



Biological actions of drug solvents

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Abbreviations:

AChE – acetylcholinesterase
AP – action potential
APAP – acetaminophen
CNS – central nervous system
DMSO – dimethylsulfoxide
i.p. – intraperitoneally
i.v. – intravenously
LPS – lipopolysaccharide
NF-κB – nuclear factor kappa B
NK – natural killer
NO – nitric oxide
PG – propylene glycol
TF – tissue factor
TNF-α – tumor necrosis factor alpha
TXA₂ – thromboxane A₂

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Abstract

Many biologic agents are weakly soluble in water. Therefore, they should be dissolved in organic lipophilic solvents (vehicles). A drug vehicle is a substance of no therapeutic value that is used to convey an active biological agent to the site of its action. Ideally, it should be biocompatible, 100% reliable, with no biological effect per se. However, presently used vehicles have pleiotropic effects, which are often unknown to researchers, and often cause misleading conclusions. In this review we summarize data on biological effects of the three most commonly used lipophilic drug vehicles dimethylsulfoxide (DMSO), propylene glycol (PG) and ethanol. Besides in experimental models, the data, where available, are shown on effects of solvents in therapeutic use in humans. In conclusion, some recommendations are given on the use of drug solvents in experiments.

INTRODUCTION

Many biologic agents are weakly soluble in water. Therefore, they should be dissolved in organic lipophilic solvents (vehicles). A drug vehicle is a substance of no therapeutic value that is used to convey an active biological agent to the site of its action. Ideally, it should be biocompatible, 100% reliable, with no biological effect per se. However, presently used vehicles have pleiotropic effects which are often unknown to researchers, often causing misleading conclusions. The aim of this review is to summarize data on biological effects of the three most commonly used lipophilic drug vehicles: dimethylsulfoxide (DMSO), propylene glycol (PG) and ethanol. The main effects of these agents are briefly summarized in Table 1.

DMSO

DMSO is one of the most common solvents used experimentally to dissolve hydrophobic substances for *in vivo* and *in vitro* purposes. The toxicity of DMSO is low: LD₅₀ in mice is 6.2 mL/kg (when applied intraperitoneally (i.p)) or 3.7 mL/kg (applied intravenously (i.v.)), LD₅₀ in rats is 9.9 mL/kg (i.p) or 7.4 mL/kg (i.v.) (1). However, DMSO by itself has a variety of biological actions which can confound the effects of drugs when DMSO is used as a vehicle. The highest given dose of DMSO (when used as a vehicle) which we found in literature was 6 mL/kg (2). It is noteworthy that some of the investigations cited in this review, found a substantial effect even with doses as low as 0.1 mL/kg (3).

Through its anti-inflammatory, anti-coagulant and reactive oxygen species scavenger activity, DMSO affects many organs, but it also has some organ-specific effects. In the central nervous system (CNS),

DMSO attenuates traumatic brain (4) and spinal injury (5), brain edema (6) and ischemic brain injury (7, 8). Neuroprotective effects of DMSO may be the result of synergy (interplay) of various contributing factors: it suppresses inflammation, scavenges free radicals, it is a potent diuretic, blocks Na⁺ channel activation and attenuates Ca²⁺ current, it decreases tissue factor (TF) expression and reduces thrombus formation... (9-12).

In contrast to this, one investigation in rats showed that DMSO, if given consecutively for 10 days, can cause reduction in nerve conduction velocity (in lower dose) and myelin disruption with uncompacted myelin lamelle (in higher dose) (13). Kleindienst *et al.* reported that DMSO may promote brain edema through increasing permeability of blood brain barrier (14).

Antinociceptive effects of DMSO have also been reported. DMSO has analgesic effect comparable to morphine, but the effect is not mediated through its action on

opioid receptors because it cannot be blocked by naloxone (15). Coadministration of DMSO with morphine interferes with antinociceptive effect of morphine; it seems that acute administration of DMSO enhances, and chronic administration, inhibits the effects of morphine (16). Matheus *et al.* showed in rats that DMSO, injected into the dorsal periaqueductal grey, acts anxiolytically (17).

