Effect of L-Type Calcium Channel Blockers,Nifedipine, on the levels of serum Cholecystokinin in domestic rabbits.

Urooj Yahya[1], Shahid Ali Shah[2], Inayat Shah[3], Shehryar Mohibullah[4], Afsheen Mehmood[5], Munir Mengal[6], Naveed Afzal Khan[7]

Abstract:

Background: Cholecystokinin (CCK) is a small polypeptide. It that functions as a neurotransmitter in the CNS and as a hormone in the gastrointestinal tract. The release of CCK is from the STC-1 cells of the gut lining mainly by dietary proteins.CCK plays a major role in the release of bile into the duodenum. Bile emulsifies fats and helps in their absorption. This shows that CCK may affect lipid homeostasis. A luminal cholecystokinin releasing factor (LCRF) has been recently discovered. It is known that LCRF is dependent on calcium for its release.Furthermore, LCRF release is inhibited by L-type calcium channel blocker drugs. Thus Nifedipine, a calcium channel blocker, may affect the release of CCK. This study was carried to investigate the effect ofNifedipine on serum CCK levels in healthy domestic rabbits.We intervened with Nifedipine for four weeks and checked for changes in serum CCK and Lipids.

Objective: The objectives of this study were to assess the effect of Nifedipine on serum CCK (Orryctolaguscuniculus).

Methodology: This study was arandomized controlled trial. Final preparation and analysis of the data was done by Microsoft excel and GraphPad Prism version 5.

Results: This study showed no significant change in serum CCK concentration.

Conclusion: We conclude from this study that there is no significant relationship between the Nifedipine and serum CCK. Further studies using hypertensive animal models is suggested to get more clinically useful information in this regard as the drug is widely used for treating hypertension.

Introduction

Although used for and effective in treating certain disease conditions, nifedipine use could cause unwanted pharmacological effects and check the synthesis of useful cellular molecules like CCK which is dependent on calcium channels for its release (1, 2).

CCK function as a neurotransmitter in the CNS and a hormone (Jorpes and Mutt, 1960). It is secreted by luminal mucosal cells present all over the small intestine. These cells are endocrine in nature. Ingestion of food causes the release of CCK into blood.

Many studies have reported activation of CCK by fat and proteins.

In humans, CCK is encoded by the gene located on chromosomes 3p22-p21.3. It is produced through many steps of processing of a large peptide precursor (3).CCK is synthesized and producedby G cells from the antrum of stomach and I-cells from the upper GIT respectively. (4).

On the basis of pharmacology and loacation,“alimentary” i.e. Type-A and “Brain” i.e. Type-B receptors have been discovered. The alimentary receptors for cholecystokinin have been found on the cells of the colon, ileum, oesophageal sphincter, stomach, gall bladder and pancreas. The Type-B receptors were first found in the brain and peripheral nerves (5-9).

Releasing factors (LCRF which was unknown at that time) that are endogenously produced are secreted into the lumen of the gut and stimulate CCK secretion, and also mediate the secretion of CCK by negative-feedback.Immunoabsorption of LCRF with specific LCRF antiserum eliminated CCK-releasing activity. But the direct action ofLCRF on CCK cells is not confirm. The effect of LCRF on stimulation of CCK release in humans is also not known.

The release of CCK has been examined in freshly isolated, partially enriched intestinal CCK cells.When plasma membrane is depolarized it blocks the potassium channels so as a result, L-type calcium channel are activated causing increase in calcium influx and hence resulting CCK secretion. So it can be concluded that secretion of CCK is dependent on calcium so release of CCK stops when calcium is blocked.

Nifedipine blocked calcium current with a half maximal inhibitory concentration (IC50) of 0.3 μM; the IC50 was reduced to 50nM when voltage was held at -40 mV, this indicates that Nifedipine preferentially block the L type Calcium channels. This blockage of calcium channels is concentration dependent and is faster if amount of drug is replenished fast keeping its half-life in view (10).

