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Ethical Observations on the Choice of Parenteral Solvents

Choice of Parenteral Solvent…

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The parenteral administration of insoluble drugs leads to the use of biologically active solvents inducing effects associated with ethical cause of concern including pain and pharmacological interactions. Selected vehicles currently used were ethically and scientifically reviewed. Our investigations allowed reinforcing the formulation decision tree with an ethical point of view. The last generation of cyclodextrin appears to be the safest solvent. Second choice could be lipidic emulsions, third choice being co-solvents, and finally non-ionic surfactants because of their hypersensitivity reactions. Screening tests including pH, osmolality measurements, cytotoxicity, and hemotoxicity, should allow to check the formulation tolerance before the animals' administration.

Keywords solvents; toxicology; parenteral administration; adverse effects; ethical considerations

INTRODUCTION

The solubilization of the pharmaceutical active ingredient (AI) is a major problem for the parenteral administration. This physico-chemical aspect is not generally a cause of concern during the first stages of development of new molecules, because the evaluation of new medicines often begins with screening tests in vitro for which the choice of the solvent is not restrictive. The problem of tolerance of the solvent used in formulations appears later, throughout preclinical studies during which it will be necessary to resort to various methods to obtain a well-tolerated injectable aqueous solution. To select the solvent, the formulator has to take several precautions. The vehicles used can be biologically active and cause experimental variations because of the toxicity of the solvent and\or an interaction with the drug. Besides, the method of solubilization must not induce pains or stress for the animal during the administration of the formulation to avoid observing reactions because of the pain rather than because of the AI itself. The various committees of ethics for animal experimentation often stress the importance of the choice of the solvent to have the best possible study conditions while respecting the 3 R. principle (reduce, refine, replace, that is, reduce the number of used animals, improve the methodology of the tests, and replace by in vitro tests when it is possible). The objective of this article is to support the choice of solvent during the preclinical studies, with a "formulation decision tree" that overviews the main classically used solvents from the most ethically acceptable (based on the toxicity) to the least tolerable. On the basis of ethical criteria, this approach could be useful in the selection of the best tolerated vehicle, at least during these indispensable preliminary stages in the evaluation of a new molecule.

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FORMULATION DESIGN

It is generally considered that there are four different methods to increase the solubility of the active hydrophobic ingredients: pH adjustment of the solution, use of co-solvents, and use of additives such as surfactants or cyclodextrins (CDs), and finally creation of lipidic emulsions (Strickley, 1999). The pH adjustment of the solution between 3.5 and 9 aims at ionizing the molecule by playing on its pK_a to improve its solubility. This physico-chemical modification of the AI is applied only in some very specific cases, according to the characteristics of the molecule. However, because this modification can alter the

efficiency of the active molecule, other methods of solubilization will be preferably chosen.

Co-solvents, also named non-aqueous organic solvents, such as ethanol, propylene glycol (PG) or polyethylene glycols (PEG 300 or 400) are helpful in developing hydrosoluble solutions by decreasing the dielectric constant of the resulting binary solvent-AI system. Although they are very common, these co-solvents are known to cause hemolysis. The hemotoxicity has been investigated in several studies by determining the hemolytic activity. The LD 50 value for lysis of the red blood cell (RBC) was expressed as a percentage of co-solvent in whole blood. Half of the RBC is lysed as 5.7% of PG or 21% of ethanol or 30% PEG 400 (Reed & Yalkowsky, 1985). An association of 20% of PEG 400 and ethanol or PG may alleviate these effects (Fu, Lidgate, Whatley, & McCullough, 1987).

Co-solvents, especially PG, are also responsible of myotoxicity (Brazeau & Fung, 1989), but this aspect is not relevant for the intravenous administration.

In vivo, the main target organs of these solvents are the central nervous system (CNS), the cardiovascular system, and the lungs. The effects on the CNS, especially for ethanol and PG, are often translated by an excitement followed by a depression with a decrease of the physical tonus, prostration, and drowsiness (Heintz & Boymond, 1989). A reduction of cardiac frequency and the amplitude of the complex QRS have been observed both in humans and in animals further to the administration of ethanol (Pagala, Ravindran, Amaladevi, Namba, & Grob, 1995).

Additives of solubilization such as surfactants (Cremophor, polysorbate 80, Solutol HS15®) are another way to solubilize the AI: they act by decreasing water surface tension by accumulation in the interface air–water through micellization at the critical miscible concentration (CMC). The main toxicity observed in human or dog involves a pseudo-anaphylactoïd reaction characterized by pruritus, erythema, (cutaneous) rash, or generalized urticaria (EMEA, 2003; Tije, Verweij, Loos, & Sparreboom, 2003). The origin of these pseudo-allergic reactions has been the object of several hypotheses. The oleic acid, free fatty acid found in Cremophor or in polysorbate 80, could provoke a release of histamine directly from mast-cells (Dorr, 1994). This hypothesis has been partially clinically confirmed because premedication with antihistamines and corticosteroids decreases the incidence of hypersensitivity reactions throughout paclitaxel therapy (an anticancer agent formulated in Cremophor).