DMSO inhibits acetylcholinesterase (AChE) activity; 1) it can potentiate effects of acetylcholine on the heart (18) and 2) it can reverse neuromuscular blockade caused by tubocurarine (19).

Cardioprotective effects of DMSO in myocardial ischemia have also been reported (20, 21). Most probable mechanisms of cardioprotection include its powerful inhibitory effect on platelet aggregation and suppression of TF expression, but other mechanisms, such as blockade of Ca²⁺ and Na⁺ influx, can also contribute to cardioprotection (10).

TABLE 1

Brief summary of the reported effects of DMSO, PG and ethanol on various organs.

Organ	DMSO	PG	Ethanol
CNS	protects from traumatic or ischemic brain and spinal injury (4, 5, 7,8) Antinociceptive and anxiolytic effect (15, 17) AChE inhibition (18, 19)	Protects from seizures caused by hyperbaric oxygen (63) or strychnine (64) anxiolytic effect (65)	CNS depressant (91-93) Low doses protect from traumatic or ischemic brain injury (91, 96, 97), high doses are detrimental (94, 95)
Heart	Protects from myocardial ischemia (10)	Reduces arrhythmias induced by strophanthin, barium chloride, aconitine or adrenaline (79, 80).	Effect on ischemia/reperfusion injury is controversial (109-115) Decreases action potential amplitude (116).
Liver	Induces CYP450 (22, 23) Protects from APAP, LPS, halothane and chloroform toxicity (3, 24, 27, 31) Protects from ischemia-reperfusion injury (32)	Inhibits CYP2E1 Protects from APAP (69) toxicity (25, 69, 81)	Given acutely protects from LPS and APAP (135-136, 139), when given chronically has hepatotoxic effect (137, 138, 140), Induces CYP450 (140)
Kidney	Protects from mercuric-chloride (34) and gentamicin toxicity (35)	In high doses causes proximal tubular cell injury (83)	Protects from salt-induced hypertension (144) Potentiates toxicity of methyl mercury (145, 146)
Gastrointestinal system	Protects from mucosal injury caused by cysteamine (37), NSAD (38-40), stress (38, 41) or ethanol (40) gastric irritant (42)		Gastric irritant (42)
Immune system	Mainly immunoinhibitory: inhibits NF-kB (31, 43), induced lymphocyte apoptosis (46), protects from sepsis (43, 45) Activates NK and NKT cells in liver (47)	Immunoinhibitory: inhibits macrophage, NK cell and neutrophil function (84, 86) Reduces carrageenin-induced edema (85)	Immunoinhibitory: inhibits function of various leukocytes (147-150)
Blood and circulation	inhibits platelet aggregation (10) suppresses tissue factor expression (10)	Causes pulmonary hypertension (74) Induces Heinz body formation in cats (87).	Inhibits platelet aggregation (126-128) Dilates splanhic blood vessels (117) Vasoconstriction in other organs (110-123)

DMSO treatment strongly induces expression and activity of phase 1 (CYP450) and phase 2 (UDP glucuronosyltransferases) drug-metabolizing liver enzymes (22, 23). However, the presence of DMSO during the drug activation often inhibits drug metabolism in the liver, probably by inhibiting the interaction of enzymes and xenobiotics (23, 24). Due to this effect, DMSO treatment can protect the liver from hepatotoxic agents that require bioactivation by CYP enzymes.

