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Single- and Repeated-dose Local Toxicity in the Nasal Cavity of Rabbits after Intranasal Administration of Different Glycols for Formulations Containing Benzodiazepines

ROLF K. HJORTKJÆR, ERIK BECHGAARD*, SVEINBJÖRN GIZURARSON†, CAROL SUZDAK‡, P. MCDONALD AND R. J. GREENOUGH§

*Danish Toxicology Centre, ATV, Kogle Allé 2, DK-2970 Hørsholm, *Department of Pharmaceutics, The Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen Ø, ‡Novo Nordisk A/S, Novo Allé, DK-2880 Bagsværd, Denmark, †Department of Pharmacy, University of Iceland, Hagi vid Hofsvallagotu, PO Box 7171, 127 Reykjavik, Iceland and §Inveresk Research, Tranent EH33 2NE, UK*

Abstract

To furnish a systemic effect after intranasal administration, a formulation must contain the therapeutic dose in no more than 150 μL, the maximum volume that can be applied as a single administration in one nostril in man. The objectives of these studies were to examine the local toxicity of formulations containing benzodiazepines and to document the effects to support clinical trials in man.

After stability, pharmacological and pharmacokinetic studies of several benzodiazepine formulations, we studied nasal toxicity after single and repeated administration to rabbits of poly(ethylene glycol) 200, tetra(ethylene glycol), glycofurolum and mixtures of these vehicles both with and without benzodiazepines. Single-dose studies with examinations 5 or 10 min after application were undertaken with poly(ethylene glycol), tetra(ethylene glycol), glycofurolum and tetra(ethylene glycol)–glycofurolum in the ratio 95:5; the reactions were similar to that after physiological saline. A 14-day repeated-dose study was conducted with diazepam, lorazepam and flunitrazepam formulations in poly(ethylene glycol), and flunitrazepam in poly(ethylene glycol)–glycofurolum in the ratio 70:30; the two vehicles without any benzodiazepine were also examined. Microscopic study revealed mild changes only in the treated groups. A final four-week study was conducted with repeated administration of clonazepam formulated in tetra(ethylene glycol)–glycofurolum in the ratio 95:5; microscopy revealed mild changes after three 150-μL doses daily, but no abnormalities after one or three 100-μL doses daily.

It was concluded that these three solvents individually or as mixtures resulted in only mild local toxicity and might be acceptable as vehicles in nasal preparations of benzodiazepines and other non-irritating drugs for short-term use in man.

Investigations have demonstrated that the nasal mucosa might be a potential site of absorption of drugs (reviewed by Chien (1994) and Chien & Chang (1987)). Although intranasal administration of drugs can be used to obtain fast systemic absorption of molecules up to the molecular size of small peptides, local irritation can arise after administration into a sensitive organ such as the nose. The cost of possible local irritation or toxicity should be weighed against the potential benefits. Thus, emergency treatment of a life-threatening condition such as epileptic seizures with a toxic formulation might be considered acceptable, whereas the same formulation will not be acceptable for sedation before elective surgery.

Benzodiazepines are insoluble in water. Previous work has shown that the pharmacological response and plasma kinetics after intranasal administration of different formulations to rabbits depend on the vehicle. Thus, solutions in organic solvents, but not suspensions containing water, resulted in rapid absorption and acceptable bioavailability (Bechgaard et al 1995, 1997a). The vehicles examined in the studies described below were regarded as pharmaceutically, pharmacologically and

Correspondence: R. K. Hjortkjær, Danish Toxicology Centre, ATV, Kogle Allé 2, DK-2970 Hørsholm, Denmark.

pharmacokinetically suitable (Bechgaard et al 1995, 1997a, b).

The objectives of the studies described were to examine the local toxicity of different formulations intended for short-term treatment, and to document the effects in support of clinical trials in man. Therefore, the studies were conducted in compliance with the EU guideline ''Nonclinical Local Tolerance Testing of Medicinal Products''. Accordingly, conventional macroscopic and microscopic pathological examination of the upper respiratory tract was performed after intranasal administration to rabbits. Benzodiazepines were formulated in different glycols in which a therapeutic dose of the diazepines could be dissolved in amounts of the glycols suitable for intranasal administration, i.e. a maximum of 150 μL (approx.) for a single application. The vehicles and benzodiazepines were selected on the basis of previous work on their pharmaceutical and pharmacokinetic properties (Bechgaard et al 1995). The studies were performed with rabbits, because this species is suitable for pharmacological, pharmacokinetic and local toxicological investigations after intranasal administration (Gizurarson 1990; Illum 1996).

