Simulation of colitis with the use of food fat

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Abstract
In recent decades, the consumption of fats, especially animal fats, has increased significantly. It has been established that high-fat nutrition leads not only to the development of obesity, liver steatosis, type 2 diabetes mellitus, but also underlies the pathogenesis of dysbiotic syndrome. The latter is the main pathogenetic mechanism of the most widespread noncommunicable diseases.

It has been established that the biological effect of various dietary fats largely depends on their fatty acid composition and, in particular, on the content of saturated fatty acids, especially palmitic acid. It was shown that triglycerides containing palmitic acid are difficult to hydrolyze by lipase of lipoproteins, which complicates their utilization by tissues.

The authors studied the effect of dietary fats with different fatty acid composition on the condition of the mucous membrane of the colon of rats.

The inflammatory process in the colon mucosa (colitis) develops only after consuming butter and palm oil. Partially, it is increased after the consumption of ordinary sunflower oil (the level of only one marker increases - MA).

The development of colitis is inversely related to the degree of decrease in the antioxidant-prooxidant API index. Thus, the consumption of palm oil causes the strongest decrease in the API index and the highest increase in the level of inflammation markers.
Key words: fat diet; palmitic acid; colon mucosa; colitis; dysbiosis.

In recent decades, the consumption of fats, especially fats of animal origin, has increased significantly [1, 2]. It has been established that high-fat nutrition leads not only to the development of obesity, liver steatosis, type 2 diabetes mellitus, but also underlies the pathogenesis of dysbiotic syndrome [3, 4]. The latter is the main pathogenetic mechanism of the most widespread noncommunicable diseases [5-7].

It was established that the biological effect of various dietary fats largely depends on their fatty acid composition and, in particular, on the content of saturated fatty acids, especially palmitic acid (C16:0) [8, 9]. It was shown that triglycerides containing palmitic acid are difficult to hydrolyze by lipase of lipoproteins [10], which complicates their utilization by tissues.

Now in the food industry, and, consequently, in the nutrition of people, palm oil, in which palmitic acid is more than 40%, is widely used.

Given all of the above, we set ourselves the task of studying the effect of dietary fats with different fatty acid composition on the condition of the mucous membrane of the colon of rats.

Materials and research methods

The following fats were used:
- Unrefined sunflower oil (Smak Sontsya, Marchenko V.V., Ukraine);
- high-oleic sunflower oil "Olive" (TU U 15.4-13903778-36-2002, NPA "Odessa Biotechnology", Ukraine);
- peasant butter 72.5% fat (VKF "Agromarin", Ukraine);
- palm oil (Dukecs RBD, Malaysia);
- Coconut oil brand "Bess" (PGFO Edible oils SDN BHD, Malaysia).

The fatty acid composition of these fats was determined using a gas chromatographic method on a Shimadzu mass spectrometer [11].

Fat feeding experiments were carried out on white Wistar rats (males, 8–9 months old, live weight 240–260 g), distributed into 6 groups of 6 animals each. The first group received a standard diet of vivarium (fat content 5%), the second received food containing 15% sunflower oil, the third - food containing 15% high-oleic sunflower oil "Olive", the fourth - with 15% butter, the 5th with 15% palm oil and the 6th with 15% coconut oil. The duration of feeding was 64 days.

After animals were euthanized under thiopental anesthesia (20 mg / kg), blind and ascending parts of the transverse colon were isolated from the heart by total bloodletting, washed from the contents with cold 0.9% NaCl solution, and the mucous membrane was scraped, which was stored until minus 30 °C.
The level of inflammation markers [12] was determined in the mucous homogenate [12]: elastase activity [13] and content of malonic aldehyde (MA) [14], antioxidant enzyme activity of catalase [15], microbial seeding activity, urease activity [16] and non-specific immunity level lysozyme activity [17]. The antioxidant-prooxidant API index was calculated by the ratio of catalase activity and MDA content [12], and the degree of dysbiosis according to A. P. Levitsky [18] by the ratio of the relative activities of urease and lysozyme.