Another hypothesis demonstrated that Cremophor can cause a complement activation clearly concentration-dependent with a minimum threshold of 2 μL of Cremophor/mL of plasma (in vivo) (Tije et al., 2003) or 2.2 mg/mL (in vitro) (Szebeni, Muggia, & Alving, 1998). This threshold may be widely exceeded during perfusion; however, the plasma clearance of the Cremophor is time-dependent and increases significantly with prolongation of the infusion duration. For 1, 3, or 24 h of perfusion, the clearance of the Cremophor is 160, 300, and 400 mL/h/m^2 respectively. This leads to an increase in systemic exposure and thus an increase of allergic reactions with a shortening of the infusion duration. Therefore, slower perfusion performed over 3 h rather than 1 h should be preferred for this type of solution, which, in these conditions, seems to be less toxic (Tije et al., 2003). Besides the incidence of peripherical neuropathy observed, such as paraesthasia or loss of proprioception, is lower with the treatment including polysorbate 80 than with Cremophor.

During the FDA excipient workshop (September 2004), BASF chemical company compared Soluol® HS15 to some Cremophor and polysorbates. It appeared that Solutol® HS15 (PEG 660 hydroxystearate) could induce a lower histamine release compared with Cremophor and polysorbate (Quadir, 2004) so this surfactant could be the best tolerated and its use should be preferred to solubilize the AI.

CDs have recently been marketed and today appear to be new useful formulation vehicles. They are cone-like cavities that increase the solubilization of the AI by forming an inclusion complex with the hydrophobic interior core of the CD. The most common CDs accepted for the parenteral use are the β-CDs, among which we can mention the methyl-β-CD (RAMEB), the sulfobutylether-β-CD (Captisol®), or the hydroxypropyl-β-CD. Various chemical groups are substituted for the primary or secondary hydroxyl to improve the water solubility and the tolerance of these molecules. A dose of 30 mg/kg/day RAMEB (randomly methylated-beta-CD) causes severe kidney failure characterized by an osmotic nephrosis with renal cortical swelling and vacuolization of the proximal convoluted tubules and an acute renal failure. The hydroxypropyl-β-CD 11.5% administered at 225 mg/kg during 7 days also induces a renal cortical vacuolization and a macrophage infiltration of the lungs. When the dose and the duration of the treatment are increased, whatever the species, the signs are associated to biochemical disorders (increase of transaminases and bilirubin) and hematological disorders (decrease of the hematocrit and the number of erythrocytes) (Gould & Scott, 2005). For a close chemical structure of β-CD, Captisol® seems devoid of toxicity because of the presence of a sulfobutylether group, which facilitates its renal elimination and prevents its accumulation (data from the supplier).

Concerning the lipophilic ingredient, the last technique to improve its solubilization is based on the creation of lipidic emulsions composed of 10–20% soybean oil, 2% glycerol, and 1% egg lecithin (Strickley, 1999). The toxic effects of these oil-in-water emulsions are mainly because of the chemical composition of the formulation. The risks of embolism sometimes mentioned in articles are related to the galenic formulation and are reduced by limiting the size of drops to approximately 1 μm (Bury & Boymond, 1990). Emulsions provide lipids that can induce an overload syndrome characterized by hepatosplenomegaly with or without icterus and hematological reversible disorders, especially for young children (Goulet et al., 1986). Fat deposits in the liver are also observed,

whatever the animal species, but the associated inflammatory reaction seems specific to dogs (Izzo et al., 1984). The presence of soybean oil in the formulation can sometimes induce hypersensitive reactions for people prone to allergy. Seeing that it also promotes the risks of bacterial contamination, it is therefore necessary to respect strict conditions of asepsis.

CRITERIA OF CHOICE OF THE VEHICLE

During preclinical studies, the experimenter has to use high concentrations of AI. So, it is indispensable to carefully choose the best tolerated vehicle for the slightly soluble or water insoluble molecule. Among the various methods of solubilization, the pH adjustment seems the most ethically acceptable one, because it does not need the addition of solvent. Nevertheless, it is necessary to examine the pH to obtain the ionization of the drug; extreme values being at the origin of necrosis or pains. Morton et al. (2001) suggest respecting a working range of pH included between 4.5 and 8, whatever the way of administration. As products administered by intravenous (IV) route are immediately diluted in the blood (and as blood has a high buffer capacity), it can be suggested to widen appreciably this range to 3.5–9 for the IV administration only. In practice, a molecule having a pK_a lower than 3 or superior to 11 cannot be solubilized by this method because the pH conditions to obtain the desired concentration will not be acceptable. For these products, it is then recommended to resort to another method of solubilization.

