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Research Article

EVALUATION OF CISPLATIN-INDUCED PICA BEHAVIOUR IN RATS BY MEASURING FAECAL CARMINE-DYE EXCRETION: AN IMPROVED EXPERIMENTAL MODEL TO SCREEN SAMPLES WITH ANTI-EMETIC PROPERTIES

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ABSTRACT
The objective of the present study is to evaluate the Cisplatin-induced pica behaviour in rats by measuring faecal carmine dye excretion and to evaluate the anti-emetic effect of drugs on Cisplatin-induced pica behaviour in rats.
Thirty-two rats were divided into 4 groups of 8 animals each. Rats from group I and II received DM water (10ml/kg p.o). Rats from group III and IV received Himalaya Anti-emetic Tablets (HAT) 250 mg/kg p.o. and ondansetron 4mg/kg p.o, respectively. After one hour of the assigned treatment, all the animals except in group I were injected with Cisplatin 3mg/kg i.p. Rats in group I were injected with saline (1ml/kg i.p.). All the animals were fed with normal as well as kaolin pellets (impregnated with carmine dye). The faeces of each rat was collected after 72 hrs of drug administration and analysed for the carmine content. Cisplatin injection (3mg/kg) caused a significant increase in kaolin consumption, which was indicated by increased carmine dye excretion in faeces compared to control. Pre-treatment with HAT and ondansetron significantly suppressed kaolin consumption induced by Cisplatin.
The present findings showed that the exact kaolin consumption can be quantified by measuring the faecal excretion of carmine, which was added in kaolin pellets and this can be a sensitive model to study the anti-emetic potential of drugs, overcoming the inherent disadvantages of measuring direct kaolin intake. Pre-treatment with ondansetron and HAT significantly decreased kaolin consumption in rats-induced by Cisplatin injection, which was further shown by decrease in faecal excretion of carmine, indicating anti-emetic potential of tested drugs.
KEY WORDS: Pica behaviour, Cisplatin, Carmine, Ondansetron, Kaolin, Himalaya Anti-emetic tablets (HAT), Chemotherapy.

INTRODUCTION

Nausea and vomiting are amongst the most common symptoms encountered in medicine. They occur together or separately in a diverse range of diseases. Nausea and vomiting are the components of body's defensive response to toxins accidentally ingested with food and also associated with both current drug treatment and novel drug therapies.
Chemotherapy induces nausea and vomiting in cancer patients[1] and these side effects lead to dehydration, compromised patient compliance or refusal of continuation chemotherapy, and therefore need to be treated. Cisplatin a chemotherapeutic agent is known to cause significant nausea/vomiting[1,2]. A number of studies have shown that Cisplatin causes generation of free radicals and release of reactive oxygen species[3-5]. This enhanced oxidative stress in gastrointestinal tract could cause injury to enterochromaffin cells and other cells and result in release of serotonin (5-HT). The released serotonin cause the stimulation of vagal afferent sensory nerves and chemoreceptor trigger zone in the brain stem ultimately results in emesis[6,7].
To evaluate the anti-emetic drugs in animals, rats and mice are rarely used, since they lack vomiting centre. However it has been reported that rats react to nausea/vomiting stimuli by altered feeding habits, manifested as increased consumption of non-nutritive substances like kaolin (a type of clay), known as pica.
Pica behavior in rats is mediated by mechanisms and receptors involving serotonin and dopamine, similar to those in humans and other species. The rat pica model has been used extensively and validated in several studies researching anti-emetic drugs[8,9]. But it was very difficult to determine the exact kaolin consumption as it is based on weighing of spillage and remaining kaolin. Due to the above said inherent practical problem, the conventional experimental model involving the method of exact kaolin consumption has its own limitation of being less accurate.
Carmine, a complex of carminic acid and aluminium has been widely used in food stuffs and drugs as a colouring agent. In addition, it is clinically used in assessing the transit time in children and in mice[10,11]. A mice model of pica behaviour was established earlier by using carmine[12]. In the present study, an attempt was made to establish experimental model of pica in rats by measuring faecal excretion of carmine dye, which was impregnated with kaolin.

MATERIALS AND METHODS

Experimental Animals

Inbred Wistar male rats (225-250g) were used in this study. Animals were housed in standard isolation cages under environmental conditions of temperature (22 ± 2°C), relative humidity (60 ± 5%) and light (12 h light/dark cycle). Rats were allowed free access to water, standard laboratory rat chow (Provimi India, Bangalore) and kaolin pellets *ad libitum*, which were placed in separated compartments throughout the experiment. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the CPCSEA guidelines.

Drugs and Chemicals

HAT (Himalaya Anti-emetic tablets, The Himalaya drug Company, Bangalore), whose main ingredients are extracts of ginger and lemon is used as an herbal reference standard. Ondansetron (Cipla Ltd, India), a 5-HT_3 receptor antagonist was used as reference anti-emetic.

