



Balancing Tricks and Mini-pigs: Steps along the Road to Propofol

J.B. Glen

I grew up on a 50-acre farm on the Island of Arran in the Firth of Clyde in Scotland. We looked across to the Mull of Kintyre, and on a clear day, we could see the coast of Northern Ireland. It was a quiet life, and I can recall the day in 1952 when mains electricity reached our farm. We had cattle, pigs, sheep, and hens, and I decided to study veterinary medicine at Glasgow University. At that time, I envisaged a life driving round the countryside examining and treating farm animals. However, by the time I had completed the undergraduate course in 1963, I decided that I would like to stay within the university environment and begin an academic career, attracted by the opportunity that the university facilities provided to investigate cases more thoroughly. My first opportunity was to join Professor Ian McIntyre and a group from Glasgow University who were running a “conversion course” for diplomates from the veterinary college in Kenya to allow them to upgrade to internationally recognized degree status. My role there was to provide a veterinary service to local Kikuyu farmers and to identify suitable cases to be brought into the college for clinical teaching. I enjoyed playing rugby with the Impala club in Nairobi and played for a Kenyan team against Tanzania in Dar es Salaam. After a year, I returned to Glasgow as a house surgeon in

Biology research group. Sue Hunter is on the left, Iain Glen in foreground, and Alex Jamieson on the right with Roger James, the chemist who submitted ICI 35,868 for test. Missing is Steve Strong, who was conducting tests in mice on May 23, 1973.

CC(C)C1=CC=C(C=C1)O
No. 35865/1

C₁₂H₁₈O

Preparation 10 mg/ml in 10% a/cemphor. Dissolved with distilled water
Yellow solution.

Route i.v. 0.1ml/100cc

Animal PRLD^o mice (18-57g) 15-23 mos **Date** 23.5.73

I.V. Anesthetic Screening Test

Dose mg/Kg	Vol. ml	Wt gms	Duration of SP	Av. No. In SP	Deaths					Remarks	
					1st	2nd	3rd	4th	5th		
100	0.02	20	30" - D	3/3	3/3	-	-	-	-	3/3	
		22	45" - D								
		21	1'15" - D								
10	0.02	19	8'30" - 9'15" 4'5"	5/5	0/5	-	-	-	-	0/5	Immediate SP released, no analgesia, mild tremor
		20	10'15" - 11'10" 5'5"								
		20	10'50" - 12' 1'10"								
		22	12'5" - 13'5" 1'								
		23	12'50" - 14'20" 1'30"								
5	0.01	20	-	5/5	0/5	-	-	-	-	0/5	Very mild anaesthesia
		18	-								
		18	-								
		21	-								
15	0.05	18	19'30" - 20'5" 2'45"	5/5	1/5	-	-	-	-	0/5	Immediate SP released, no analgesia, mild tremor
		20	20'10" - 21'35" 1'25" 1'30"								
		18	21' - 22' 1'								
		20	21'45" - 23'30" 1'45"								
		18	23'30" - 25'50" 1'20"								
40	0.08	20	25'45" - 27'50" 4'5"	5/5	1/5	-	-	-	-	1/5	up to 12' after SP admin analgesia on 1, mild tremor
		21	26'50" - 29'45" 2'55" 5'30"								
		20	28'15" - D								
		20	29'30" - 33'30" 4'5"								
		20	30'30" - 36'40" 6'10"								

Laboratory record obtained on May 23, 1973, when the anesthetic activity of 2,6-diisopropylphenol was first observed in mice.

the small animal surgery department at the university. The Professor of Veterinary Surgery, Sir William Weipers, had been a president of the Royal College of Veterinary Surgeons and was instrumental in the introduction of specialization within the veterinary profession, one of the first diplomas offered being in veterinary anesthesia. With further study, assisted by attendance at physics, pharmacology, and physiology lectures at the Royal Infirmary in Glasgow provided for junior doctors specializing in anesthesiology, I obtained the diploma in veterinary anesthesia in 1968. This allowed me to remain in the university environment as a lecturer in veterinary anesthesia, and I became responsible for clinical veterinary anesthesia at the university veterinary hospital, dealing with both large and small animals. Where general anesthesia was required, the principal agents used were thiopentone for induction and inhalation of halothane for maintenance.

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A number of new drugs had been introduced for use in human patients. These included the anesthetics ketamine and propanidid, the steroid mixture of alphaxalone and alphadolone, the neuroleptic droperidol—used as a sedative—and the analgesic fentanyl. I became interested in the clinical evaluation of these agents in animals and in the development of methods to evaluate and compare the effects of these agents on circulation and respiration. This interest prompted me to apply for a position as a research biologist in the anesthetic project team at ICI pharmaceuticals division, and in 1972, I headed 350 km down the road to Cheshire to join this team of four graduate chemists looking for new anesthetic agents. ICI at that time was a major chemical company in the UK with interests in many specialty areas, including paints, pharmaceuticals, and agrochemicals. The pharmaceuticals division had achieved earlier success in this area with the development—by James Raventos in 1956—of the inhalational agent halothane, which, as a rapidly acting,

non-flammable agent, soon replaced the use of ether. My role was to head the biology group responsible for the evaluation of both potential new inhalational and intravenous anesthetic agents synthesized by project chemists, and my staff included two experimental officers and one laboratory assistant. While some new fluorinated inhalational compounds were found to have anesthetic activity, none were considered to be candidates for clinical development, and a new intravenous agent became our principal target.