Materials and Methods

Materials

Clonazepam, flunitrazepam and glycofurolum 75 were kindly provided by Hoffman-La-Roche (Basel, Switzerland). Tetra(ethylene glycol) was commercially available from Fluka (Basel, Switzerland), propylene glycol and poly(ethylene glycol) 200 were from Mecobenzon (Copenhagen, Denmark). All other materials used were of either pharmaceutical or analytical grade and were commercially available.

Test and control formulations were prepared before each study.

Animals

New Zealand White rabbits (Novo Nordisk, Bagsværd, Denmark) and New Zealand/lop ear cross rabbits (Inveresk Research, UK), 3 kg (approx.), were used in all experiments. They were left to acclimatize for five days before each experiment. The animals were housed in single cages. Temperature was maintained at $20 \pm 3^{\circ}$C and relative humidity at 30–70%, with a 12 h–12 h light–dark cycle. Rabbits at Novo Nordisk A/S were fed Altromin formula 2110 rabbit chow, those at Inveresk Research standard rabbit diet supplied by Special Diet Service, Stepfield, Witham, Essex, UK. Drinking water was freely available via an automatic watering system.

Study design

Four individual studies were undertaken to evaluate the local toxicity of the preparations to the nasal mucosa and the nasal cavity. The two single-dose and the four-week repeated-dose studies were performed at Novo Nordisk, and the 14-day repeated-dose study at Inveresk Research. The intranasal doses were administered by direct instillation of the specified test substances into the nostril using laboratory repetitive pipettes and tapered adaptors. In the two single-dose studies the post-mortem observations were performed 5 and 10 min after dosing. This time interval was chosen to enable examination for immediate signs of irritation such as hyperaemia which might disappear within a short time if the irritant effect was only minor. In the repeated-dose studies the post-mortem observations were undertaken 24 h after the last dose. Findings after this time represent more long-lasting effects.

Single-dose study with 5-min observation

Eighteen rabbits were randomly allocated to six equal groups. The groups received 50 μL of the formulations 0·9% saline, poly(ethylene glycol), tetra(ethylene glycol), glycofurolum, tetra(ethylene glycol)–glycofurolum, 95:5, or Locasyn vehicle (Table 1), either into the left or the right nostril. The other nostril of each animal was left untreated. The rabbits were killed by an intravenous dose of pentobarbital 2 min after the application, and 5 min after the application the nasal cavity was opened and both sides evaluated macroscopically. The evaluator was unaware of the dosing scheme.

Table 1. Locasyn vehicle.

Component	Amount (%)
Propylene glycol	5
Poly(ethylene glycol) 400	20
Tween 20	2·5
Benzalkonium chloride	0·035
Disodium EDTA	0·01
Butylated hydroxytoluene	0·01
Citric acid	0·005
Sodium citrate, $2H_2O$	0·00765
Sorbitol	2
Water	To 100

The pH of the vehicle was 5·2.

Single-dose study with 10-min observation
Sixteen rabbits were randomized and divided equally into four groups. The groups were administered a single dose of 50 μL 0·9% saline, poly(ethylene glycol), tetra(ethylene glycol), or glycofurolum in both nostrils. The rabbits were killed 10 min after dosing, and their right nasal cavity opened and evaluated macroscopically. The evaluator was unaware of the dosing scheme. The left nasal cavity was processed and subjected to histopathological evaluation.

Fourteen-day repeated-dose study
The 14-day study was conducted to examine possible differences between vehicles and benzodiazepine formulations and to support single or a few administrations in man. Thirty rabbits were divided randomly into five groups. The animals received different treatments to the left and right nostrils. The left nostrils were given 30 μL once a day of either 0·9% saline or one of the solutions containing a benzodiazepine. The animals receiving 0·9% saline in the left nostril were also administered 100 μL 0·9% saline in the right nostril three times a day. Animals receiving solutions containing a benzodiazepine were given increasing doses of the vehicles in the right nostril. The different vehicles and doses were allocated evenly with regard to the treatment given to the left nostril. Although this design might seem complicated, it enabled balanced treatment with four benzodiazepine formulations at one dose level, each of the two vehicles at three dose levels, and controls, by use of only 30 rabbits. The animals were observed daily for clinical signs. Body weights were recorded twice weekly and food consumption at weekly intervals. After completion of 14 days dosing, all animals were necropsied and the brain and nasal cavities were examined histopathologically.