Preparation of Kaolin Pellets

Kaolin pellets were prepared based on the method described previously[11]. Briefly, pharmaceutical grade kaolin acacia and carmine were mixed at a ratio of 98.5:1:0.5. Distilled water was used to form a thick paste of this mixture. The paste was rolled and cut into pieces that resembled regular rat chow pellet. The pellets were dried at room temperature for 72 hrs.

Experimental protocol

32 rats were divided into 4 groups of 8 animals each. Rats from group I and II received DM water (10 ml/kg) and served

as normal and positive control, respectively. Rats from group III received Himalaya Anti-emetic tablet formulation (HAT) at the dose of 250 mg/kg b.w p.o and rats in the group IV received Ondansetron at the dose of 4mg/kg b.w p.o. Rats were adapted to experimental environment for three days prior to study, during this period animals were placed in individual cages to allow access to both regular and kaolin pellets. On the day of experiment, 1 hr after the assigned treatment, all the rats except in group I were injected with Cisplatin intraperitoneally at a dose of 3 mg/kg b.w. Rats from Group I were injected with normal saline i.p (1ml/kg). The faeces of each rat was collected after 72 hrs of drug administration and stored at -20^{o}C in refrigerator until analysis.

Quantification of kaolin consumption

Faecal samples were dried completely before the analysis. They were weighed, soaked in distilled water (9.5 ml/g) for 2 hrs and homogenised by sonication. 3N NaOH (0.5ml/g) was added to mixture and mixed vigorously by vortexing. The homogenate was centrifuged at 10,000 X *g* for 15 mins. An 80 µl aliquot of the supernatant was diluted with 920µl of distilled water. 200 µl of this dilution then placed in 96 well microplate and the absorbance was read at 520nm using Biotek Synergy-HT reader.

For the faecal blank, the faeces of rats fed only standard chow pellets were collected and extracted as mentioned above. The standard carmine curve was established by preparing carmine solution in faecal extract (400 µg/ml).

Recovery rate of carmine from faeces

To determine the recovery rate of carmine from faeces, 1g kaolin pellets containing 5 mg of carmine was administered orally through oral gavage (n=5) and the carmine contents were quantified in the faeces excreted during the following 2 days.

Statistical analysis

The results of study were expressed as mean ± SEM and analyzed statistically using One Way ANOVA followed by Dunett's multiple comparison test to find out the level of significance. The minimum level of significance was fixed at 95% confidence limit. The analysis was performed using Graph pad Prism software package (Version 4.0).

RESULTS

Calibration curve for carmine

The calibration curve of carmine was obtained by plotting the absorbance of carmine in faecal extract at 520nm against the concentration of carmine (µg/ml). The equation derived from the standard curve was y=0.007x-0.010 with the correlation coefficient of 0.998 (**Fig 1**).

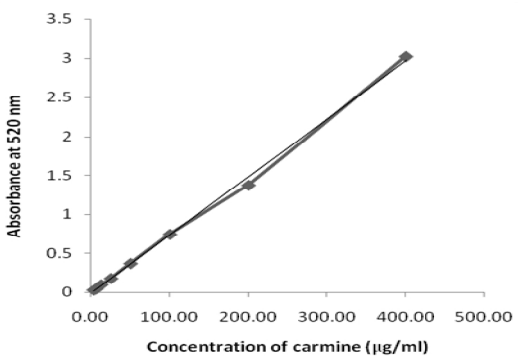

Fig.1. Calibration curve of carmine in faecal extract

Recovery rate of carmine from the faeces

The recovery rate of carmine from the faeces was found to be 80% after 2 days.

Effect of Cisplatin injection on kaolin consumption (pica) in rats

Cisplatin injection (3mg/kg i.p) significantly increased kaolin consumption during 72hrs post injection (**p<0.01) compared to normal control (saline 1ml/kg i.p). The above finding indicates a pica behaviour in rats treated with Cisplatin **(Fig 2)**.

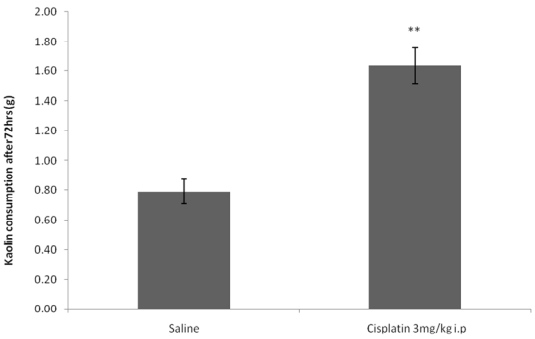

Fig 2: Effect of Cisplatin injection on kaolin consumption (pica) in rats estimated by the faecal carmine content (**p<0.01; n=8)

Effect of Himalaya anti-emetic tablets (HAT) and ondansetron on Cisplatin-induced pica behaviour in rats

Anti-emetic tablet manufactured by The Himalaya Drug Company, Bangalore **(HAT)** was tested for its effect on pica behaviour in rats-induced by Cisplatin. Ondansetron, a standared antiemetic drug used in the management of chemotherapy-induced nausea and vomiting was also tested as a reference standard. The results indicates, significant suppression of both the tested drugs on the kaolin consumption in rats-induced by Cisplatin during the 72 hrs after the Cisplatin injection **(Fig 3 & 4)**.