The effect of DMSO varies in different models. DMSO strongly protects against acetaminophen (APAP)-induced liver injury if applied before APAP, but it does not protect when applied 2 h after APAP (when bioactivation of APAP is completed) (3, 24, 25). On the other hand, it protects from halothane and chloroform toxicity even when applied 10–24 hours after poisoning, thus in this model the hepatoprotection cannot be solely explained by the blockade of bioactivation (26–28). Interestingly, DMSO has no, or very low, effect on carbon tetrachloride toxicity (23, 29); this is explained by the high affinity of carbon tetrachloride for the lipophilic CYP enzyme site (23). The protective effect of DMSO was also shown in models of liver injury caused by D-galactosamine (30), by simultaneous injection of D-galactosamine and lipopolysaccharide (LPS) (31) (in this model, high doses of DMSO are required for protection; we achieved no protection with a dose of 0.6 mL/kg (25) and by ischemia-reperfusion (32). In these models there is no need for P450 metabolism, thus not all hepatoprotective activity of DMSO can be explained by CYPs inhibition. Protection in these models seems to be mediated through reduced nuclear factor kappa B (NF- κ B) activation and anti-oxidative activity (31). DMSO also blocks the metabolism of various barbiturates and prolongs the sleeping time caused by these drugs (33).

Nephroprotective actions of DMSO have been reported in different models: it protects kidneys from mercuric-chloride (34) and gentamicin-induced injury (35). Nephroprotection in these models is probably the result of its anti-inflammatory and antioxidant effects. Protection against succinimide-induced kidney injury seems to be the result of the blockade of succinimide biotransformation in to its active metabolites N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) and N-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (NDHSA), since it was shown that DMSO is unable to protect kidneys against injury caused by direct injection of these metabolites (36).

Various investigations showed that DMSO protects stomach and duodenum from mucosal injury caused by cysteamine (37), by non steroid analgesic drugs (38–40), by stress (38, 41) and by ethanol (40). The proposed mechanism is scavenging of hydroxyl radicals (OH) (39, 41). However, Sorbye et al. reported that DMSO per se is a gastric irritant and causes mucosal-gastric injury and proposed that the protection achieved by DMSO in previously mentioned models is the result of »adaptive cytoprotection« rather than of an oxyradical scavenger effect (42).

It has been generally accepted that the effect of DMSO on the immune system is anti-inflammatory, through the inhibition of NF- κ B it suppresses tumor necrosis factor alpha (TNF- α) formation and reduces Inter-Cellular Adhesion Molecule 1 (ICAM-1) expression (31, 43, 44). Its protective role was shown in various models of sepsis (43, 45). Intraperitoneal treatment of rats with DMSO induced lymphocyte apoptosis in the thymus, spleen, and Peyer's patches (46). However, the immunostimulatory effect of DMSO was observed in a few studies. For example, it activated natural killer (NK) and natural killer T (NKT) cells in mice liver (47) and augmented the immune response to Sindbis virus infection in a mouse model by increasing the antibody titers and serum interferon gamma (IFN- γ) levels (48).

DMSO induces differentiation in many tumor cell lines (49–51), most notably HL-60 (from promyelocytic leukemia cells to mature granulocyte), probably by down-regulation of c-Myc expression (51). However, an opposite effect was described in a few cell lines: DMSO inhibited differentiation of human trophoblast cells and promoted invasiveness and metastatic potential of epithelial-cell lines. This is possibly mediated through increased methylation of DNA; methyl radicals are generated by the reaction of OH⁻ radicals with DMSO (52, 53). DMSO is not a good solvent for cisplatin, because these two substances react and form an adducted compound with reduced cytotoxicity (54).

In humans, the use of DMSO was considered as therapy in various pathological conditions such as amyloidosis, gastrointestinal disorders, brain edema, stroke, brain and spinal injury, rheumatoid arthritis, schizophrenia (55–58). The only indication currently approved by the United States Food and Drug Administration (FDA) is treatment of interstitial cystitis by intravesical instillation (59). DMSO is also used as a cryoprotectant in the freezing and long-term storage of embryonic stem cells and hematopoietic stem cells. Side effects described after DMSO treatment include hemolysis, vomiting, nausea, rash, flushing, bronchospasm and (very rarely) cardiac arrest, stroke, encephalopathy, pulmonary edema or renal failure (60). However, these side effects (except hemolysis) (61). are mainly described on occasions when human stem cells were transplanted with DMSO as a carrier. Thus, for the most of the reported cases it is not clear whether the side effect was caused by DMSO, or by some other contributing factor. DMSO administration causes garlic-like breath due to the pulmonary excretion of its breakdown product dimethyl sulfide. This can cause problems in double blinded studies, since the patients who received DMSO can be detected by odor (60).