Four-week repeated-dose study
This study was conducted to support repeated administration in man. Twenty-four rabbits were divided equally into four groups. The rabbits were dosed into the left nasal cavity. Group 1 received 150 μL 0·9% saline with 0·01% benzalkonium chloride and 0·0005% sodium acetate three times a day. Groups 2 and 3 received 100 μL tetra(ethylene glycol)–glycofurolum, 95:5, with 0·5% clonazepam once and three times a day, respectively. Group 4 received 150 μL tetra(ethylene glycol)–glycofurolum, 95:5, with 0·5% clonazepam twice (at 0900 h and 1400 h, respectively) plus 150 μL tetra(ethylene glycol)–glycofurolum, 95:5, once daily (at 1200 h). The animals were observed daily for clinical signs, and body weights were recorded on days 1, 3, 8, 15, 22 and 29. After completion of the 28 days of dosing, all animals were killed on day 29 (i.e. the day after the last day of dosing) and the nasal cavities were examined histopathologically.

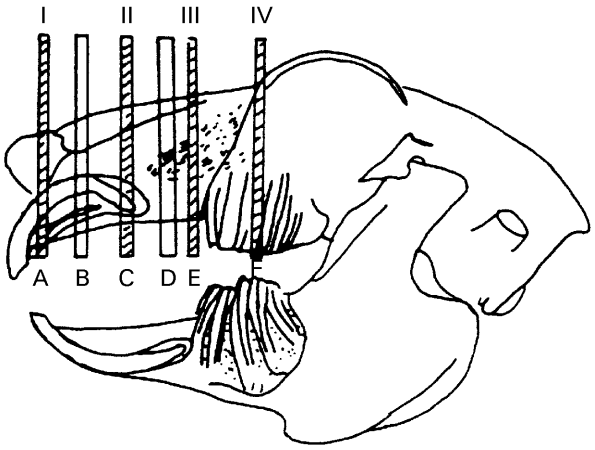

Figure 1. The regions of the nasal cavity from which sections were taken for histopathological examination.

Terminal observations
All animals were killed by intravenous overdose of sodium pentobarbitone. For histological evaluation, tissues were preserved in buffered formalin without opening, and thus internal inspection, of the nasal cavity.

Samples were processed and stained with haematoxylin and eosin. Figure 1 indicates the regions of the nasal cavity from which the sections for histopathological examination were taken. Examination was performed at levels I–IV in the single-dose study with 10 min observation and the 14-day repeated-dose study, and at levels A–F in the four-week repeated-dose study.

Results

The macroscopic findings in the single-dose study with 5 min observation are given in Table 2. The only finding which might be considered pathological was mucosal hyperaemia. Considering the distribution of this finding, it seems reasonable to conclude that an equivocal response was seen after treatment with Locasyn vehicle, and no pathological changes were seen after treatment with the negative control (0·9% saline) or the test vehicles poly(ethylene glycol), tetra(ethylene glycol), glycofurolum, and tetra-(ethylene glycol)–glycofurolum, 95:5.

The macroscopic and microscopic findings in the single-dose study with 10 min observation are given in Table 3. The macroscopic lesions

Table 2. Single-dose study with 5 min observation. Design and post-mortem observations 5 min after dosing.

Test material (50 μL)	n	Macroscopic findings	
		Treated side	Untreated side
0·9% Saline	12	NP[a]	
	13		
	17		
Poly(ethylene glycol)	5	NP	
	8		NP
	14		NP
Tetra(ethylene glycol)	25		
	26		
	28		
Glycofurolum	16	NP	
	10		
	15		
Tetra(ethylene glycol)–glycofurolum, 95:5	11		
	18		
	20		
Locasyn vehicle	7		
	9	Hyperaemic mucosa	
	19	Hyperaemic mucosa	Hyperaemic mucosa

[a]Blood vessels slightly more visible than in the other cavity; not pathologically. If no indication is given, no abnormalities were detected. n = number of rabbits.

Table 3. Single-dose study with 10 min observation. Design and post-mortem observations 10 min after dosing.

