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Article · December 2015

DOI: 10.4172/jhgd.1000105

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Effect of Total Extract of Milk Thistle Fruits on the Absorption of Glycine in the Rats Small Intestine under Physiological Conditions

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Received date: December 1, 2015, Accepted date: December 8, 2015, Published date: December 17, 2015

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Abstract

Objectives and method: To determine the effect of total extract of milk thistle fruits on the velocity of glycine absorption in the chronic experiments under physiological condition with no operation trauma, pain, narcosis and atrophy of the small intestine.

Results: In the presence of total extract of milk thistle fruits the glycine absorption velocity increases during one hour of perfusion. Absolute values of glycine absorption are higher in the presence of milk thistle fruits extract than in its absence. We observed no dissolution of perfusate with gastrointestinal fluids in the small intestine functioning part indicating that absorption of water in this fragment of small intestine prevails in the presence and in the absence of milk thistle extract both.

Conclusions: Total extract of milk thistle fruits activates the velocity of absorption of glycine in the functioning fragment of the rats' small intestine under physiological conditions.

Keywords: Glycine; Absorption; Perfusion; Small intestine; Physiological condition; *In vivo*; Milk thistle

Introduction

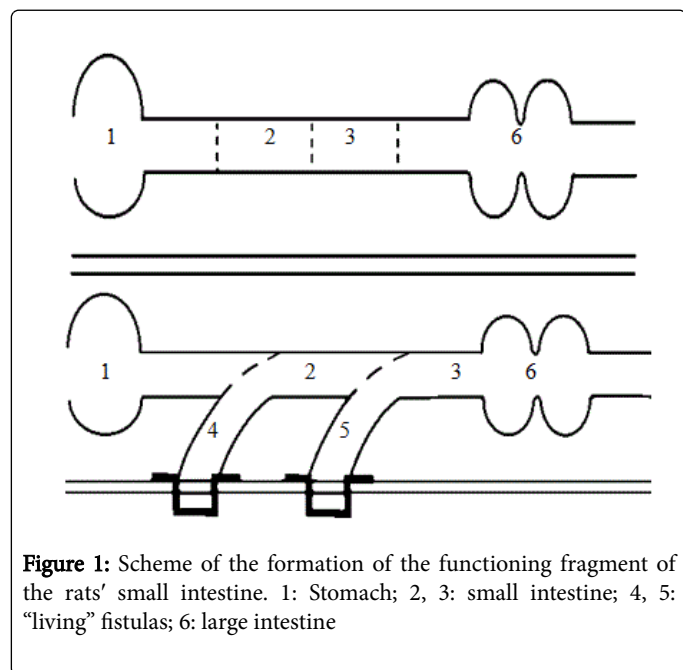
Due to environmental pollution, changes occur in the metabolism of the human body, and its adaptive regulatory mechanisms. The small intestine, in which the final stages of digestion and the formation of monomeric nutrients take place, is a barrier between the environment and the internal environment of the body and therefore it is sensitive to the presence of various pollutants that can both penetrate the enteral barrier and affect the intestinal epithelium. Thus, the existence of the whole organism to a certain extent depends on the functional activity of small intestine. Recently, for the correction of metabolic disorders in humans often use herbal remedies that are gentle action and mild side effects on the background of a broad therapeutic range [1-6]. The natural complex of biologically active substances - milk thistle - more than 2 thousand years uses to treat a range of diseases - from inflammation and arthritis to prostate carcinoma and breast cancer [3-6]. The most famous it is as a hepatoprotector: on the basis of its main active ingredient - silymarin-Carsyl, Hepabene, Legalon, Galstena and other mono- and multicomponent hepatoprotective drugs were created. Hepatoprotective effect of silymarin consists primarily reparation of the hepatocyte membrane, stimulation of protein synthesis by the activation of r-RNA synthesis and changes in the qualitative composition of the membranes by increasing the amount of phospholipids and fatty acids [3]. Considering membranotropic properties of silymarin we can expect a similar effect on the enterocytes. Therefore, the idea to explore the effect of the total extract of milk thistle containing both water- and lipid-soluble active ingredients, on the functional activity of small intestine appears. Earlier in the experiments *in vitro* we investigated the effect of the total