Nifedipine also decreases resting gallbladder tone and

1. Dept. of Physiology, Kabir Medical College, Peshawar
2. Dept. of Physiology, Khyber College of Dentistry
3. Dept. of Physiology, Khyber Medical University
4. Mphil Scholor of Pharmacology, Khyber Medical University,Peshawar
5. Dept. of Physiology, Khyber Girls Medical College
6. Jhalawan Medical College, Khuzdar
7. Dept. of medical education, Khyber Girlsmedical college

Address for Correspondence:

Dr.Shahid Ali Shah,
Dept. of Physiology, Khyber College of Dentistry, Peshawar
Contact: +923335044097
Email: dr_s.a.shah@live.com

abolishes spontaneous inter-digestive gall bladder contraction. (11).

It is evident from the above details that CCK, CCK-receptors, calcium ions and channels and the calcium channel blocking medications are all inter-related and have broad effects on each other. The aim of this study is to look into the effect of Nifedipine on serum CCK levels in laboratoryrabbits. As one of the functions of secreted CCK is the removal of cholesterol from the blood and its excretion in the form of bile components. Thus it is possible that CCBs may interfere with cholesterol homeostasis indirectly. Thus changes in serum CCK due to the use ofNifedipine were assessed.

Materials and methods

This is randomized Controlled trial with random allocation of experimental animals into three groups.The study was conducted at Veterinary research centreKohat city of Khyber Pakhtunkhwa province in Pakistan. The animals were tagged after being assigned into groups. Male and female rabbits were kept separately.

Rabbits were distributed randomly amongst three groups, n=6 in each of the three groups. An extra of two similar rabbits were kept in each group to avoid reduction in sample size in case of unwanted illness or death of a study animal. Each group was given a different colour tag.

Simple random sampling was carried out using adult 3-6 month aged, 2-3 Kilograms, healthy domestic rabbits (Orryctolaguscuniculus). Rabbits having problems with vision, skin issues, teeth or lethargy for any other reason as determined by the veterinarian were exclude from the study.

The experimental protocol was approved by the Khyber Medical University advanced studies and research board and ethical approval was obtained from Khyber Medical University (KMU) institutional ethical committee.

A four weeks period was approved as ample by KMU advanced study and research board for this study. Another week was added at the start of the regimen to acclimatize the rabbits to the new living environment.

Animals were kept under a full time surveillance in the research centre. The breathing rate of each rabbit was assessed intermittently for a general assurance of health. A twelve hour dark and light cycle was observed.

A standardized diet including breads, vegetables and milk that average out to be a 100gm/rabbit was allowed to the animalsduring this study with water available as per desire. The rabbits were fed in this way for five weeks, the first week being for animal acclimatization.

Animals from each labelled section of the animal house were brought out one at a time and fed with the medication. All animals from one section were fed first and then re-housed before the next section of the animal house was approached.

A solution comprising of 5 milligrams of Nifedipine (Pfizer Ltd) in 40 milli-litres ofpropylene glycol prepared and fed to the intervention group animals orally in a dose of 2ml per kilogram i.e. 0.25 mg/kg/day orally(12).

An initial and a final sample were taken after an overnight fast, in the start and end of the study period in this manner. Blood was taken in a serum separator tube (SST) and was allowed to clot for half an hour at ambient temperature before their centrifugation for 10 minutes at three thousand revolutions per minute (3000rpm) in a centrifuge machine (AJ-IE, China). Serum was removed as clear transparent supernatant. The clear serum was transferred as aliquots into labelled Eppendorf tubes with the help of droppers. The samples were kept at -20°C until the analysis. The storage time was 4 weeks and two days.

Calculations and Statistics

Microsoft Excel Spreadsheets were used to organize all the data and to calculate the serum CCK concentrations from the ODs given by the plate reader. A standard graph was drawn taking known concentrations of the standards on the horizontal axis and their measured optical density value on the vertical axis. This generated a formula for the mathematical relation of the ODs and concentration. The concentrations of the study samples were calculated from their ODs using this formula. The measurements thus calculated were expressed in pg/ml.

Data thus sorted in Microsoft Excel was then exported to Graph-pad prism for analysis. The data was analysed usingstudent's paired t-test for the same group andt-test was used for comparison between the groups.Graph-pad Prism was also used for plotting graphs and charts.

