg), and after 30 min they received phenobarbitone (50 mg/kg, i.p.). The sleeping time was defined as the sleep-time period from the loss of the righting reflex to its complete recovery (Piel et al. 1969; Henderson et al. 2003; Chan et al. 2009).

**Statistical analysis**

Data are presented as means ± SE. Statistical significance was evaluated by one-way ANOVA followed by Dunnett’s multiple comparison tests. Results were considered statistically significant at P < 0.05.

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**Table 1. Effects of DMSO, DMF, ETOH, PG, and Tween 20, given at a dose of 0.6 mL/kg, on APAP-induced liver injury.**

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>APAP (0.6 mL/kg)</th>
<th>ALT (U/L)</th>
<th>GSH (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>300</td>
<td>6747.5±2290.4</td>
<td>9.8±1.26</td>
</tr>
<tr>
<td>DMSO</td>
<td>300</td>
<td>178.6±96.9*</td>
<td>53.2±1.7*</td>
</tr>
<tr>
<td>DMF</td>
<td>300</td>
<td>135±47.7*</td>
<td>56.1±1.0*</td>
</tr>
<tr>
<td>ETOH</td>
<td>300</td>
<td>4633.8±2066.4</td>
<td>19.6±5.1</td>
</tr>
<tr>
<td>PG</td>
<td>300</td>
<td>441.4±317.8*</td>
<td>46.2±5.7*</td>
</tr>
<tr>
<td>Tween 20</td>
<td>300</td>
<td>5268.6±2512.6</td>
<td>9.4±2.5</td>
</tr>
<tr>
<td>Saline</td>
<td>—</td>
<td>20±1.5</td>
<td>64.9±0.5</td>
</tr>
<tr>
<td>DMSO</td>
<td>—</td>
<td>30.7±5.7</td>
<td>65.8±0.9</td>
</tr>
<tr>
<td>DMF</td>
<td>—</td>
<td>27±1.5</td>
<td>57.1±0.28*</td>
</tr>
<tr>
<td>ETOH</td>
<td>—</td>
<td>32.7±5.2</td>
<td>63.8±1.3</td>
</tr>
<tr>
<td>PG</td>
<td>—</td>
<td>28.3±4.4</td>
<td>65.1±0.7</td>
</tr>
<tr>
<td>Tween 20</td>
<td>—</td>
<td>37.3±7.1</td>
<td>64.5±2.4</td>
</tr>
</tbody>
</table>

*Note: Mice were treated with DMSO, DMF, ETOH, PG, Tween 20, or saline intraperitoneally 30 min before APAP administration (300 mg/kg, i.p.) or only with DMSO, DMF, ETOH, PG, Tween 20, or saline. GSH and ALT levels were determined 7 h after APAP. Each value represents the mean ± SE of 6–8 mice. GSH content was measured as total hepatic nonprotein sulfhydryl content. *, significantly different from mice treated with saline before APAP administration (ANOVA followed by Dunnett’s multiple comparison test, p < 0.05); †, significantly different from mice given only saline (ANOVA followed by Dunnett’s multiple comparison test, p < 0.05). APAP, acetaminophen; ALT, alanine aminotransferase; GSH, reduced glutathione; DMSO, dimethylsulphoxide; DMF, dimethylformamide; ETOH, ethanol; PG, propylene glycol.

**Results**

**Effects of drug vehicles on APAP-induced liver injury**

In the first experiment, drug vehicles were given 30 min before APAP administration (300 mg/kg, i.p.). As shown in Table 1, DMSO, DMF, and PG at a dose of 0.6 mL/kg almost completely abolished APAP toxicity; ALT levels were 37, 45, and 15 times lower, respectively, than in the control group (which received saline before APAP administration). ETOH only slightly reduced ALT levels, and Tween 20 had no influence. APAP depleted GSH from liver, and DMF, DMSO, and PG reduced this depletion. The protective effect of drug vehicles was clearly visible in histopathological analysis (Fig. 1); centrilobular necrosis, which was seen in mice given saline (or Tween 20) before APAP administration (Fig. 1A, 1C), was almost absent in mice given DMF (Fig. 1B, 1D) or DMSO before APAP administration.

