Comparison of the hemodynamic effects of intravenous administration of IQB-9302 and bupivacaine in anaesthetised rats

DIRECTOR OF THE STUDY: Prof. Antonio G. García
Professor of Pharmacology

CO-ORDINATOR OF THE PROJECT: Francisco Abad Santos

REALISATION OF THE EXPERIMENTS: Sonia Gallego Sandín
Jesús Novalbos Reina

LOCATION OF THE STUDY: Instituto de Farmacología Teófilo Hernando
Dpto. de Farmacología y Terapéutica
Facultad de Medicina
Universidad Autónoma de Madrid
C/ Arzobispo Morcillo, 4
28029 Madrid
Tel. 91-397 53 88
Fax: 91 397 53 97

DATE OF THE STUDY: February 1999 - July 1999

ARCHIVE OF DATA: Dpto. de Farmacología y Terapéutica
Facultad de Medicina
Universidad Autónoma de Madrid

SPONSOR: Dr. Alvaro Galiano
Instituto de Investigación y Desarrollo Químico Biológico

Number of pages of the report: 38

The present study was performed in compliance with the rules and regulations of Good Laboratory Practices published by OECD (1981) and according to the Real Decreto 822/1993 BOE (May 1993). There were no incidences that could affect reliability of data.

Director of the study:

Antonio G.García, M.D., Ph.D.
Prof. of Pharmacology Date: / / 1999

Co-ordinator of the study:

Francisco Abad Santos, M.D., Ph.D. Date: / / 1999

Realisation of the experiments:

Sonia Gallego Sandín, Biologist Date: / / 1999

Jesús Novalbos Reina, Biologist Date: / / 1999

INTRODUCTION

IQB-9302 [1-(methylcyclopropyl)-N-(2,6-dimethylphenyl)-2- piperidinecarboxamide; dl-1-(methylcyclopropyl-2',6'-pipecoloxylidide, hydrochloride)] is a new local anaesthetic agent developed by INSTITUTO DE INVESTIGACION Y DESARROLLO QUIMICO-BIOLOGICO, SA (IQB, Madrid) and LABORATORIOS INIBSA.

The anaesthetic effects of IQB-9302 were studied by three standard tests (infiltration anaesthesia in the guinea pig, palpebral anaesthesia in the rabbit and the guinea pig and the sciatic nerve block in the rat) in comparison with mepivacaine and with bupivacaine. Results of these tests demonstrated a long lasting effect much longer than mepivacaine and at least equivalent to that of bupivacaine. A blind randomised study in the beagle dog demonstrated that epidural IQB-9302 induces sensory and motor blockades similar to those induced by bupivacaine. Nevertheless, the sensory response induced by IQB-9302 0.5% is similar to that induced by bupivacaine 0.75% and sensory response induced by IQB-9302 0.25% is similar to that produced by bupivacaine 0.50%. However, sensory and motor blockades are similar after IQB-9302 whereas bupivacaine exhibited a longer motor than sensory blockade.

Comparative experiments of IQB-9302 and bupivacaine on isolated rat aorta have shown that they both have little effect on the basal tone of isolated rat aorta at concentrations ranging from 10^{-7} to 10^{-5} M. At 10^{-4} M bupivacaine increased basal tone, whereas no effects were elicited by IQB-9302. At concentrations ranging from 10^{-7} to 10^{-5} M, small effects were shown by both drugs on contractions evoked by phenylephrine or KCl 35 mM. Higher concentrations of IQB-9302 and bupivacaine induced a similar and significant vasorelaxant effect.

No differences were observed in the systemic intravenous toxicity of IQB-9302 and bupivacaine in rats. In vitro experiments to test the impairment of electrical conduction in isolated rat ventricle showed that bupivacaine was significantly more toxic than IQB-9302. Total blockades were more frequent in tissues perfused with bupivacaine and both amplitude and delay of action potentials were significantly more affected by bupivacaine.

Although no significant differences were observed as far as anaesthetic effects are concerned between bupivacaine and IQB-9302, a trend to a deeper anaesthesia and longer duration of action was definitively observed for IQB-9302. No differences in systemic toxicity were found between bupivacaine and IQB-9302, but impairment of cardiac conduction was significantly less for the latter.

The aim of the present study was to compare the hemodynamic effects produced by the intravenous administration of IQB-9302 and bupivacaine in anaesthetised rats. This study is relevant to shape the safety profile of the compound.

MATERIALS AND METHODS

IQB-9302.HCl, control number 9810034, was obtained from LEBSA. Bupivacaine.HCl and sodium pentobarbital were obtained from Sigma, sodium heparin from Laboratorios Leo, and sodium chloride 0.9% from Laboratorios Grifols (Spain). Sodium pentobarbital was dissolved in ethanol/sodium chloride 0.9% 20:100. IQB-9302 and bupivacaine were dissolved in sodium chloride 0.9% at the required concentration to administer always the same volume (250 µl).

We used 18 three months-old Sprague-Dawley rats, weighing 250 to 350 mg, supplied by Facultad de Medicina (Universidad Autónoma de Madrid). Four of them were out of the weight limits (see table 1). Every day we took two rats of the same sex and age that were anaesthetised with 60 mg/kg intraperitoneal sodium pentobarbital. If the level of anaesthesia was not enough, and extra dose of 10 mg/kg sodium pentobarbital was administered. Everyday a different investigator randomised the sex of the rats and the drug that will be administered to the first rat (the second rat will receive the other drug). In this way, 8 rats received IQB-9302 and 8 rats bupivacaine. Two other drugs only received sodium chloride (control rats) in each dose.