Test material (50 μL)	n	Macroscopic findings[a]		Microscopic findings (left side)
		Haem focus	Abrasion	Focal haemorrhage
0·9% Saline	3			IV
	6	II		II
	12	(6 × 3 mm)		II
	13			
Poly(ethylene glycol)	4			
	5	II		II
	11	(2 × 2 mm)		II
	14	II		
Tetra(ethylene glycol)	1	(2 × 2 mm)		
	7			II
	10			
	15		II (pp)	II
Glycofurolum	2			II
	8			II, III, IV
	9			
	16	II (4 × 3 mm)		II

[a]II, III and IV indicate lesions present in regions II, III and IV, respectively (cf. Figure 1). The values in parentheses is the size of the lesion (pp = pinpoint). If no indication is given, no abnormalities were detected. n = number of rabbits.

observed, i.e. a haemorrhagic focus or a pinpoint abrasion, were distributed almost evenly over the control and test groups. The focal nature and the anterior location of the lesions correspond to abrasion from the tip of the applicator pipette. Therefore, the lesions observed are ascribed to application trauma and not to the test materials.

In the 14-day repeated-dose study some animals receiving flunitrazepam tended to be subdued or ataxic, or both, after dosing over the first few days of the study. No adverse clinical signs of toxicity were seen. Body-weight profiles were generally satisfactory; a slight reduction in food intake was seen for some of the treated males. No gross pathological findings were considered related to treatment. No microscopic abnormalities were detected in the brain. Inflammatory changes were seen in the nostrils of a number of animals. There were no notable differences among the treatments. The microscopic findings in the nasal cavities are given in Table 4. The presence of a few inflammatory cells in the middle part of the nasal cavity was seen more frequently after treatment with the test articles than with the controls. Flattening of the olfactory epithelium was noted after treatment with the test articles only. No difference was found among the effects of test articles, and no dose-relation was seen in the effects caused by the different doses of the two vehicles without benzodiazepines. The changes seen are considered to be mild.

In the four-week repeated-dose study, a dose-related increase in sluggishness was seen. No occasions of real sedation or sleeping were observed. There were occasional sneezes after dosing in the treated groups. Body weights and food consumption were normal in all groups. The microscopic findings are given in Table 5. No abnormalities were found in the control group or in the groups given 0·5% clonazepam in tetra(-ethylene glycol)–glycofurolum, 95:5, 100 μL × 1 or 100 μL × 3 daily. Foci of mucosal hyperplasia occurring mostly over boney spicules in the middle part of the nasal cavity were found in the last group given tetra(ethylene glycol)–glycofurolum, 95:5, with or without clonazepam 150 μL × 3 daily. The findings were foci as opposed to areas and were considered to be mild.

Discussion

These studies were performed as part of a development project with the principal objective of finding suitable benzodiazepine preparations for intranasal administration.

In the single-dose study with 5 min observation, Locasyn vehicle was used as reference control; this

Table 4. Fourteen-day repeated-dose study. Design and post-mortem nasal observations 24 h after last dosing.

Test material	Dosage daily for 14 days	n	Left or right	Macroscopic findings[a]					
				Inflammatory cells	Purulent exudate	Flattening of olfactory epithelium	Focal inflammation of the olfactory epithelium	Cyst in respiratory epithelium	Increase in goblet cells
0·9% Saline	30 μL × 1	1	L						
		2	L						
		3	L						
		4	L	III					
		5	L						
		6	L						
	100 μL × 3	1	R						
		2	R						
		3	R						
		4	R						
		5	R						
		6	R	III					
3% Diazepam in poly(ethylene glycol)	30 μL × 1	7	L						
		8	L						
		9	L	III					
		10	L	III					
		11	L	III					
		12	L						
5% Lorazepam in poly(ethylene glycol)	30 μL × 1	13	L						
		14	L						
		15	L	III		IV			
		16	L						
		17	L	III					
		18							
1% Flunitrazepam in poly(ethylene glycol)	30 μL × 1	19	L			IV			
		20	L						
		21	L			IV			
		22	L			IV			
		23	L						
		24	L						
1% Flunitrazepam in poly(ethylene glycol)–glycofurolum, 70:30	30 μL × 1	25	L						
		26	L						
		27	L				III		
		28	L						
		29	L	III		IV	IV		
		30	L						
Poly(ethylene glycol)	30 μL × 1	7	R						
		13	R						
		22	R	III		IV			
		28	R						
	100 μL × 1	8	R						
		14	R						
		23	R						
		29	R	III		IV			
	100 μL × 3	9	R	III					
		15	R	III		IV			
		24	R						
		30	R					III	
Poly(ethylene glycol)–glycofurolum, 70:30	30 μL × 1	10	R	III					
		16	R	III					
		19	R			IV			
		25	R						
	100 μL × 1	11	R	III	II				
		17	R						
		20	R						
		26	R						
	100 μL × 3	12	R						
		18	R					II	
		21	R						
		27	R				II		