The results of the study were subjected to standard static processing [19].

**Research results and discussion**

The table 1 shows the results of determining the fatty acid composition of the used edible fats. Only the main fatty acids were selected, amounting to 84.2-98.9% of all fatty acids.

Table 1 - Fatty acid composition of used fats (%)

| Fatty acids       | Short formula | Sunflower oil | "Olive" | Butter | Palm oil | Coconut oil |
|-------------------|---------------|---------------|---------|--------|----------|-------------|
| Lauric acid       | C_{12:0}      | 0             | 0       | 3,0    | 0,2      | 46,6        |
| Myristic acid     | C_{14:0}      | 0,1           | 0       | 10,4   | 1,2      | 22,7        |
| Palmitic acid     | C_{16:0}      | 9,7           | 4,4     | 27,9   | 42,0     | 11,7        |
| Stearic acid      | C_{18:0}      | 3,9           | 3,1     | 12,7   | 4,9      | 13,6        |
| Oleic acid        | C_{18:1}      | 30,6          | 88,7    | 26,6   | 40,9     | 0,3         |
| Linoleic acid     | C_{18:2}      | 53,5          | 1,2     | 3,1    | 9,5      | 0           |
| Linolenic acid    | C_{18:3}      | 0             | 0,1     | 0,5    | 0,2      | 0           |
| Sum               |               | 97,8          | 97,5    | 84,2   | 98,9     | 94,9        |

For ordinary sunflower oil, the main fatty acid is linoleic, which contains two double bonds and belongs to the $\omega$-6 series of polyunsaturated fatty acids (PUFAs). In high-oleic sunflower oil "Olive", the main acid is monounsaturated oleic acid. In butter, the sum of three saturated fatty acids (myristic, palmitic and stearic) is 41%, in palm oil, the sum of these acids is 48.1%, in coconut oil - 48%, but the main acid is medium chain lauric acid, which is 46, 6%

In studies, the effect of various high-fat diets (HFD) on the condition of the mucous membrane of the colon of rats was studied. Fat was introduced into the standard low-fat diet (fat content less than 5%) in an amount of 15%. After 9 weeks, the rats were euthanized and elastase level and malondialdehyde content were determined in the homogenates of the colon mucosa. The relevant data are presented in table 2.

From the data it is seen that a significant increase in the level of biochemical markers of the
colon is carried out only by two edible fats: butter and palm oil.

Table 2 - The level of markers of inflammatory-dystrophic processes in the mucous membrane of the colon of rats that received a high-fat diet

| Groups          | Elastase, mk cat / kg | MA, mmol / kg |
|-----------------|-----------------------|---------------|
| The control     | 60.4±7.6              | 4.32±0.26     |
| Sunflower oil   | 76.0±12.3             | 5.24±0.29     |
|                 | p>0.1                 | p>0.05        |
| "Olive"         | 62.7±4.8              | 4.49±0.20     |
|                 | p>0.3                 | p>0.3         |
| Butter          | 83.2±3.5              | 6.62±0.19     |
|                 | p<0.05                | p<0.01        |
| Palm oil        | 118.7±8.1             | 8.08±0.54     |
|                 | p<0.001               | p<0.01        |
| Coconut oil     | 66.2±3.6              | 4.70±0.24     |
|                 | p>0.3                 | p>0.2         |

Notes: p - in relation to control.

So, in rats that consumed butter, elastase activity increased by 38%, and the content of malondialdehyde by 53%. Palm oil increased elastase activity by 97%, and the content of malondialdehyde by 87%. That is, the presence and degree of dysbiosis depends more on the composition of fats than on their total amount. A feature of these two edible oils is that they contain more than 30% palmitic acid (45% palm oil). There is evidence of the harmful effects on the body of excessive consumption of this fatty acid. That is, highly unsaturated fats, which are consumed worse in the body, possibly entering in large quantities into the large intestine, induce processes of inflammatory damage to the mucous membrane.