PG

The toxicity of PG is low; LD₅₀ in mice is 9.3 mL/kg (when applied i.p) or 6.4 mL/kg (i.v.), in rats LD₅₀ is 13 mL/kg (i.p) or 6.2 mL/kg (i.v.) (1). The highest given dose of PG (when used as a vehicle) which we found in the literature was 5 mL/kg. (62) The effects described in our review occur with doses lower than this.

Although there are much fewer reports about biological effects of PG than for DMSO, PG is not completely inert. PG has been shown to have inhibitory effects on CNS: it reduces locomotor activity in mice; pretreatment with PG delays the onset of seizure caused by exposure to hyperbaric oxygen in cats (63) and by injection of pentylenetetrazol or strychnine in mice (64). Lin *et al.* showed in rats that PG acts as an anxiolytic agent, but its anxiolytic potency was weaker than that of ethanol (65). Prolongation of hypnotic effect of pentobarbital in rodents (66) and enhancement of the sedative effect of diazepam in humans (67) have also been reported, but these two effects are more likely caused by inhibitory effect of PG on CYP2E1 (68, 69) enzymes in the liver (which metabolize diazepam and pentobarbital) than by PGs' inhibitory effects on the CNS activity. In an ischemic stroke model of rat brain (70) Sood *et al.* found that PG has protective effect on blood brain barrier after a stroke. Hattori *et al.* reported that PG induces skeletal muscle excitation in frogs and mice. In these experiments, PG caused an increase in twitch tension and in amplitude of the endplate potential. The authors assumed that PG exhibits this action by facilitating transmitter release from nerve terminals and by raising the acetylcholine sensitivity of the muscle endplate (71, 72). However, another explanation for this could be the inhibitory effect of PG on AChE activity. Such inhibitory effect has already been reported for DMSO and some other organic solvents (19, 73), but the effect of PG on AChE activity has, to our best knowledge not been investigated.

PG causes pulmonary hypertension in sheep, possibly through the release of thromboxane A₂ (TXA₂) (74). Black *et al.* reported (1984) that administration of PG via a nebulizer and face mask can prevent bronchoconstriction induced by methoxamine in asthmatic patients (75). On the contrary, a recent study by Choi *et al.* found that children exposed to PG and PG ethers in bedroom air have 1.5-fold greater likelihood of asthma (76). Similarly, Wieslander *et al.* showed that short exposure to PG mist from artificial smoke generators may cause acute ocular and upper airway irritation in non-asthmatic subjects and that a few of them may react with cough and slight airway obstruction (77). Except for these three reports, there are, to our best knowledge no other studies on the influence of PG on asthma. Al-khudhairi *et al.* reported that intravenous administration of PG to dogs causes a transient decrease in heart rate and in arterial pressure, possibly because of reflex stimulation of vagus (78). PG prolongs effective refractory period and can reduce arrhythmias induced by strophanthin, barium chloride, aconitine, and adrenaline (79, 80).

PG strongly protects the liver from APAP toxicity because of its inhibitory effect on CYP2E1 enzymes which are important for bioactivation of APAP to its toxic product-NAPQI. It appears that PG does not inhibit CYP1A2 (25, 69, 81). As DMSO, PG blocks the metabolism of various barbiturates and prolongs the sleeping time caused by these drugs (33, 64). Because some immunosuppre-

ssive effects of PG (see below) have been reported, we investigated the effect of PG on the model of liver injury induced by D-galactosamine and LPS, but found no effect. However, our investigation was conducted with a low dose (0.6 mL/kg) of PG (25). The effect of PG on hepatotoxicity of some other substances that require bioactivation (halothane, chloroform, carbon tetrachloride) has not been investigated. Investigations about the influence of PG on other models of liver injury are also lacking. PG is metabolized by the liver to form lactate, acetate, and pyruvate, lactic acidosis may occur after treatment with PG (82).