For a long time, the use of CDs was difficult because of their toxicity. However, the effects of the last generation of CDs, in particular the renal disorders, have lessened because of the chemical modifications performed on the basic structure of these molecules. Therefore, sulfobutylether-β-CD (Captisol®®) seems to be the best tolerated solvent among all those found in this study.

The second most appropriate solvent for lipophilic products only is lipidic emulsions. The adverse effects of these emulsions are generally moderate and reversible, but it is still necessary to pay attention to the allergies they can sometimes induce because of their soya composition, and the risks of secondary bacterial infections connected to the nature of their environment. The use of co-solvents, adequately concentrated, could be suggested in the third place, providing the hemolytic and cytotoxic effects they can engender are monitored. Many studies consider that the least toxic co-solvent is PEG 400 because it exhibits the greatest substantial protective effect to muscular or blood cells when it is associated to other usual co-solvents. On the basis of the tolerance data, the worst solvent that could be used would be surfactant, especially Solutol®. To limit the allergic effects, it is advised to carefully shake the solution before administration, in order to homogenize micelles and prolong the infusion. Antihistamic premedication has proved successful to decrease these effects on humans, although it does not eliminate them completely, but it is inconceivable during preclinical studies because of the risks of experimental deviation due to the addition of new molecules in the experimental protocol.

Once the selection of the solvent has been made and the solubilization of the drug obtained, the tolerance of the formulation could be estimated by different tests. First of all, the measure of pH and osmolality of the solution, even if approximate, would permit an action on the osmolality by adding buffers. Besides, the realization of in vitro tests would help to determine the hemocompatibility and the risks of cellular alteration, which might be induced by the formulation. The classical tests of cytotoxicity performed on cultured endothelial cells monolayer (HUV-EC) would assess the risks of venous hurts and of extravasations induced by a prolonged contact with these solutions (Medlicott, Foster, Audus, Gupta, & Stella, 1998). These type of tests seem particularly interesting to compare various formulations prepared with variable proportions of solvent and so help in the choice of the best tolerated mixture.

CONCLUSION AND DISCUSSION

Today, medicine development studies still have to be performed on laboratory animals in the absence of alternative methods. The toxic effects are tested parenterally using high doses of AI, which can be a problem when the product is weak or not soluble in water. It is then necessary to use potentially biologically active solvents, but if not chosen sensibly, these will induce scientific consequences characterized by pharmacological and kinetic interactions and effects associated with ethical cause of concern including pain/stress for the animals. Reviewing the main advantages and disadvantages of the currently used selected vehicles, a reinforced formulation decision tree has been established, based on both scientific and ethical considerations (Figure 1). This theoretical proposal is based on ethical considerations and is intended to improve the conditions of the preclinical studies.

As for water insoluble AI whose pK_a is too weak (pK_a < 3.5) or too high (pK_a > 11) to alter the pH of the solution, the CDs and more particularly Captisol®, which presents a weak toxicity and a strong solubilization ability, seem to be not only the most interesting excipients but also the most expensive. The second choice would be the administration of lipidic emulsions, mainly for the neutral and highly lipophilic molecules, because they permit pH and tonicity adjustment of the solution. However, it is necessary to strictly respect the rules of asepsis, and this kind of formulation may be difficult to manufacture. Co-solvents, which are generally used, would be our third position. PEG appears to be the least toxic and is often associated to ethanol and sometimes to Tween®. Propylene glycol is commonly used but presents an important hemolytic and myotoxic activity. Therefore, it could be proposed to replace it by PEG 400, which has been shown to have equivalent solubilizing power and less adverse effects. When the use of the solvents

