INTRODUCTION

Inflammatory Bowel Diseases (IBD) are chronic, remitting and relapsing inflammatory disorders encompassing Crohn’s disease and ulcerative colitis. Trinitrobenzene sulphonic acid (TNBS) induced colitis remains one of the most common methods used for studying IBD in animal models, although the efficacy of TNBS to induce pain hypersensitivity can be variable. The aim of the present study was to optimize experimental conditions and to validate the model of colorectal distension (CRD) with phloroglucinol, a clinically used antispasmodic.

MATERIAL & METHODS

 Animals: Male Wistar (Han) rats, weighing 160-200 g at the beginning of the experiment, supplied by Janvier Labs, France.

 TNBS sensitization and colorectal distension:

- After overnight food-deprivation, rats are anesthetized (isoflurane) and injected with TNBS (20 mg/rat) or saline into the colon.
- After a recovery period of 14 days, the testing phase (colonic distension) is performed:
  - On the testing day, rats are again anesthetized and a latex balloon of 2 cm in length tightly attached to a catheter is introduced to a depth of 5 cm from the anal margin. Anesthesia is then immediately discontinued.
  - Stage 1 of the CRD: 75 minutes later, the balloon is filled with 0.4 ml of water and the number of abdominal cramps is counted for 10 minutes.
  - Stage 2 of the CRD: At the end of the stage 1, 0.4 ml of water is added (total volume = 0.8 ml) and the number of abdominal cramps is again counted for 10 minutes.
  - Stages 3 and 4: following the observations at stage 2, two other stages of distention are performed using 0.2 ml of water (final volume: 1.2 ml at the end of the experiment, total time of observation: 40 minutes).

- At the end of the testing phase, rats are sacrificed and the position of the balloon is verified.

 Drugs

Trinitrobenzene sulphonic acid (TNBS), prepared in 20% ethanol is administered by intracolonic route (0.56 ml/rat). Phloroglucinol (1,3,5-Trihydroxybenzene dihydrate 97%), dissolved in physiological saline, was administered by p.o. 60 min before the test on Day 14 (5 ml/kg).

 Statistics

Inter-group comparison was performed for the test substance using a Kruskall-Wallis test, followed by Mann-Whitney U tests in case of significant group effect.

RESULTS

During the first period of observation (probe filled with 0.4 ml of water), presence of TNBS do not increase the number of cramps, indicating that the presence of a minimal volume of distension is needed to observe an effect of the TNBS sensitization.

During the 3 following periods of observation, the number of cramps is significantly increased in the vehicle control group sensitized with TNBS as compared to a non-sensitized control group receiving intracolonic saline only.

Phloroglucinol at 30 and 100 mg/kg p.o. significantly decreases the number of cramps in TNBS-sensitized rats as compared with the vehicle control. The intermediate dose displays a similar trend.

CONCLUSION

These results suggest that the effects of TNBS are robust when the cramps are induced by incrementing CRD. The efficacy of phloroglucinol, used in the clinic, suggests that the model has a translational value. Our TNBS-induced colitis model may be particularly useful for evaluating the efficacy of drugs against visceral pain in the rat.

ACKNOWLEDGEMENTS: special thanks to Arnaud Godeau and Olivier Pouessel for their assistance in data management of this poster.