When given alone (i.e., without APAP treatment), none of the vehicles had a significant effect on the ALT level; that is, the concentration of ALT in plasma was similar to that in plasma from mice given saline. However, DMF slightly reduced the GSH level in comparison to that in mice given saline, while other vehicles had no such effect (Table 1).

At a dose of 0.2 mL/kg (second experiment), DMF and DMSO were similarly effective; ALT levels were, respectively, 15 and 18 times lower than in the control group. PG also reduced ALT concentration and blocked GSH depletion, but not significantly. ETOH and Tween 20 had no influence. DMF alone had no influence on GSH levels (Table 2).
Protective effect of drug vehicles was not dependant on the administration route

In previous experiments, both vehicle and APAP were injected intraperitoneally, with short intervals between the 2 injections, which might cause physical or chemical interference of the agents. To exclude this, we conducted an experiment in which APAP was administered per os by gastric lavage. As shown in Fig. 2, the effect of solvents on concentration of ALT in plasma was very similar to that when APAP was given intraperitoneally (comparison of data in Table 1 and Fig. 2).

Protective effect of DMF was dependant on time of DMF application

To examine the effect of time, DMF was given at various time points before or after APAP administration. DMF completely blocked APAP toxicity if given 2 h before APAP administration; it was also effective if given 1 h after APAP administration, but ineffective 2.5 or 4 h after APAP (Fig. 3).

Phenobarbitone pretreatment had no influence on protective effect of DMSO

It is known that phenobarbitone induces microsomal enzymes and therefore stimulates formation of a toxic metabolite of APAP, NAPQI (Belardinelli et al. 2008). Mice were pretreated for 7 days with phenobarbitone in drinking water (300 mg/L) and then received DMSO (0.2 mL/kg, i.p.) or saline prior to APAP (300 mg/kg, i.p.). As shown in Fig. 4, DMSO was also highly protective in this model (Fig. 4).

DMF and DMSO prolonged phenobarbitone sleeping time

Sleeping time was measured in mice injected with saline or drug vehicles (0.6 mL/kg) 30 min before phenobarbitone administration (50 mg/kg, i.p.). DMSO and DMF significantly increased phenobarbitone sleeping time. PG pro-

Fig. 1. Effects of drug vehicles on histopathological changes in liver. Hematoxylin–eosin stained liver sections. (A, C) Typical liver sections (A: 100× original magnification; C: 200× original magnification) from a mouse treated with saline 30 min before APAP administration (300 mg/kg) showing extensive necrosis of centrilobular hepatocytes. The ALT level measured in the plasma of this mouse was 6480 U/L. Liver sections of mice treated with Tween 20 before APAP administration looked similar. (B, D) Typical liver sections (B: 100× original magnification; D: 200× original magnification) from a mouse treated with dimethylformamide (0.6 mL/kg) 30 min before APAP administration (300 mg/kg) showing essentially normal histology. The ALT level measured in the plasma of this mouse was 150 U/L. Liver sections of mice treated with PG or DMSO (0.6 mL/kg) before APAP treatment looked similar.
longed it only slightly, while Tween 20 and ETOH had no influence (Table 3).

Effects of drug vehicles on liver injury induced by GalN and LPS
Treatment of mice with GalN and LPS highly increased plasma ALT levels (Fig. 5). None of the vehicles had a sig-

Table 2. Effects of DMSO, DMF, ETOH, PG, and Tween 20, given at a dose of 0.2 mL/kg, on APAP-induced liver injury.

