British Journal of Anaesthesia **111** (5): 825–32 (2013)
Advance Access publication 25 June 2013 · doi:10.1093/bja/aet218

BJA

Efficacy comparison of the novel water-soluble propofol prodrug HX0969w and fospropofol in mice and rats

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Editor's key points

- Fospropofol is approved by the Federal Drug Administration but its metabolite, formaldehyde, is not considered to be ideal.
- In this study, a similar water-soluble compound HX0979w was synthesized, which had gamma-hydroxybutyrate as a metabolite.
- HX0969w released propofol and was as effective as fospropofol in rats and mice.
- Further studies are needed, but HX0969w may be a safer alternative to fospropofol.

Background. HX0969w is a novel water-soluble prodrug designed to release propofol and gamma-hydroxybutyrate (GHB) and has a sedative–hypnotic effect. This study was performed to compare the efficacy of HX0969w with fospropofol in mice and rats.

Methods. We performed hydrolysis studies in the plasma from mice and rats. The half-maximal effective doses (ED_{50}) and half-maximal lethal doses (LD_{50}) of fospropofol and HX0969w were determined. A pharmacodynamics comparison of these two compounds was also performed. Time to loss of righting reflex, time to return of righting reflex, recovery time, and adverse effects were recorded.

Results. The hydrolysis studies demonstrated that HX0969w released propofol as expected. HX0969w ED_{50} values in mice and rats were 133.03 and 53.79 mg kg^{-1}, respectively, and LD_{50} values were 607.11 and 283.79 mg kg^{-1}, respectively. The calculated therapeutic index (TI), safety index (SI), and certain safety factor (CSF) of HX0969w were 4.56, 3.33, and 2.92 for mice, and 5.28, 3.94, and 3.49 for rats, respectively. The pharmacodynamic comparison studies suggest that HX0969w has a longer onset time and shorter duration than fospropofol.

Conclusions. Similar to fospropofol, HX0969w is an effective, water-soluble prodrug that is capable of inducing a sedative–hypnotic effect in mice and rats. Unlike fospropofol, HX0969w releases GHB instead of formaldehyde. Further studies regarding the efficacy and safety of HX0969w are necessary.

Keywords: anaesthetics i.v., propofol; gamma-hydroxybutyrate; potency, anaesthetic, ED_{50}; water-soluble prodrug

Accepted for publication: 17 April 2013

Propofol, an i.v. sedative–hypnotic agent with rapid onset and recovery, is widely used to induce and maintain general anaesthesia and long-term sedation in intensive care units (ICUs). However, its lipid-based formulation has disadvantages, including emulsion instability, hyperlipidaemia, and injection pain.[1–5] New propofol formulations that do not have those disadvantages are currently being developed (e.g. aqueous formulations of propofol prodrugs).

Propofol phosphate is a water-soluble propofol prodrug that can be enzymatically converted to propofol and inorganic phosphate, but its long onset and duration make it unsuitable for anaesthesia.[6 7] Fospropofol disodium (Lusedra®) is the only water-soluble propofol prodrug approved by the Federal Drug Administration (FDA) in the USA. Though its metabolite, formaldehyde, is an endogenous compound which has been confirmed not to be accumulated in the body after administration of fospropofol, it is considered better for the propofol prodrug not to have formaldehyde as a metabolite.[1 8–14] Therefore, novel propofol prodrugs that do not incorporate formaldehyde have been synthesized. Ethyl dioxy phosphate is a water-soluble propofol prodrug with a metabolite of acetaldehyde, which is considered not as detrimental as formaldehyde.[8]

Unlike formaldehyde and acetaldehyde, gamma-hydroxybutyrate (GHB) is an endogenous neurotransmitter that is rapidly and completely converted into CO_2 and H_2O through the Krebs cycle.[15 16] Thus, GHB is considered a more suitable metabolite of a propofol prodrug than formaldehyde or acetaldehyde. We synthesized HX0969w, a novel water-soluble prodrug composed of propofol, GHB and a phosphate group, with intramolecular cyclization reactions.[17 18] On the basis of these reactions, we hypothesized that HX0969w would release propofol and a GHB by-product (Fig. 1) and induce a sedative–hypnotic effect similar to that of fospropofol.

In this study, we assessed the efficacy of HX0969w in mice and rats. Hydrolysis studies in the plasma were performed to ascertain whether HX0969w can release propofol as hypothesized and to compare it to fospropofol. The median effective