Additionally, studies were conducted on the effect of these two fats (palm and butter) on the colon of rats, which received a semi-synthetic fat-free diet (FFD) with the addition of 20% of the studied fats. After 2 months of feeding, the level of markers of inflammatory-dystrophic processes was determined in the mucous membrane of the colon. The relevant data are presented in table 3.

It was found that palm oil increases elastase activity by 39%, and the content of malondialdehyde by 25%. Butter increased elastase activity by 96% and malondialdehyde content by 23%. Especially pathogenic is the increase in butter in the diet.

In the experiment, the level of biochemical markers of inflammatory-dystrophic processes in
the mucous membrane of the colon of rats, which received palm oil or palm oil against dysbiosis, was studied using a semi-synthetic fat-free diet (HFD).

Table 3. - The level of markers of inflammatory-dystrophic processes in the mucous membrane of the colon of rats that received high-fat diets

| Groups                  | Elastase, mk cat / kg | MA, mmol / kg |
|-------------------------|-----------------------|---------------|
| The control, FFD        | 109,8±10,3            | 7,48±0,46     |
| Palm oil, 20 %          | 161,8±17,8 p<0,05     | 10,20±0,38 p<0,05 |
| Butter, 20 %            | 215,1±28,2 p<0,01     | 9,19±0,57 p<0,05 |

Notes: p - in relation to control.

As a comparison, we used a diet containing high-oleic sunflower oil "Olive". The duration of feeding was 38 days after euthanasia of animals in the homogenate of the mucous membrane of the colon was determined the activity of elastase and the content of malondialdehyde. The relevant data are presented in table 4.

Table 4 - The level of markers of inflammatory-dystrophic processes in the mucous membrane of the colon of rats that received high-fat diets (HFD)

| №№ | Groups                           | n  | Elastase, mk cat / kg | MA, mmol / kg |
|-----|----------------------------------|----|-----------------------|---------------|
| 1   | FFD                              | 7  | 79,2±7,2              | 4,25±0,24     |
| 2   | HFD with 15% "Olives"            | 7  | 97,1±12,5 p>0,05     | 4,47±0,67 p>0,3 |
| 3   | HFD with 15% palm oil            | 7  | 114,7±10,6 p<0,05    | 5,06±0,56 p>0,05 |
| 4   | HFD with 15% palm oil + lincomycin 60 mg / kg for 5 days | 7  | 120,8±9,7 p<0,05     | 5,49±0,55 p<0,05 |

Notes: p - in relation to control.

As can be seen from these data, the use of palm oil increases the level of elastase and malondialdehyde by 45% and 21%, respectively. The use of palm oil against a background of
dysbiosis further increases the level of markers: elastase by 53%, malondialdehyde by 29%. That is, we observe the summation of the pathogenic effect of palm oil and dysbiosis. At the same time, the use of "Olives" did not significantly increase the level of inflammation markers. This confirms, on the one hand, that it is not excess fat that is pathogenic, namely palmitic acid, and unsaturated oleic acid, on the contrary, exhibits a significantly lower pathogenic effect, that is, its effect can be considered prophylactic.

Thus, based on our studies, we can conclude that high-fat nutrition using oils with a high content of palmitic acid (creamy and palm) causes a decrease in nonspecific immunity in the colon mucosa, an increase in bacterial seeding, and an increase in the degree of dysbiosis.

However, the inflammatory process in the colon mucosa (colitis) develops only after consuming butter and palm oil. Partially, it is increased after the consumption of ordinary sunflower oil (the level of only one marker increases - MA).

The development of colitis is inversely related to the degree of decrease in the antioxidant-prooxidant API index. Thus, the consumption of palm oil causes the strongest decrease in the API index and the highest increase in the level of inflammation markers.

It seems that the development of colitis is more dependent on lipid peroxidation processes, which reflects the content of MA, than on the level of non-specific immunity.

Conclusions

1. High-fat nutrition using fats with a high content of palmitic acid (butter and palm oil) causes the development of colitis, dysbiosis, and increased lipid peroxidation.

2. The development of inflammation in the colon mucosa is more dependent on the activity of lipid peroxidation than on the level of non-specific immunity.

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