At that time, thiopentone remained the gold standard for induction of anesthesia. The new intravenous agent sought was one that would reproduce the quality of anesthesia provided by thiopentone but would undergo more rapid metabolism such that anesthesia could be maintained by repeated injections or by continuous infusion without the penalty of delayed recovery. Two properties of an anesthetic are to some extent incompatible: water solubility is desirable to facilitate intravenous injection, but to gain access to the brain, the compound has to be relatively lipophilic. Two recently introduced anesthetics, propanidid (Epontol, Bayer) and the steroid mixture of alphaxalone and alphadolone (Althesin, Glaxo) were poorly soluble in water and were presented in a formulation that contained 20% of a surfactant, polyoxyethylated castor oil (Cremophor EL, Bayer). The availability of this solubilizing agent now allowed poorly water-soluble compounds to be tested for anesthetic activity in animals. With knowledge of the clinical profile of existing agents in domestic animals, I devised and refined a series of tests in laboratory animals to detect compounds exhibiting desirable properties and to eliminate from further testing those which failed to meet the required profile. All compounds were given in the first place to laboratory mice by intravenous injection. In most cases, no effect was observed, but if anesthetic properties were detected, the test compound entered a cascade of secondary tests in mice and rabbits to determine in greater detail hypnotic potency, speed of onset, duration of effect, speed of recovery, and side-effect profile. In early 1973, we detected hypnotic activity in ICI 43,117 (2,6-diethylphenol), one of a number of poorly soluble compounds selected by Roger James, a project chemist, from ICI's existing compound collection. Compounds synthesized for other projects were retained in this collection, and a number of substituted phenols had been made and tested as possible antibacterial agents. Because induction of anesthesia was slow, this compound was not taken further, but it provided a lead to follow. A range of related alkyl phenols were then tested, and on May 23, 1973, when I was in Scotland to attend my mother's funeral, the anesthetic activity of propofol (ICI 35,868; 2,6-diisopropylphenol) was first observed in mice. A degree of hypnotic activity was observed in many of these compounds, but propofol was selected as the only compound with the optimum balance of properties and acceptable effects on respiration and circulation. In comparison with thiopentone, propofol could be given by repeated injection without prolonging recovery time, and full recovery of coordination also occurred much more quickly. We could show that the recovering propofol-treated mice were much more adept when asked to balance on a horizontal rod compared to those in the thiopentone-treated group.

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Pure propofol is a highly lipophilic oil, and difficulty in finding an acceptable i.v. formulation led to a 13-year delay before the new agent could be marketed. The clinical acceptance of Epontol and Althesin, both formulated in Cremophor, allowed use of this agent for initial trials with propofol. However, a number of anaphylactoid reactions had been reported with both of these agents, and clinicians began to regard Cremophor with suspicion. As such, it was determined that a non-Cremophor formulation would be sought before marketing propofol.

In parallel with the search for an improved formulation, pharmacology studies continued to examine anesthetic effects in other animal species, potential drug interactions, and alternative routes of administration. Oral doses of propofol up to 300 mg/kg failed to induce anesthesia in mice, indicating rapid first-pass metabolism. Results in animal models indicated that propofol was unlikely to induce known undesirable effects of other agents, such as the adrenocortical depression, malignant hyperthermia, or porphyria. Cremophor induces histamine release and marked hypotension in dogs. We therefore conducted studies to examine the haemodynamic and respiratory effects of propofol, thiopentone, and Althesin in mini-pigs. These relatively small animals, with chronically implanted carotid artery and jugular vein cannulae, could be readily trundled along to our lab in a wheeled crate and connected to monitoring equipment without the need for restraint. In the process of this work, a suspected anaphylactoid reaction occurred when a second injection of Althesin was given to a pig. This observation led to a systematic evaluation of the effects of Cremophor and Cremophor-containing agents in this species. This work confirmed



Members of the group responsible for the clinical trial evaluation of the final emulsion formulation of propofol. From the left: Iain Glen, Katie Hopkins, Sue Hunter, Kate O'Conner, Ron Stark, and Sue Binks (missing is Vera Dutka).

that a second administration of Cremophor or one of the Cremophor containing agents, given 7 days after an uneventful first exposure, produced a marked anaphylactoid response in the mini-pig. When propanidid was administered in this way in a non-Cremophor solvent (propylene glycol and alcohol), no adverse response was observed. With alphaxalone and alphadolone in this same non-Cremophor solvent, some reactions were still seen, suggesting that these steroids could play a contributory role. The reactions seen in pigs were not classical hypersensitivity responses, as a subsequent challenge after a positive response was uneventful. One feature of the response was a marked but transient reduction in blood polymorph count, suggestive of complement C3 activation with the production of anaphylatoxins. With this model, it was now possible to examine alternative solubilizing agents for propofol, and a synthetic polyoxyethylene/polyoxypropylene surfactant (Synperonic PE39/70) manufactured by another division of ICI produced no adverse response in the mini-pig. Pharmacology and toxicology studies were repeated with a Synperonic formulation of propofol, but histological changes in liver tissue prevented further progress in this direction.