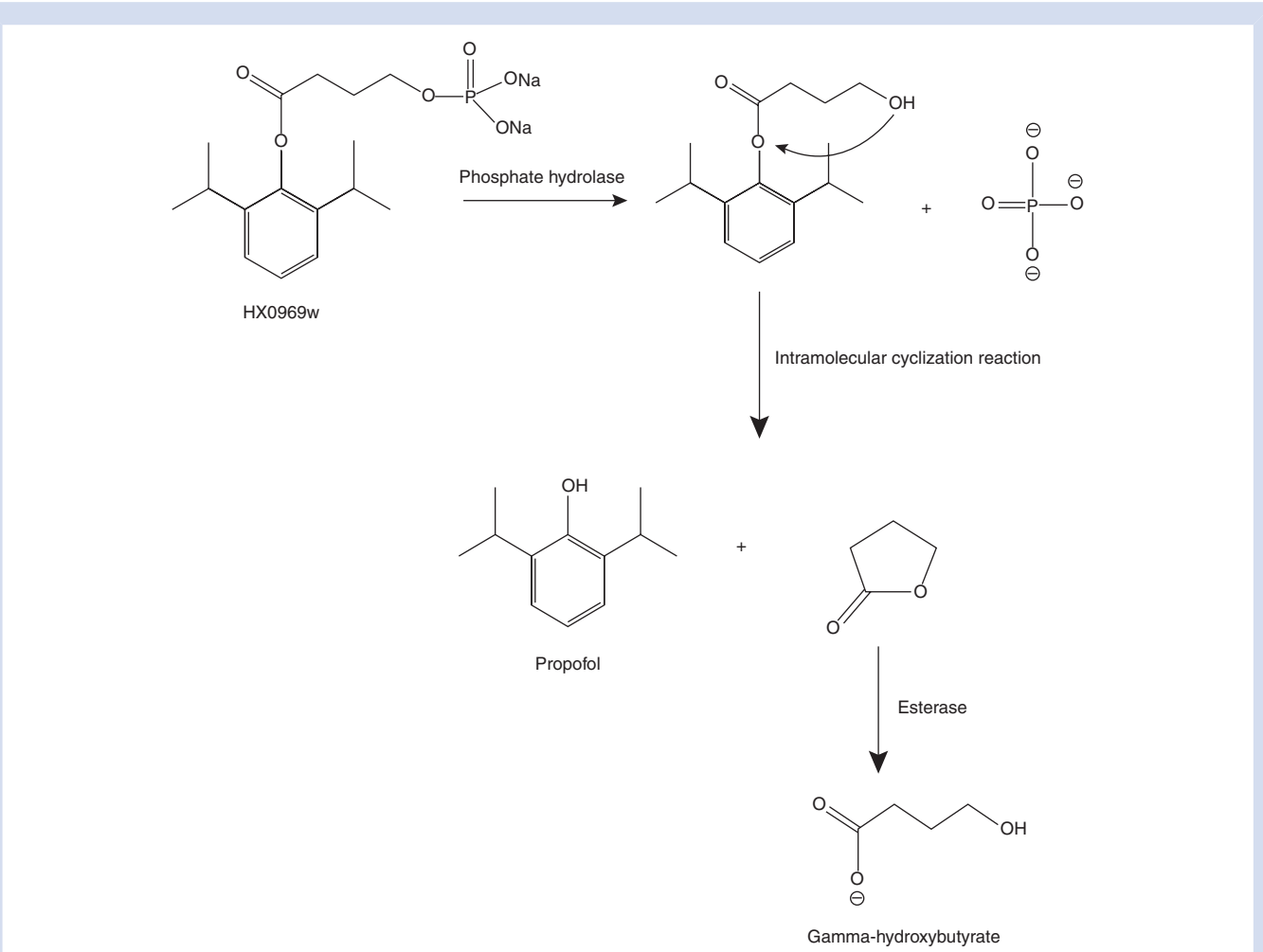

Fig 1 Chemical structure and the possible enzymatic hydrolysis path of HX0969w.

dose (ED_{50}) and median lethal dose (LD_{50}) of both compounds were evaluated and compared. Likewise, we compared the pharmacodynamic properties of equivalent doses of HX0969w and fospropofol in mice and rats.

Methods

Chemicals and reagents

HX0969w (molecular weight, 389.11 g mol^{-1}) and fospropofol (molecular weight, 332.28 g mol^{-1}) were synthesized according to the protocols described in their patents.[19]

Solutions of HX0969w (10, 20, and 80 mg ml^{-1}) and fospropofol (10, 15, and 50 mg ml^{-1}) were prepared using 0.9% normal saline.

In vitro studies

Hydrolysis study in rodent plasma

The objective of the hydrolysis study was to investigate the release of propofol from HX0969w and fospropofol, respectively. Stability of HX0969w and fospropofol in normal saline was determined at 37°C for 5 h before study. HX0969w (10 mg ml^{-1}, 0.97 ml) or fospropofol (10 mg ml^{-1}, 0.83 ml) was added to pre-heated mouse or rat plasma, respectively, and the mixture (5 ml, 5 μmol ml^{-1}) was shaken in a 37°C water bath for 3 h.[20] The samples (100 μl) withdrawn from the mixture were added to 900 μl of methanol in order to deproteinize the plasma at 0, 1, 3, 5, 7, 10, 20, 30, 60, 120, and 180 min. Then, the mixtures underwent vortex for 5 s and centrifugation for 13 min at 12 000×*g*. The supernatants (10 μl) were, respectively, collected for propofol analysis with high-performance liquid chromatography (HPLC) (Agilent 1100 series, Agilent Technologies, Santa Clara, CA, USA). The mobile phase consisted of methanol–water (70:30), on an Eclipse Plus C_{18} reversed-phase column kept at 25°C (150×4.6 mm, particle size of 5 μm, Agilent Technologies) with a guard column (Phenomenex, Torrance, CA, USA). The flow rate was 1 ml min^{-1}, and the UV absorbance detector was set at 272 nm. Both the limit of quantification and detection for propofol were 0.2 μg ml^{-1}. The retention time for propofol was ~7 min. The standard curve ranged from 0.2 to 100 μg ml^{-1}, with $R^2 \geq 0.99$. The inter- and intra-precision at three concentrations (0.4, 8, and 80 μg ml^{-1}) were in the range