extract of milk thistle on glycine transport into the accumulating mucosal preparations [7,8]. Often, however, data from different methodological conditions vary considerably, not only in absolute terms, but also in direction, so the purpose of the work was to study the effect of the total extract of milk thistle on the absorption of glycine in the small intestine under physiological conditions in the absence of the effect of anesthesia and surgical trauma. We have developed an original technique for the formation of a functioning fragment of the small intestine directly included in the digestive system and preserving innervation, blood supply and passage of chime. That allows to explore the functional activity of the small intestine in chronic experiments on non-anaesthetized animals in the absence of surgical trauma and stress - i.e., under physiological conditions. Previously, using this technique, we determined the dynamics of absorption of neutral non-essential amino acids - glycine [9]. The aim of this paper was to study the effect of the total extract of milk thistle *Silybum Marianum* (G.) on this process.

Materials and Methods

The experiments were performed on male rats of Vistar breed weighted 170-180 g that were held out on the standard ration of vivarium and were not fed for 18-24 hours prior to the experiment. There were five rats in the group with functioning part of the small intestine. During the surgery we formed two "V"-shaped contacts by connecting two anastomoses using "end to end" principle and placing the free ends of intestine in the side of animal ("living" fistulas), secured with standard metallic fistulas (Figure 1). Including "living" fistulas, the length of the investigated area was 10 cm. 4-5 days after the operation, the animals were perfused by peristaltic pump "Zalimp"(Poland). Velocity of perfusion was 0.6 ml/min. For the perfusion, we used 25 mmol/l solution of glycine on the Ringer

solution and 25 mmol/l solution of glycine on the Ringer solution with summary extract of milk thistle fruits. Summary extract of milk thistle fruits was dried under 20°C water-alcohol extract of milled milk thistle fruits (3 gr of fruits+10 ml of distilled water+20 ml of ethanol), then it was dissolved in the Ringer solution (10 ml of extract dried under 20°C and 100 ml of Ringer solution added). Then to this solution glycine was added (pH=7.4 to of the perfusion solution=37°C) [10]. We added an unabsorbed marker polyethylene glycol (PEG-400) to the perfusion solution to control possible dilution of perfusion solution with the liquids of digestive tract (saliva, gastric, intestinal and pancreatic juices, bile or reflux from the next part of intestine). The concentration of glycine was determined using method described in ref. [11] colorimetrically on photoelectron colorimeter – CFC-2MP, $\lambda=540$ nm. The concentration of PEG was determined based on modified method colorimetrically on CFC-2MP, $\lambda=465$ nm [12]. All experiments were conducted in accordance with scientific/practical recommendations regarding animal care and work with them and in compliance with the positions of "European convention about defense of the vertebrates used for experimental and scientific aims" [13]. The statistical processing of the obtained data was conducted using "Primer Biostatistics" software.



Results and Discussion

Previously, we showed absorption dynamics of its free glycine 25 mmol/l solution - absorption rate for 60 min perfusion tended to increase from about 2 to 5 mmol/l [9]. In the presence of total extract of milk thistle in the perfusion solution the absorption velocity of free glycine also increased during perfusion, but absolute values were (with one exception indicator) higher than in the absence of extract - from about 3 to 6 and above mmol/l (Figure 2). Because of analyzing of the dynamics of absorption of glycine in the presence and in the absence of milk thistle extract, it can be seen that after a 20-minute perfusion trends of both curves are virtually identical (Figure 2). Therefore, presence of milk thistle extract in perfusion solution does not influence on the character of absorption of glycine, but significantly (with the exception of 2 points on the curve perfusion) increases its rate of

absorption. As in the case of perfusion of the small intestine by the solution of free glycine, the rate of absorption of glycine in the presence of milk thistle extract at the end of the perfusion was significantly higher than at the beginning ($p < 0.0001$: 6.81 ± 0.15 versus 2.99 ± 0.40 mmol/l correspondingly). The average rate of absorption of glycine in the presence of milk thistle extract significantly (22.3%) exceeded that in the absence of the extract ($p < 0.0001$). Thus, it was found that the total extract of *Silybum marianum* promotes the activation of absorption of glycine in the functioning part of the rat small intestine.