<table>
<thead>
<tr>
<th>Vehicle (0.2 mL/kg)</th>
<th>APAP (mg/kg)</th>
<th>ALT (U/L)</th>
<th>GSH (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>300</td>
<td>8767±1663</td>
<td>9.7±0.9</td>
</tr>
<tr>
<td>DMSO</td>
<td>300</td>
<td>585±397**</td>
<td>44.9±1.7**</td>
</tr>
<tr>
<td>DMF</td>
<td>300</td>
<td>470±188**</td>
<td>44±2.9**</td>
</tr>
<tr>
<td>ETOH</td>
<td>300</td>
<td>899±2048</td>
<td>12.1±4.5</td>
</tr>
<tr>
<td>PG</td>
<td>300</td>
<td>5830±142</td>
<td>17.2±5.5</td>
</tr>
<tr>
<td>Tween 20</td>
<td>300</td>
<td>8774±1762</td>
<td>11.2±2.2</td>
</tr>
<tr>
<td>Saline</td>
<td>—</td>
<td>25.6±1.6</td>
<td>67.5±1.8</td>
</tr>
<tr>
<td>DMSO</td>
<td>—</td>
<td>29±1.3</td>
<td>65±4.0</td>
</tr>
<tr>
<td>DMF</td>
<td>—</td>
<td>34.6±3.5</td>
<td>65.7±0.7</td>
</tr>
<tr>
<td>ETOH</td>
<td>—</td>
<td>24.6±1.9</td>
<td>65.8±1.9</td>
</tr>
<tr>
<td>PG</td>
<td>—</td>
<td>20.3±0.2</td>
<td>65.3±1.8</td>
</tr>
<tr>
<td>Tween 20</td>
<td>—</td>
<td>29.6±3.3</td>
<td>64.1±2.1</td>
</tr>
</tbody>
</table>

Note: Mice were treated with DMSO, DMF, ETOH, PG, Tween 20, or saline intraperitoneally 30 min before APAP administration (300 mg/kg, i.p.) or only with DMSO, DMF, ETOH, PG, Tween 20, or saline. ALT levels were determined 7 h after APAP. Each value represents the mean ± SE of 6 mice. GSH content was measured as total hepatic nonprotein sulfhydryl content. **, significantly different from mice treated with saline before APAP administration (ANOVA followed by Dunnett’s multiple comparison test; p < 0.01). APAP, acetaminophen; ALT, alanine aminotransferase; GSH, reduced glutathione; DMSO, dimethylsulphoxide; DMF, dimethylformamide; ETOH, ethanol; PG, propylene glycol.

Fig. 2. Effects of DMSO, DMF, ETOH, PG, and Tween 20 on concentration of ALT in plasma, when APAP was given orally. Mice were treated with DMSO, DMF, ETOH, PG, Tween 20, or saline (0.6 mL/kg, i.p.) 30 min before APAP administration (300 mg/kg, per os). ALT levels were determined 7 h after APAP administration. Each value represents the mean ± SE of 6 mice. *, significantly different from mice treated with saline and APAP (ANOVA followed by Dunnett’s multiple comparison test; p < 0.05 and p < 0.01, respectively). DMSO, dimethylsulphoxide; DMF, dimethylformamide; ETOH, ethanol; PG, propylene glycol; ALT, alanine aminotransferase; APAP, acetaminophen.

Fig. 3. Effect of varying time of DMF application on ALT plasma levels. Mice were treated with saline or DMF (0.6 mL/kg, i.p.) at indicated times prior to or after APAP (300 mg/kg, i.p.). ALT levels were determined 7 h after APAP. Each value represents the mean ± SE of 6 or 7 mice. *, **, significantly different from mice treated with saline and APAP (ANOVA followed by Dunnett’s multiple comparison test; p < 0.05 and p < 0.01, respectively). DMF, dimethylformamide; ALT, alanine aminotransferase; APAP, acetaminophen.

Fig. 4. Effect of DMSO on ALT levels in mice pretreated with phenobarbitone. Mice were pretreated for 7 days with phenobarbitone in drinking water (300 mg/L) and then treated with DMSO (0.2 mL/kg, i.p.) or saline prior to APAP (300 mg/kg, i.p.). ALT levels were determined 7 h after APAP administration. Each value represents the mean ± SE of 8 mice. **, significantly different from mice treated with saline before APAP administration (Student’s t test; t < 0.001). DMSO, dimethylsulphoxide; ALT, alanine aminotransferase; APAP, acetaminophen.

Fig. 5. Effect of GalN and LPS on plasma ALT levels. Mice were treated with saline or GalN/LPS at indicated times prior to or after APAP (300 mg/kg, i.p.). ALT levels were determined 7 h after APAP. Each value represents the mean ± SE of 6 or 7 mice. *, **, significantly different from mice treated with saline and APAP (ANOVA followed by Dunnett’s multiple comparison test; p < 0.05 and p < 0.01, respectively). GalN, galactosamine; LPS, lipopolysaccharide; ALT, alanine aminotransferase; APAP, acetaminophen.