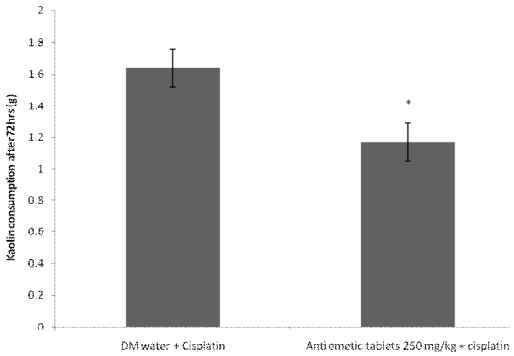

Fig 3: Effect of Himalaya Anti-emetic tablets (HAT) on Cisplatin-induced pica behaviour in rats; (*p<0.05) during the 72 hrs after injection. (n=8)

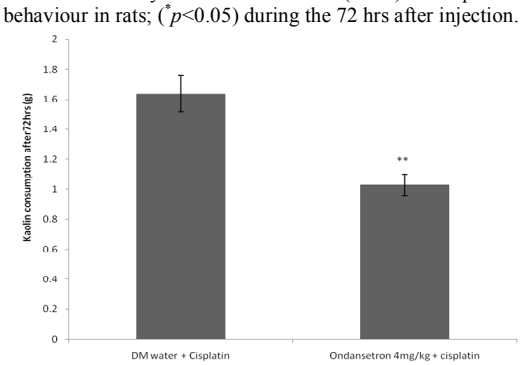

Fig 4: Effect of ondansetron on Cisplatin-induced pica behaviour (kaolin consumption) in rats; ondansetron significantly suppressed the kaolin consumption induced by Cisplatin (**p<0.01) during the 72 hrs after injection. (n=8)

DISCUSSION

Pica is a condition characterised by consumption of non-nutritive substances such as clay, dirt, wood, charcoal or soil and is a common phenomenon in animals and also observed in humans[13-15.]

Rats injected with toxins or subjected to motion, consumed non-nutritive substances that they would normally not ingest and it has been reported that pica in rats is an illness response behaviour analogous to emesis because this behaviour can be induced by several stimuli that commonly cause gastrointestinal distress or emesis in humans[8,9,13]. They have evaluated pica behaviour by measuring the kaolin consumption, but it is laborious and non-specific. To measure the exact amount of kaolin consumption, Takeda and co-workers used carmine as a marker and determined the exact kaolin consumption by measuring carmine excreted in faeces in mice. Carmine, a complex of carminic acid and aluminium, has been widely used in food stuffs and drugs as a colouring agent. It was used clinically to assess the intestinal transit time in children[10].

In the present context, an attempt was made to establish a rat model of pica behaviour using carmine as a marker. We prepared kaolin pellets mixed with carmine and quantified the exact kaolin consumption by measuring the amount of carmine excreted in faeces using spectrophotometer. The absorption spectrum of carmine in 0.15M NaOH shows two peaks at 520 nm and 550 nm. Based on these observations and on the previous report[11], we selected 520 nm for our study. Calibration curve for carmine was obtained by substracting the absorbance obtained by faecal background with that of carmine in faecal extract with correlation coefficient of 0.998.

Oral administration of 1g of kaolin pellets which contained 5mg of carmine was quantitatively recovered faeces with a recovery rate of 80%. Using this method, we characterised the Cisplatin-induced pica in rats.

Cisplatin in a single dose induced an alteration in food habit, characterised by prolonged increase in kaolin consumption[8] and it has been reported that prolonged increase in pica corresponds to a prolonged and delayed emetic response to Cisplatin[16]. Cisplatin-induced nausea/vomiting is possibly mediated via cytotoxic damage to the enterochromaffin cells in the intestine and release 5-HT. The resultant 5-HT release stimulates 5-HT_3 receptors located on the vagal afferents and initiation of the emetic reflex in the brain stem and cause nausea and emesis[17, 18].

CONCLUSION

Present study indicates the measure of Cisplatin-induced pica in rats using carmine as a marker. Kaolin pellets were prepared by incorporating carmine (0.5% W/W) and the exact amount of kaolin consumption was determined by the carmine excreted in faeces. It was found that, the recovery rate of carmine was 80%. Cisplatin at the dose of 3 mg/kg induced significant increase in the kaolin consumption compared to control, indicating pica behaviour. The present findings showed that exact kaolin consumption can be quantified by measuring the carmine added in it and can be a useful model to study the anti-emetic drugs. Further, the model was validated by commonly used anti-emetic drugs, such as ondansetron (4mg/kg.p.o) and Himalaya Anti-emetic tablets (HAT). These drugs significantly decreased the kaolin consumption in rats-induced by Cisplatin injection, which was further shown by decrease in faecal excretion of carmine. This method may further overcome the inherent disadvantages of measuring direct kaolin intake.

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