Clinical trials were begun with a formulation containing 2% propofol in 16% Cremophor EL and 8% ethyl alcohol. The first study by Kay and Rolly in Belgium in 1977 found that in unpremedicated patients, induction of anesthesia was rapid and smooth and recovery rapid. The induction dose at 1 mg/kg was smaller than I had anticipated, and several patients reported pain on injection, which had not been seen in animal studies. In view of these results, the concentration of propofol was reduced to 1%, alcohol was no longer needed as a co-solvent, and the severity of pain on injection was reduced in subsequent studies. In follow-on studies, a dose of 1 mg/kg of propofol was found to be insufficient to induce anesthesia in unpremedicated patients, and further dose-finding

studies determined an induction dose closer to my original prediction of 2 mg/kg. I have a vivid recollection of a development team meeting at that time in 1979 when, because of concerns about Cremophor, the confusion resulting from those early dose-finding studies and a view of limited commercial potential, I was on the side which voted by five votes to four to continue development.

With the demise of the Synperonic formulation, clinical studies continued with propofol in Cremophor, and at one stage in 1980, very much against my advice, it was thought that this formulation could be marketed. However, when more than 1,000 patients had been studied, a number of anaphylactoid reactions were encountered, and studies with this formulation were stopped. Earlier attempts to produce an emulsion formulation had failed, but as the clinical results with propofol continued to look promising and emulsion-manufacturing technology had recently improved, we decided in 1981 to reopen research on an emulsion formulation. A formulation containing soybean oil and purified egg phosphatide was eventually identified in collaborative studies with David Kent in our pharmaceutical department, and pharmacology and toxicology studies repeated again. These confirmed that the desirable properties observed with the propofol formulation were retained in the emulsion preparation, and no adverse response or histamine release was produced by the repeated administration of the emulsion formulation. Behavioral responses in the rat also suggested that the emulsion formulation should produce less discomfort on i.v. injection.

The clinical evaluation of the emulsion formulation began in 1983 and was planned and coordinated by an ICI physician, Ron Stark, and his team. At this time, I moved to the medical department to assist with international trials and clinical pharmacology studies. Studies were conducted in the United Kingdom, Belgium, France, and Eire. Regulatory approval was obtained, and first commercial launches, for use in induction of anesthesia and short-term maintenance in adults, occurred in 1986. Continuing clinical trials led to subsequent submissions for use in children, for long-term maintenance of anesthesia and intensive care sedation, and use for conscious sedation as a supplement to regional or local anesthesia.

I recognized that a barrier to the wider use of propofol for maintenance of anesthesia was the lack of suitable equipment, as syringe pumps available at the time had an insufficient delivery rate range. Having alerted syringe pump manufacturers to this potential opportunity, the Ohmeda 9000 produced by BOC HealthCare was the first of a new generation of computer-controllable syringe drivers, with a range of infusion rates sufficient for both induction and maintenance of anesthesia or sedation. The technique of target-controlled infusion (TCI), where a pharmacokinetic model for the drug to be infused is incorporated in the pump and a computer program calculates the infusion rate required to achieve a desired drug concentration, was introduced by Helmut Schwilden in Bonn in 1981. From 1990 onward, I hosted meetings with international groups with a research interest in this area, and by 1992 ICI agreed to develop a "Diprifusor" TCI module that could be incorporated in a compatible syringe pump to allow the infusion of propofol to achieve a target blood concentration. This module incorporated the software developed by Gavin Kenny and Martin White in Glasgow and was constructed and validated by Martyn Gray. Further clinical trials and a complex regulatory program led to the introduction of this technique in most countries from 1996 onward but not to date in the USA, where regulatory issues were encountered. The TCI technique has been applied by other groups to the administration of analgesic and sedative agents, and the ability to achieve and make proportional changes in the blood concentration of a drug may in time prove important in other areas, such as i.v. antibiotic therapy.

The development of propofol was very much a team effort, and in addition to the chemistry, biology, and clinical groups, there was a need for close involvement with teams focusing on pharmacokinetics, metabolism, toxicology, pharmaceutical development, and regulatory and commercial activities. Propofol has now, to a large extent, replaced the use of thiopentone as an induction agent and is also used in combination with analgesic drugs for maintenance of anesthesia, for sedation for surgery conducted with local anesthetic techniques, and for sedation to facilitate artificial ventilation in patients receiving intensive care. With its rapid recovery and compatibility with the laryngeal mask developed by Dr. Archie Brain and introduced into anesthetic practice about the same time, it has greatly facilitated ambulatory surgery. In the UK alone, a recent survey indicated that propofol was used in more than 2 million procedures in 1 year.