Results

A total of 24 healthy rabbits having 12 males and 12 females were recruited into the study. The rabbits were then randomly distributed into three groups in such a manner that the number of males and females in each group is equal.

The Baseline Data

The data was analysed by Kolmogorov-Smirnov test for normality and was found to have a normal distribution.The baseline data of the animals in the study groups was similar. This is suggestive that the animals recruited into the study were similar in demographics. Thus a change in these parameters after the study period could be easily linked to an intervention.The demographics and serum CCK levels of the rabbits are given in the table.

Demographics and CCK of the study animals in the start of the study.

Variable	Vehicle only	Vehicle + Nifedipine	Control	Mean	P
Age (weeks)	10.2 ±1.5	9.7 ± 1.6	9.2 ±1.3	9.7	0.57
Weight (kg)	2.18 ±0.2	2.3 ±0.3	2.4 ±0.2	2.29	0.65
RR(Br/m)	60 ±3.1	61 ±2.5	62 ±2.7	61	0.32
CCK (pg/ml)	693 ± 172	701 ± 201	634 ± 188	676	0.95

Table 3 1the serum CCK levels, age, RR-respiratory rate, and weight of the rabbits measured before treatment.

The measurements after the study period

The results suggest that Nifedipine has no significant effect on the serum Cholecystokinin (P >0.05) of domestic rabbits when given in the recommended dose. The rabbits in the Nifedipine treated group showed a slight decrease in their weight. Same variables were measured after four weeks of the trial. The t.test was used for comparison between the groups. The results after the study have been summarized in the following table.

Demographics and CCK after four weeks					
	Vehicle only	Vehicle + Nifedipine	Control	Mean	P
Age (weeks)	14.2 ±1.33	13.7 ±1.63	13.2 ±1.33	13.7	0.57
Weight (kg)	2.1 ±0.16	2.1 ±0.29	2.4 ±0.24	2.2	0.72
RR (breaths/m)	60 ± 1	60 ± 2	62 ± 3	60.67	0.34
CCK (pg/ml)	695 ±152	694 ±151	619 ±97	669.33	0.99

Table 3 2 the serum CCK levels, age, RR-respiratory rate, and weight of the rabbits measured before treatment.

Discussion

The current literature is lacking in studies on the effects of CCBs on serum CCK. Thus our study could not be properly compared to many other studies. The findings and comparisons of this study to other studies are discussed in this section as follows.

Our study suggests that Nifedipine has no significant effect on the levels of the serum CCK. No such study was found after a thorough literature search. However,in our study we measured CCK levels in serum from blood collected after an overnight fasting period. As CCK is a hormone that is produced in response to food. An overnight measurement would only be suggestive of any long term effects of the medicine on serum CCK.

The intestine shows a response if food is present in the intestine where CCK secreting cells are present. The negative findings (no changes in serum CCK) are suggestive of the fact that CCK is a transiently active hormone which is produced at the time of feeding. The levels of CCK are not maintained for long periods of time.

Tanaka and Liou reported that the ingestion of fat also induces secretion of the gut peptide hormone cholecysto kinin (CCK) through the long-chain FFA receptors GPR120. This secretion of CCK by FFA was abolished either by removal of extracellular Calcium or by the L-type Calcium channel blocker, Nicardipine. Their results indicate that long-chain FFAs induce CCK secretion through GPR120-coupled Calciumsignalling.(Tanaka et al., 2008 , (13)

That is why, it was proposed that CCBs might affect the CCK release into circulation. However the finding suggest that there is no effect of oral CCBs on CCK.

Conclusion

CCK has an effect on satiety lasting for a few hours during the presence of food in the small intestine. This suggests that food intake has a transient effect on serum CCK which may not last for an overnight fasting period.Our study suggest that fasting serum CCK is not altered by Nifedipine. It might have short term effects the satiety hormone but its affects do not last long as the hormone has a transient releasemediated by a variety of nutritional elements.Studies on the transient effects of CCBs on CCK are hence warranted to investigate their instantaneous effects.

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