Administration of high doses of PG to humans can cause acute kidney injury, probably because of proximal tubular necrosis; it was shown that proximal tubular cell injury occurs in cultured human cells exposed to PG (83).

Several immunoinhibitory actions of PG have been reported. It inhibited the LPS activation of rat peritoneal macrophages (84) and carrageenin-induced edema and pleurisy, as well as granulomatous tissue formation (85). Denning *et al.* showed that PG has inhibitory action on NK cell and neutrophil function (86).

PG administration induced Heinz body formation in cats and decreased red blood cell lifespan in adult cats in a dose dependent manner (87).

In humans, several cases of PG poisoning have been described after a treatment with a drug dissolved in PG (typically lorazepam). Toxic effects include hyperosmolality, increased anion gap metabolic acidosis (due to lactic acidosis), acute kidney injury, and sepsis-like syndrome (88). In dermatology, it is known that topical PG can cause allergic contact dermatitis (89).

Ethanol

Effects of ethanol are numerous and well investigated. We will limit our review on actions which are most likely to interfere with outcome in commonly used experimental models. LD₅₀ in mice is 4 mL/kg (when applied i.p) or 2.8 mL/kg (i.v.), in rats LD₅₀ is 6.3 mL/kg (i.p) or 2.3 mL/kg (i.v.) (1). The highest given dose of ethanol (when used as a vehicle) which we found in the literature was 1.65 mL/kg (90). Some of the effects described in our review were obtained in investigations with doses higher than this. We decided to indicate such investigations rather than to omit them because in most of them the lower dose of ethanol was not tested; it is therefore not possible to exclude that the similar effect would be obtained with a lower dose.

Ethanol is the CNS depressant, acting probably by increasing the effects of the inhibitory neurotransmitter GABA, although exact mechanisms are not fully understood (91-93). The effect of ethanol on CNS-experimental models varies from protective to detrimental, depending on the experimental model and the dose of ethanol applied. In a model of traumatic brain injury in rats, detrimental effect of the high doses of ethanol (higher than 2 mL/kg) was shown through an increase in cerebral ede-

ma (94, 95). However, for lower doses of ethanol, most investigations showed protective effect, possibly through a decrease in pro-inflammatory cytokine production (96, 97). Persson *et al.* investigated the effect of acute ethanol ingestion on cerebral-stab wounds in rats and found aggravated injury in ethanol pretreated rats due to the increased blood brain barrier permeability (98). It was considered that nimodipin and diazepam can protect against cerebral hypoxia (99), but Moursi *et al.* showed that the protection was actually mediated by ethanol which was used as a vehicle for these drugs (100). Titova *et al.* showed that ethanol protects against intracerebral hemorrhage induced by collagenase injection (101). Iida *et al.* showed that ethanol suppresses initial cerebral vasoconstriction caused by cigarette smoking, possibly by attenuating the smoking-induced TXA₂ production (102).

A study by Obregon *et al.* which compared the effect of various solvents per se on the cerebral AChE activity of rats found that ethanol had no significant effect on AChE activity even at 10% concentration and proposed ethanol as the best organic solvent choice (73). However, Rosenberg *et al.* reported that ethanol exposure (1% in volume) during 1 h increased AChE activity in zebrafish brain (103) and Owasoyo *et al.* reported that acute dose of ethanol significantly decreased AChE activity in the cerebral cortex of mice (104).