Discussion
Although vehicles are generally considered relatively inert, they can induce biological effects that are often overlooked. AILI is shown to be very sensitive to bias caused by solvents (Jaeschke et al. 2006; Masson et al. 2008). Our results show that DMSO and DMF greatly suppress AILI even at small doses (0.2 mL/kg), PG is somewhat less protective, and it seems that doses of ETOH (0.2 mL/kg) and Tween 20 (0.2 and 0.6 mL/kg) have no effect. None of the vehicles appears to be hepatotoxic itself at the above-mentioned doses. The efficacy of vehicles in vivo was not dependent on the route of administration (Fig. 2); that is,
Table 3. Effects of DMSO, DMF, ETOH, PG, and Tween 20 (0.6 mL/kg) on phenobarbitone sleeping time.

<table>
<thead>
<tr>
<th>Vehicle (0.6 mL/kg)</th>
<th>Sleeping time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>28.5±5.8</td>
</tr>
<tr>
<td>DMSO</td>
<td>60±5*</td>
</tr>
<tr>
<td>DMF</td>
<td>55.5±5.4*</td>
</tr>
<tr>
<td>ETOH</td>
<td>27±7.2</td>
</tr>
<tr>
<td>PG</td>
<td>34.5±6.3</td>
</tr>
<tr>
<td>Tween 20</td>
<td>26.7±3.5</td>
</tr>
</tbody>
</table>

Note: Mice were treated with DMSO, DMF, ETOH, PG, Tween 20, or saline intraperitoneally 30 min prior to phenobarbitone treatment. Each value represents the mean ± SE of 4 mice. *, significantly different from mice treated with saline (ANOVA followed by Dunnett’s multiple comparison test, *p < 0.05). DMSO, dimethylsulphoxide; DMF, dimethylformamide; ETOH, ethanol; PG, propylene glycol.

DMSO, DMF, and PG were protective to a similar extent regardless of whether APAP was administered orally or intraperitoneally. The most probable means of protection is interference with the liver microsomal metabolism of APAP. This is supported with our findings that PG, DMSO, and DMF inhibited APAP-induced GSH depletion and prolonged phenobarbitone sleeping time. Yoon et al. proposed that DMSO inhibited microsomal formation of NAPQI in a competitive manner (Yoon et al. 2006). Therefore, we hypothesized that an experimental model in which microsomal enzymes are induced prior to APAP administration could be less sensitive to DMSO. However, our results showed that mice that received phenobarbitone for 7 days in drinking water (Guarner et al. 1988; Belardinelli et al. 2008) were equally protected by DMSO. Aside from its effect on APAP metabolism, each of these vehicles can change several other parameters that play a role in AILI. For example, DMSO shows antioxidative activity (Jeffery et al. 1988; Dunphy et al. 2007), and several antioxidants protect against AILI (Marotta et al. 2009; Reisman et al. 2009). It also demonstrated adjuvant-like actions on the immune system (Kunze 1975; Yamasaki et al. 1988), which were the cause of bias in the previously mentioned natural killer cell investigation.

Because DMSO is so frequently a source of bias, we supposed that DMF might be a useful alternative (some of the substances that we intend to investigate in the future can be dissolved in both DMSO and DMF). However, DMF showed a protective effect similar to that of DMSO. There are no reports about the effect of DMF on oxidative stress or the immunological system. On the other hand, DMF shows anticoagulant-like effects (Imbriani et al. 1986), and some anticoagulants are able to protect from AILI (Ganey et al. 2007). Since this is the first report about the protective effect of DMF, we further investigated the effect of time of administration. It showed a typical pattern for substances that interfere with APAP metabolism, a very strong protection if given before or 1 h after APAP administration, but no protection if given 2.5 h after APAP administration, when biotransformation of APAP is essentially complete. DMF inhibits CYP2E1, most probably by suicidal enzyme inactivation (Tolando et al. 2001).