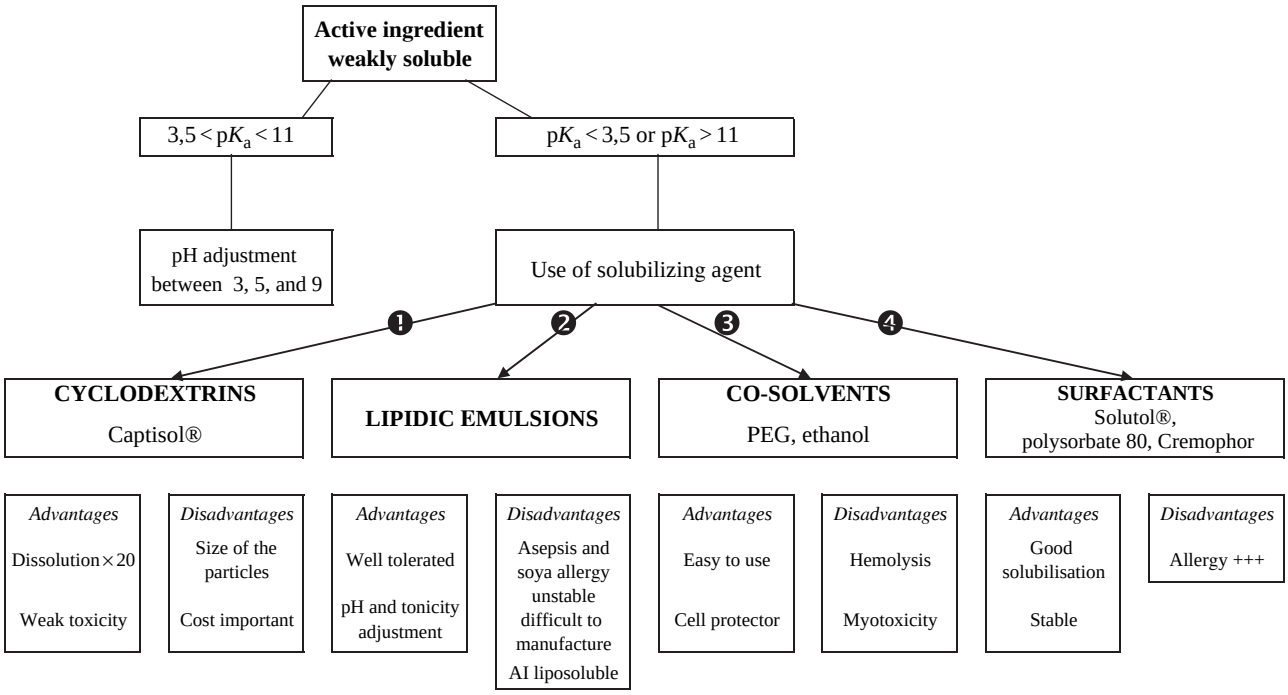

FIGURE 1. Proposal for an ethical choice of a solubilization method.

mentioned above is not possible, the last option would be to use surfactants, preferably Solutol®. However, this class of solvents will be used carefully because of strong allergic reactions engendered by these compounds.

On a large scale, manufacturing and cost may modify this classification. The use of CD would induce patent and royalty fee implications. Moreover, because of the weak stability of the emulsion, the formulation prepared with this method should not long be stored and would involve new materials to measure the size of the particles. Surfactants induce high allergic reactions; nevertheless, many anticancer agents are commonly solubilized on Cremophor. Most of the tolerance studies found investigate Cremophor, and a few of them focus on polysorbates.

Because surface active agents are widely used to stabilize biopharmaceutical solutions despite the sensitive reactions they induce, it is difficult to consider them as a last resort; however, their use should be avoided as much as possible. When there is no other solution, the hypersensitivity should be reduced by using slow rate of perfusion or by shaking the formulation before administration.

Currently, several strategies to develop formulations of surfactant-free chemotherapeutic agents have been developed. Nanoparticles are new solvents and seem to be a good alternative to replace the surfactant. Approved by the FDA, Abraxane® is a nanoparticle colloidal suspension, prepared "by high-pressure homogenization of paclitaxel in the presence of human serum albumin at a concentration of 3–4%, similar to that of albumin concentration in the blood." Devoid of Cremophor, this nanoparticle colloidal suspension has a shorter administration time, and a lower incidence of hypersensitivity reactions. Moreover, these nanoparticle drug carriers have been known to preferentially accumulate in tumor beds and facilitate the partitioning of nab paclitaxel into tumor tissue (Hennenfent & Govindan, 2005).

Dispersed systems such as liposome (phospholipid unilamellar or multilamellar vesicles) and nanosphere [solid colloidal particles that incorporate drug through dissolution encapsulation in polymer matrix or IDD (insoluble drug delivery] are also novel systems that use phospholipid as a surface-modifying agent to form micron size particles for solid or oily drugs. These approaches allow to decrease the adverse effect of the solvent with an excellent safety and efficacy profile. Pace, Pace, Parikh, and Mishra (1999) reported that "most phospholipids are accepted by regulatory agencies for parenteral administration because of their endogenous nature in mammalian system and their history of use and their safety." There are several novel formulations, including albumin, nanoparticles, or microsphere encapsulation to improve the solubilization of the drugs. However, whichever way is retained, it would be important not only to estimate systematically the pH and the osmolality but also to perform in vitro classical tests of cytotoxicity and hemotoxicity before any administration of the final formulation to the animal. The importance of these recommendations would help to compare the various possible formulations and choose the most effective and best tolerated one. Finally, it is important to consider that a change in the formulation might alter the pharmacokinetic parameters of the AI. This

implies checking systematically the physico-chemical characteristics of the prepared solution, case by case, according to the considered molecule because a study performed with well-tolerated solvent ensures reliable results.

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