Low to moderate doses of ethanol are protective in a model of ischemia followed by reperfusion in the CNS and also in some other organs (small bowel, liver...) (91, 105-108), whereas the effect on ischemia/reperfusion injury in heart remains controversial. Different mechanisms for protective effect in this model of injury were proposed. One of the possibilities is that ethanol stimulates production of oxygen species which can induce organs to initiate various mechanisms of protection. This theory is supported by findings of Wang *et al.* that the protective effect of ethanol in CNS can be inhibited by apocynin, a specific inhibitor of NADPH oxidase (106). Similarly, Yamaguchi *et al.* reported that the protective effect of ethanol against ischemia/reperfusion injury in the small bowel of mice can be abrogated by administration of some common antioxidants (108). On the other hand, results of the experiments conducted by Qi *et al.* suggest that ethanol exhibits its protective effects in the CNS by enhancing GABA release and subsequently decreasing c-Jun N-terminal kinase 3 (JNK3) activation (91, 107).

The effect of ethanol on ischemia/reperfusion injury in the heart remains controversial. The chronic administration of low and moderate doses of ethanol is considered to be protective against ischemic or ischemia/reperfusion injury in the heart. Zhou *et al.* showed that the protective effect of chronic ethanol administration on ischemia/reperfusion injury of the heart in rats can be blocked by inhibition of protein kinase C epsilon (109), and Pagel *et al.* showed the same for a blockade of the mitochondrial K (ATP) channel (110). However, the role of acute ethanol administration is controversial. Chen *et al.* showed that a brief exposure of isolated adult rat cardiac myocytes to 10–50 mM ethanol protects against the dam-

age induced by prolonged ischemia, and that this effect could be blocked by inhibition of protein kinase C epsilon (111). Churchill *et al.* reported a protective effect of ethanol in vivo (0.5 g/kg, i.p.), when given 60 min prior to a 30-minute transient ligation of the left anterior descending coronary artery in rats (112). Similarly, Gross *et al.* showed the protective effect of ethanol (0.25–0.5 g/kg, i.v.) in dogs (113). However, Weber *et al.* could not reproduce these results (114). The difference between these two experiments is that in the former dogs were anesthetized and in the latter awake. The authors hypothesized that the anesthesia could be the confounding factor (113, 114). Hale *et al.* found no protection with acutely administered ethanol in rabbits (115).

Beberova *et al.* investigated the changes of the action potential (AP) configuration and ionic membrane currents in rat cardiomyocytes under ethanol exposure and found decelerated upstroke velocity, decreased AP amplitude and inhibition of the fast sodium current and I-type calcium current and also a transient inhibition of outward potassium current (116).

It is well known that ethanol acts in vasodilatory manner on splanchnic blood vessels (117). However, it seems that effects on blood vessels in brain, lungs, and possibly in some heart vessels are mainly constrictive (118-120). Doekel *et al.* showed that ethanol potentiates hypoxic pulmonary vasoconstriction in dogs (121), and Lu *et al.* showed that this effect can be blocked with a lipoxygenase inhibitor (122). Vasoconstrictive effect of ethanol on pulmonary circulation has also been reported (123). Most reports about effects of ethanol on cerebral blood vessels suggest vasoconstrictive effect (119, 120); however, a few reports (Iida *et al.*, for example) reported a vasodilatory effect (102). Haves *et al.* showed that ethanol caused epicardial coronary artery vasoconstriction in dogs (124). Vasoconstriction of carotid artery and aortic ring after ethanol exposure was also reported (118, 119). Baraona *et al.* investigated the effect of ethanol on blood flow in various organs in rats; ethanol (3 mg/kg, p.o.) increased portal blood flow, but did not change the hepatic, splenic or pancreatic arterial blood flows, it quadrupled the coronary blood flow, doubled the renal flow and increased cardiac output, but no significant changes in pulmonary, cerebral or testicular flows were found. All these effects of ethanol could be blocked with the nitric oxide (NO) synthase inhibitor (125). Ethanol is known to inhibit platelet aggregation, possibly through inhibition of phospholipase A₂, resulting in a decrease in arachidonate metabolites, including TXA₂, a potent stimulant of platelet aggregation (126, 127). Ethanol inhibits platelet aggregation mainly via transplasmalemmal Ca²⁺ entry-independent mechanisms (128).

