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Potential confounding effects of benzyl alcohol as a formulation excipient support the elimination of the abnormal toxicity test from pharmacopoeias

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ABSTRACT

Benzyl alcohol is an excipient used in many drugs as a stabilizer. Depending on the amount present in drug formulations there might be confounding findings in the Abnormal Toxicity Test (ATT). The ATT is utilized as a quality control (QC) release test to detect extraneous contaminants according to national pharmacopoeias. Our study assessed the effects of benzyl alcohol as defined in ATT designs. This study – the first thorough evaluation of the confounding effects of benzyl alcohol on the ATT – was conducted in relation to particular health authority questions and was part of the root-cause analyses resulting from some transient behavioral findings observed in the test. Two strains of mice, CD-1 & Kunming, plus Hartley guinea pigs were administered intraperitoneally (ip), subcutaneously (sc), or intravenously (iv) with benzyl alcohol at dose level defined in the ATT design. In both mice and guinea pigs, only after ip administration, minimal behavioral changes were observed transiently within 2–3 min after administration. Therefore, the presence of benzyl alcohol in the product batch may confound the ATT results. This study provides further evidence to question the validity of the ATT for its intended use.

1. Introduction

The abnormal toxicity test (ATT) is also referred to as a general safety or innocuity test. According to a number of national pharmacopoeias or other regulatory requirements for certain product classes, it is currently still used as a quality control (QC) release or import test to detect extraneous contaminants. The test principle consists of injecting a certain volume of the relevant product batch into guinea pigs and/or mice. The exact test design varies slightly between respective national pharmacopoeias (European Pharmacopoeia, 2013; US Food and Drug Administration, 2013; World Health Organization, 1990; Russian State Pharmacopoeia, 2007; China Pharmacopoeia, 2010). Typically, a product batch passes the test if the findings follow these criteria: 1. animals survive the test period; 2. animals do not exhibit any abnormal response or signs of toxicity; and 3. animals do not weigh less at the end of the test period than that at the time of injection. In the mid-1990s the Paul-Ehrlich-Institute published a retrospective analysis of several thousand ATT results. The authors provided strong evidence that the ATT is, in principle, not suited to predict or detect harmful batches because it lacks specificity and reproducibility. Additional factors, including high concentrations of active drug or formulation components, can lead to misinterpretation of results (Duchow and Kramer, 1994; Kraemer et al., 1996). Very recently, on July 2nd 2015, the FDA published a final rule 'amending the biologics regulations by removing the general safety test (GST) requirements for biological products' (FDA, 2015). The reasons for the removal seem to correlate with what the authors from the Paul-Ehrlich Institute published and another recent review of the ATT (Garbe et al., 2014).

Benzyl alcohol is a member of the fragrance structural group alkyl alcohols and is a primary alcohol. It is used in a wide variety of cosmetic or medicinal formulations as a fragrance component, preservative, solvent, and viscosity-decreasing agent. In medicinal products it is not only used intramuscularly in antibiotics, anti-inflammatory or neuroleptic medicines or intravenously in anti-cancer drugs or cardiovascular drugs, but also topically in

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antifungal and anti-inflammatory products. The European guideline "Excipients in the label and package leaflet of medicinal products for human use" (European Commission, 2003) issued in 2003 defines the thresholds for various excipients, in which the one for benzyl alcohol by parenteral administration is 90 mg/kg daily. Acceptable daily intakes were established by the World Health Organization at 5 mg/kg for benzyl alcohol (Nair, 2001). Compared with what is administered to humans, irrelevantly high levels of benzyl alcohol as a vehicle component may be injected into rodents via the ip route per ATT design. This may cause untoward responses that could be misinterpreted as false positive results. In the present study, the effects of benzyl alcohol, at a concentration of 10 mg/mL used in some marketed drugs, was assessed in both mice and guinea pigs with a corresponding dose level of approximately 250 mg/kg in mice or 200 mg/kg in guinea pigs as by ATT design according to national pharmacopoeias. This study was conducted in relation to some health authority questions and was part of the root-cause analyses resulting from some transient behavioral findings observed in the ATT. This was the first thorough evaluation on the confounding effects on ATT test by benzyl alcohol, a widely used formulation excipient in many drugs.

2. Materials and methods

2.1. Test substance

Benzyl alcohol (C_7H_8O, CAS No. 100-51-6, Lot No. BNOH128-4; purity 98–100.5%) was supplied by Sigma–Aldrich (Arklow, Ireland).