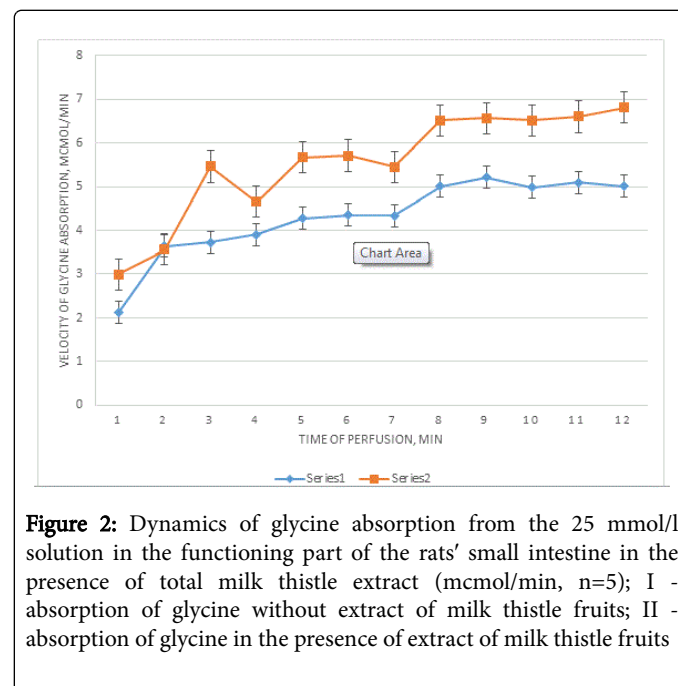


Figure 2: Dynamics of glycine absorption from the 25 mmol/l solution in the functioning part of the rats' small intestine in the presence of total milk thistle extract (mcmol/min, n=5); I - absorption of glycine without extract of milk thistle fruits; II - absorption of glycine in the presence of extract of milk thistle fruits

Attention is drawn to the fact that into the experiment adult animals weighing 170-180 grams were involved. Earlier *in vitro* experiments with the same weight rats, we found that the total extract of milk thistle fruits significantly stimulated the transportation of glycine into the accumulating intestinal mucosal preparations from adult rats only in those cases when accumulation of the substrate in the preparation does not exceed 28 mmol/l [7]. If the accumulation of glycine in the product was higher, the total milk thistle extract did not change the level of transport of the substrate [7]. Apparently, under the conditions of reduced systems *in vitro* in the area of 28 mmol/l, corresponding to approximately 3-fold accumulation of substrate from the medium (10 mmol/l of glycine) a certain transport mechanisms switching takes place and on the new level there is no stimulating effect of the milk thistle extract. This assumption is supported by the fact of inhibition of transport of glycine in the presence of the total extract of milk thistle ($p=0.001$) *in vitro* conditions in a group of animals weighing 90 ± 5 g [8]. In this group the initial value of accumulation of glycine was 68.96 ± 5.35 mmol/l versus 37.26 ± 2.19 mmol/l of glycine in the presence of a total extract of milk thistle fruits for n=8 [8]. It should also be noted that in these experiments, the incubation medium lacking bile, whose role in the transport of nutrients modification was later shown by us [14]. However, under physiological conditions the natural bile enters the perfusion solution and can influence the absorption parameters glycine.

It is interesting that in the similar studies *in vitro* on the two-month rat pups (50-65 g) the total extract of milk thistle in the presence of bile

in the incubation medium did not change the absolute values of glycine transport. In this case, the absolute baselines of transport in intact pups were almost twice higher than in intact adult rats (51.84 ± 3.62 mmol/l [15] versus 28.56 ± 2.29 mmol/l [7]). Apparently, this is because the young animal is experiencing increasing demand for amino acids more than the adult organism.

Interestingly, the milk thistle extract on the absorption of glucose in the same concentration under the same *in vivo* conditions exerted the opposite effect - it caused a significant inhibition of absorption of glucose (40%) [16]. It should also be noted that *in vitro* the absorption of glucose in the presence of a total extract of milk thistle fruits also was inhibited [17]. Those in the case of glucose transport direction of the effect persists under both *in vitro*, and *in vivo*, whereas in the case of the glycine absorption the effect of total extract of milk thistle fruits is multidirectional *in vitro* and *in vivo*. This information can be used under the formation of protein diets, and under the use of glycine as a neurotransmitter.

Conclusion

Finally, the total extract of milk thistle fruits promotes the activation of absorption of glycine in the functioning part of the rat small intestine. Stimulation of glycine transport by the help of total extract of milk thistle helps to increase its pool in the cells and tissues.

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