[a]II, III and IV indicate lesions present in regions II, III and IV, respectively (cf. Figure 1). If no indication is given, no abnormalities were detected. n = number of rabbits.

Table 5. Four-week repeated-dose study. Design and post-mortem nasal observations 24 h after last dosing.

Test material	Daily dosage for 28 days	n	Microscopic findings[a]	
			Focus of hyperplasia	Focus of metaplasia
0·9% Saline	150 μL × 3	1		
		2		
		3		
		11		
		12		
		13		
0·5% Clonazepam in tetra(ethylene glycol)–glycofurolum, 95:5	100 μL × 1	21		
		22		
		23		
		31		
		32		
		33		
	100 μL × 3	41		
		42		
		43		
		51		
		52		
		53		
0·5% Clonazepam in tetra-(ethylene glyco)–glyco-furolum, 95:5 plus tetra-(ethylene glycol)–glyco-furolum, 95:5	150 μL × 2 + 150 μL × 1	61	D	C
		62	C, E	
		63	B, C	
		71	B, C, D	
		72	D	
		73		

[a]B, C, D, E indicate lesions present in regions B, C, D and E, respectively (cf. Figure 1). If no indication is given, no abnormalities were detected. n = number of rabbits.

vehicle has fewer side effects compared with an old Locasyn formulation which has, however, been used for long-term treatment (Nielsen et al 1989). The choice of Locasyn vehicle as a positive control is regarded as very conservative, because Locasyn is accepted for long-term treatment whereas the glycols were primarily examined in respect of possible use for formulation of benzodiazepines for only one or a few administrations. No adverse macroscopic effects were found in this study, after Locasyn vehicle or after the three organic solvents. These solvents were further investigated histologically in the single-dose study with 10 min observation. As in the single-dose study with 5 min observation, there were no differences between results from the saline control and the organic solvents. Two selected vehicles (poly(ethylene glycol) and poly(ethylene glycol)–glycofurolum, 70:30) were further examined, both with and without benzodiazepines in the 14-day repeated-dose study. Mild changes were attributed to both vehicles, but no difference between the vehicles was seen, and no dose-relationship was apparent. Furthermore, there were no differences between the vehicles with and without the benzodiazepines. For the last study, the formulation tetra(ethylene glycol)–glycofurolum, 95:5, with 0·5% clonazepam was selected because of its pharmacological and pharmacokinetic properties. The dose-related mild changes seen in this study are consistent with findings when this vehicle was used with guinea-pigs (Gizurarson et al 1996). In addition, morphological examination of the rabbit nasal mucosa reported by Bindseil et al (1995) showed increasing local toxicity as follows: saline < commercial 1% ephedrine nose drops < glycofurolum < 30% acetyl-salicylic acid in glycofurolum. Besides showing the expected differences in local toxicity, the morphological findings correlate well with the findings of glycofurolum in the two single-dose studies.

From published results on eye irritation and local toxicity after injection, glycofurolum was expected to be more locally toxic than poly(ethylene glycol) and tetra(ethylene glycol), which were expected to be equally toxic (BIBRA 1992). However, significant, but reversible inhibition of the mucociliary clearance in the frog palate model has been shown for a 5% ethylene glycol solution whereas 5% glycofurolum had no effect (Gizurarson, unpublished results).

Overall, this information is consistent with our findings that no clear difference was found between the three solvents and the mixtures of the solvents. They caused only mild local toxicity after nasal application in rabbits and might be acceptable as vehicles for nasal preparations of benzodiazepines and other non-irritating drugs for short-term use in man. The results satisfy the requirement for non-clinical local tolerance testing of tetra(ethylene glycol)–glycofurolum, 95:5, with or without clonazepam for single and repeated intranasal administration to man.

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