For injection administration, the test substance was mixed into saline (0.9% sodium chloride in sterile injection water, Lot No. 130801) supplied by Shanghai Changzheng Fuming Jinshan Pharmaceutical Co. Ltd (Shanghai, China). The dose formulation was prepared at 10 mg/mL and sterilely filtered one day before dosing.

2.2. Animals and husbandry

Female outbred albino mice (CD-1 and Kunming) were supplied by Shanghai Animal Research Center (Shanghai, China). They were received at the test facility at approximate 3–4 weeks of age and acclimated for around 1 week, during which they were examined for suitability for the study, before commencing treatment. At the start of dosing, average body weight range was 18.1–20.1 g (CD-1) or 18.6–20.9 g (Kunming).

Male outbred albino guinea pigs (Crl:Hartley) were supplied by Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). They were received at the test facility at approximate 6 weeks of age and acclimated for around 1 week, during which they were examined for suitability for the study, before commencing treatment. At the start of dosing, average body weight range was 275–280 g.

All animals were SPF grade and met the requirements for ATT in terms of body weight, etc. according to the pharmacopoeias.

Animals were either group housed for mice (5 mice/cage) or individually housed for guinea pigs in plexiglass boxes with autoclaved sawdust bedding. They were maintained in an air conditioned animal room with a daily average temperature of 22 °C ± 3 °C, and a daily average relative humidity range of 40%–70%, under a 12-h light/dark cycle.

The animals received standard pelleted maintenance diet (mouse diet from Shanghai SLAC Laboratory Animal Co. Ltd., Shanghai, China; guinea pig diet from Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) ad libitum, and tap water ad libitum (in water bottles). No any diet or water contaminants which would be expected to interfere with the study were found based on the facility periodic analysis.

2.3. Study design

The study was performed at the Roche Innovation Center (Shanghai, China) rodent animal facility with a common ATT study design in accordance with several pharmacopoeias (European Pharmacopoeia, 2013; US Food and Drug Administration. rev., 2013; World Health Organization, 1990; Russian State Pharmacopoeia, 2007; China Pharmacopoeia, 2010).

Benzyl alcohol at 10 mg/mL corresponding to approximate 250 mg/kg in mice or 200 mg/kg in guinea pigs was given once by intraperitoneal, subcutaneous, or intravenous injection to female mice or by intraperitoneal or subcutaneous injection to male guinea pigs. The dose volume was selected based on what is required in most pharmacopeias, i.e. 0.5 ml for intraperitoneal injection for mice. The same volume was used in mice for subcutaneous injection. This route was used for comparison purposes. For the bolus intravenous injection to mice 0.1 ml was used according to the good practice guide for the administration of substances (Diehl et al., 2001; Gad, 2007). A dose volume of 5 ml as indicated in pharmacopeias was given to each guinea pig for both administration routes. Animals were assigned to groups using a computerized random sort program so that body weight means for each group from the same species were comparable as follows: 5 mice/group or 2 guinea pigs/group for each administration route.

In general, a blank control is not required for ATT according to most national pharmacopoeias, except China. For the purpose of comparison between two strains, 5 mice/group was employed as the blank control to each administration route and dosed with saline (0.9% sodium chloride in sterile injection water, sterilely filtered one day before dosing) in the same manner as the benzyl alcohol administration. The general study design is summarized in Table 1.

All animals were observed for 7 consecutive days after the dosing. Each test animal was weighed, and the individual body weights were recorded immediately prior to injection and daily at the similar time during the test. Each animal was observed twice daily for mortality and clinical signs. The first detail clinical observation was performed individually for each animal within the first hour post dosing. Any animal response was recorded on the same day the response was observed.

2.4. Statistical analysis

Mice body weight statistical analyses were performed by one-way analysis of variance, followed by Tukey's post-hoc tests at $p < 0.05$ ($n = 5$) for different administration routes within the same strain.

Mice body weight statistical analyses were performed by unpaired t-test ($n = 5$) at $p < 0.05$ between two strains for the same administration route.

No statistical analysis was performed for guinea pig body weight due to low sample numbers ($n = 2$).

3. Results

3.1. Mortality and clinical observations

All animals of both species survived until the end of the 7-day observation. No clinical signs were observed in any of the mice treated with saline. For the benzyl alcohol treatment, there were also no clinical signs in any of the mice administered either subcutaneously or intravenously or in any guinea pig administered subcutaneously.