Animals were placed in the supine position in a surgical table. Ten to fifteen minutes later, once the rat was asleep, a polyethylene catheter (0.5 mm internal diameter and 0.8 mm external diameter) was inserted in the left femoral artery and vein. Both catheters were full of heparinised sodium chloride (50 UI/ml). The arterial catheter was connected to the detector, and the venous catheter was used to administer several doses of the drugs.

A tracheotomy was performed to facilitate the elimination of respiratory secretions. Rectal temperature was measured before and after operation and before the administration of each dose. We tried to maintain temperature between 36.5 and 37.5 ºC with the help of a table-lamp.

Fifteen minutes after operation the register was connected and we waited 30 min to allow stabilisation of arterial pressure and heart rate. Thereafter we began the experiment. Each rat received successive increasing doses of the drug (0.1, 0.3, 1, 3, and 10 mg/kg) at 20-min intervals. Each dose was administered during 30 s in a volume of 250 µl followed by an infusion of 100 µl of heparinised sodium chloride (50 IU/ml) in 15 s. Heart rate, systolic, diastolic and mean arterial pressure were continuously recorded on a MacLad 4.e system. As there were very little differences between systolic and diastolic blood pressures, we only show in this report the results of mean arterial pressure. Results of arterial pressure are presented in mmHg and heart rate in bits per min (bpm).

Results are presented as mean and standard deviation (SD). IQB-9302 and bupivacaine were compared with analysis of variance in SPSS for Windows (version 6.1.3). Statistical significance were assumed at $p < 0.05$.

RESULTS

In table 1 we can see the characteristics of the rats enrolled in the study. All the rats completed the experiments, except one rat of bupivacaine group that died after receiving the dose of 3 mg/kg. In the IQB-9302 group, by mistake a rat received two consecutive doses of 3 mg/kg and did not receive the 10 mg/kg dose, and in other rat we lost the record of the effects of the administration of 10 mg/kg.

Tables 2a to 2c show the rectal temperature of the rats before the administration of each dose.

Tables 3 to 6 show the heart rate and the mean arterial pressure at different times after the administration of each dose of bupivacaine and IQB-9302. We can also see it in figures 1 to 4. Both bupivacaine and IQB-9302 produced a decrease of heart rate, especially at the doses of 1 and 3 mg/kg (figures 1, 5a and 5b). This decay seems bigger with bupivacaine (table 7 and figures 2b and 5b), although the difference is not statistically significant, maybe because of the small number of animals. If we calculate the sample size needed to find statistically significant differences with the data of table 7, we need 41 rats per group for the 1 mg/kg dose and 470 rats per group for the 3 mg/kg dose. This can be explained by the high variability of the results.

Both bupivacaine and IQB-9302 produced an increase of mean arterial pressure (figures 3, 6a and 6b). This increase is bigger and more sustained with IQB-9302 (table 8 and figures 6a and 6b), although the differences do not reached statistical significance, maybe because of the small number of animals. If we calculate the sample size needed to find statistically significant differences with the data of table 8, we need 25 rats per group for the 1 mg/kg dose and 62 rats per group for the 3 mg/kg dose. This can be explained by the high variability of the results.

All rats treated with 10 mg/kg bupivacaine or IQB-9302 died within two minutes and this was accompanied by a sharp decrease in heart rate (figures 2c and 5c) and mean arterial pressure (figures 4c and 6c). However, bupivacaine had a quicker effect because all rats were dead at 1.25 min after the dose whereas with IQB-9302 all rats survived more than 1.5 min (tables 3e, 4e, 5e and 6e).

DISCUSSION AND CONCLUSIONS

In this study we have administered successive doses of the drug, so the hemodynamic effects depend on the dose administered and on the remaining drug, but as half life for these drugs is very short this may not be a problem. If we consider cumulative doses the real values would be 0.1, 0.4, 1.4, 4.4 and 14.4 mg/kg, not very much different from the individual doses.

As shown in *in vitro* experiments, IQB-9302 has a smaller effect on cardiac function than bupivacaine, as the maximum decrease in heart rate was 133 bpm with bupivacaine and 114 bpm with IQB-9302. On the other hand, IQB-9302 produced a higher increase in arterial pressure: maximum increase of 20.7 mmHg versus 12.6 mmHg with bupivacaine. We think these effects are not related between them because a reduction in heart rate should produce a decrease in arterial pressure. So these drugs could act directly on the heart (negative chronotropic effect) and on the arterial wall (vasoconstriction). However, at higher doses (10 mg/kg) both drugs produced cardiac arrest and hypotension that could be related with peripheral vasodilatation.

The possible vasoconstrictive effect of IQB-9302 may be clinically important because it can prolong the anaesthetic effect when administered locally, so that it would not be necessary to associate a vasopressor drug as adrenaline.

IQB-9302 seems less toxic than bupivacaine because all rats tolerated the 3 mg/kg dose whereas in the bupivacaine group a rat died after the administration of this dose. At the higher dose (10 mg/kg) both drugs were highly toxic and all rats died immediately. Our results are congruent with the calculated lethal dose-50 of around 5 mg/kg for both drugs obtained in other studies.

We can conclude that IQB-9302 seems to be less cardiodepressor than bupivacaine, and maybe it has some vasoconstrictive effect because it produces a higher increase in mean arterial pressure and a smaller decrease in heart rate. As general anaesthetic drugs such as pentobarbital used in this study can impair the hemodynamic parameters, these results should be confirmed in freely moving concious rats.

REFERENCES

- IQB-9302 Investigator’s Brochure. http://www.iqb.es/Cpcaina/proyecto/PHOME.htm