The protective effects of PG are well known; however, these were shown in experiments in which high doses of PG (2–4 mL/kg) were used (Hughes et al. 1991; Thomsen et al. 1995). Our results demonstrated that even small doses of 0.2 mL/kg, and particularly 0.6 mL/kg can suppress AILI. For ETOH, it is generally accepted that its chronic consumption increases AILI, but acute administration, together with or shortly before APAP administration, suppresses AILI (Prescott 2000). The dose of 0.2 mL/kg ETOH was not protective, and the dose of 0.6 mL/kg only slightly decreased ALT levels. PG and ETOH can reduce inflammatory responses and, opposite to the effect of DMSO, inhibit natural killer activity (Denning and Webster 1987; Oshiro et al. 1990; Chen et al. 2006; Pan et al. 2006).

Detergents such as Tween 20 and Tween 80 are also sometimes added to increase the solubility of lipophilic drugs in AILI experiments. It appears that Tween 20 at doses of 0.2 and 0.6 mL/kg has no influence on AILI or GSH content in the liver. There are no reports about its influence on the immunological response, oxidative stress, CYP2E1 activity, or coagulation system.

In another common model of liver injury, GalN plus LPS, none of the vehicles was protective or harmful. Interestingly, there are reports that show that DMSO is protective against GalN alone in rats. However, in those experiments the dose of DMSO was much higher (2.5 mL/kg) (Iida et al. 2007).

To avoid artifacts in AILI investigations it is advisable to use, when possible, pure saline or distilled water as a drug vehicle without adding any organic solvent or detergent. This is possible more frequently than is actually done. For example, 300 mg/kg of APAP, dissolved in warm saline under mild magnetic stirring, can be safely administered in a volume of approximately 0.5 mL per mice, and this is more than enough to induce AILI. Because PG and DMSO suppress AILI when used as vehicles for APAP, the dose of
APAP must be increased to 500 or even 750 mg/kg (Oz and Chen 2008) to cause similar liver damage. The result is an unnecessary increase in the APAP dose and application of a more biologically different organic solvent. Liu et al. reported that 300 mg/kg APAP is not enough to induce AILI in C57BL/6 mice and concluded that this strain was more resistant to APAP (Liu et al. 2004). Apparently, this was because DMSO was added to the APAP vehicle. In our experience, the 300 mg/kg dose of APAP induces severe hepatitis in CBA or BALB/c mice and as shown by Harill et al. in many other mouse strains (Harrill et al. 2009). When substances with low solubility in water are investigated for their effect on AILI, the control groups should be formed more carefully. Typical investigations include a treatment group, in which the substance of interest is dissolved in an organic vehicle, and the control group, in which only the vehicle is used. It would be good to include, at least in the first experiment, one more control group, in which only pure saline or distilled water is used, to better understand the effect of the vehicle. The dose of solvent or detergent added to the vehicle should be as small as possible and explicitly noted. It is probably better to use vehicles that interfere less with APAP toxicity. This means that Tween 20 and ETOH should be considered first, and then PG. The usage of distilled water is used, to better understand the effect of the saline solution. In some general hepatoprotective mechanism is investigated, it would be helpful to see whether it also protects against the GaIN plus LPS model of hepatotoxicity, since this model is probably better to use vehicles that interfere less with APAP toxicity. This means that Tween 20 and ETOH should be considered first, and then PG. The usage of DMSO or DMF should be limited to occasions when it is inevitable. Alternatively, some water-insoluble substances become soluble if the pH of the saline solution is changed. If some general hepatoprotective mechanism is investigated, it would be helpful to see whether it also protects against the GaIN plus LPS model of hepatotoxicity, since this model appears to be more resistant to vehicle usage.

Finally, in the effort to avoid a possible experimental artifact with water-insoluble drugs, it is necessary to reduce the amount of injected biologically active organic solvent (or reduce its concentration in in vitro experiments) to the minimum dose possible, use an organic solvent with a smaller biological effect, or try to dissolve the substance in aqueous solvent with changed pH. Nonetheless, it is very important to include control groups in which the effect of the drug vehicle alone is tested.

Acknowledgements

This study was supported by funding from the Croatian Ministry of Science, Higher Education and Sports (research project No. 0108-00000000-0328). All investigations were conducted at the Department of Physiology, School of Medicine, University of Zagreb, Zagreb, Croatia.

References