Ethanol is known to trigger attacks of asthma in susceptible human individuals. Trevisani *et al.* showed that ethanol treatment caused bronchoconstriction and increased plasma extravasation in the guinea pig airways. This action could be blocked by the inhibitor of the transient receptor potential vanilloid-1 (129). Because of its immunosuppressive actions, ethanol can impair lung re-

sistance to infection caused by *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and other microorganisms (130, 131). Some possible mechanisms are negative effects on neutrophils and NK cells, attenuation of inducible NO synthase and cyclooxygenase-2 induction, up-regulation of IL-10 and downregulation of IL-12 (132, 133).

Acute ethanol treatment, performed a few hours before LPS, protects from LPS-induced liver injury; it causes tolerance of Kupffer cells due to the downregulation of toll-like receptor-4 and decreases production of TNF- α (134-136). However, chronic ethanol treatment has an opposite effect; it sensitizes Kupffer cells and aggravates LPS-induced liver injury (134, 137, 138). These effects were described for the doses of ethanol greater than 2 mg/kg. In a model of liver injury caused by simultaneous injection of D-galactosamine and LPS we found no effect of a low dose of ethanol (0.6 mg/kg) (25). To our best knowledge, there are no other reports about effects of ethanol in this model. Wong *et al.* showed that a concomitant single dose of ethanol (1 g/kg) protected mice from hepatic injury induced by APAP, probably through an inhibitory effect of ethanol on the biotransformation of APAP to the toxic intermediate, an effect similar to that of DMSO, that has been described previously (139). In our experiments the dose of 0.6 mL/kg applied 30 min before APAP had no influence. Chronic ethanol feeding increases the hepatotoxicity of APAP probably through an enhanced production of reactive metabolites since ethanol is known inductor of microsomal enzymes (140). Jaruga *et al.* showed that chronic ethanol administration increased the susceptibility of mice to concanavalin A – induced T cell-mediated hepatitis via upregulation of the NF- κ B signaling pathway (141). Acute and chronic ethanol treatment potentiates the toxicity of chloroform and carbon tetrachloride; this effect cannot be fully explained by induction of microsomal enzymes (142, 143). Yamagishi *et al.* reported that a low dose of ethanol (1 mg/kg), given 15 min before gut ischemia, attenuated ischemia-reperfusion liver injury and that a high dose (4 g/kg) had an opposite effect (105).

Vasdev *et al.* reported that the chronic consumption of low doses of ethanol protects rats from salt-induced hypertension (144). Several independent studies confirmed that ethanol, given chronically at low doses, potentiates the nephrotoxicity and neurotoxicity of methyl mercury (145, 146). We could find no report about effects of ethanol on the commonest models of kidney injury induced by gentamicin or cisplatin.

Ethanol treatment causes immunosuppression, increasing susceptibility of animals to various microorganisms. Ethanol inhibits the function of leukocytes (monocytes, neutrophils, NK cells...) (147-150). Szabo *et al.* showed that ethanol treatment reduces the levels of pro-inflammatory cytokines (TNF- α and IL-1 beta) and increases the levels of mediators with immunoinhibitory potential, including transforming growth factor-beta and IL-10 (147). Vinson *et al.* investigated whether the ethanol-caused suppression of neutrophil accumulation in

the peritoneal cavity of the rats can be explained by an ethanol-induced increase in endogenous corticosterone. However, ethanol maintained the suppressive effect even when the action of corticosterone was blocked (148).

Other drug solvents

As we pointed out in Introduction, we have not discussed other drug solvents that are rarely used in experiments or in therapeutic application of lipophilic drugs. For example, we have shown that dimethylformamide is highly protective in APAP-induced liver injury, but not in the liver injury induced by simultaneous injection of D-galactosamine and LPS (25).

In conclusion, 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 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 or ionic strength, warming and mixing the solution, etc. Nonetheless, it is very important to include control groups in which the effect of the drug vehicle alone is tested. These principles were successfully applied for investigation of influence of high lipophilic eicosanoid derivatives on APAP-induced liver injury in mice (151, 152).

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