EFFECT OF A HELIUM–NEON LASER ON THE PHYSICOCHEMICAL PROPERTIES OF BILE

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The effects of helium–neon lasers are being studied in two directions: the development of methods of laser fragmentation of gallstones by powerful lasers [4-7] and the therapeutic use of low-intensity laser radiation in inflammatory diseases of the biliary passages [1, 2]. For instance, Gausman and co-workers (1987) used the helium–neon laser (HNL) in the combined treatment of 32 patients with acute cholecystitis, and obtained a beneficial effect in 30 of them: subjective improvement, relief of pain in the right hypochondrium, reduction of leukocytosis, and disappearance of muscular rigidity. In the control group, receiving the usual conservative treatment without laser radiation, indications for emergency operations occurred in 14.3% compared with 6.3% when laser therapy was included in the treatment schedule.

The use of HNL radiation on patients with suppurative cholangitis by applying a quartz light guide through the external drainage tube from the bile duct directly into the lumen of the common bile duct (in the postoperative period), against the background of intensive antibacterial and detoxicating treatment, demonstrated the efficacy and safety of this method of treatment [2].

The only study of spectral transmission factors of bile have been undertaken by Georgadze and co-workers (1987), and the effect of low-intensity laser radiation on the physicochemical properties of bile remain unstudied.

Considering the fact that definite pathological changes, accompanied by a change in its physicochemical properties, take place in the bile of patients with diseases of the biliary system, we decided to study the effect of HNL radiation on some physicochemical properties of bile in vitro.

Bile is a multicomponent system consisting of solid and liquid parts. The solids include bile acids and their salts, cholesterol, phospholipids, bilirubin, proteins, inorganic salts, etc., and the liquid part consists of water.

As a result of the relative proportions of the above-mentioned components, healthy human bile is stable or colloidally stable. This is an important fact, preventing precipitation of the components of the bile and the formation of biliary concrements.

Among the physicochemical parameters of bile, those of the greatest importance in the genesis of changes in colloidal stability are pH, surface tension, and nucleation time. The pH of bile is one of the main parameters of its physicochemical properties. In bile, as a colloidal solution, any change of pH is an indicator of disturbances of the thermodynamic equilibrium of the system. Hence it follows that, depending on changes of pH, qualitative changes in the bile can be indirectly judged.

The value of the pH in colloidally-stable and lithogenic bile varies within wide limits, as we showed previously [3]. For clinically healthy persons the pH of the cystic bile is 6.86-7.10. In the physicochemical stage of cholelithiasis this parameter has a value of 7.2-7.5, whereas in patients with gallstones, when calculi are found in the gallbladder, the pH is 7.5-8.3. Changes in pH toward the acid or alkaline side depend primarily on the thermodynamic equilibrium and the equivalent proportions of components of the bile and its colloidal stability. If, under the influence of a certain method of treatment, the hydrogen ion concentration is corrected, this will be to a certain extent an indication of the beneficial effect of the method used.

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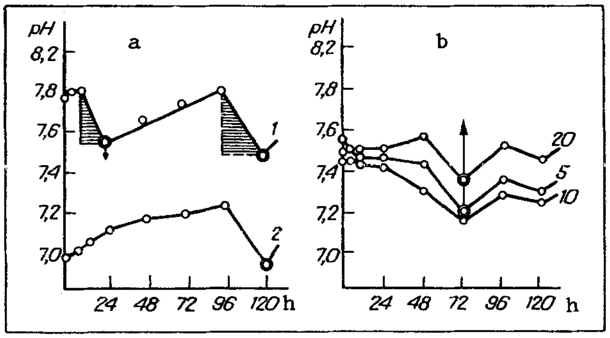

Fig. 1. Dependence of pH of bile on nucleation time under normal conditions and in patients with gallstones, before (a) and after (b) irradiation by HNL. 1) Bile from patients with gallstones, 2) bile from healthy individuals. Arrow indicates region of complete coagulation.

The surface tension occupies an important place in the chemistry of colloidal solutions, especially in the case of biological objects. Depending on the surface tension it is possible to judge between colloidally-stable and destabilized systems, and this is not only informative, but also of theoretical and practical importance. The value of the surface tension in destabilized bile varies from 116 to 118 erg $\cdot$ cm^2, but in stable bile between 72 and 74 erg $\cdot$ cm^2, i.e., the surface tension of destabilized bile is more than 1.5 times greater than that of stable bile. The reason for this difference is that in destabilized bile, because of its structuring, hydrophilic molecules are released from the micelles. Under these circumstances a certain number of molecules pass from the inner layers to the surface of the fluid, where they increase the surface tension.

Another indicator of the physicochemical properties of bile is the nucleation time, or colloidal stability. Together with others, this parameter is a basic and essential value of the colloidal system. The duration of existence of its systems, i.e., the time from the formation of the system until its destruction (the nucleation time) is a measure of the stability of the dispersed system (in this case bile). Depending on the duration of existence of the system it is possible to judge its aggregative stability: the longer this duration, the higher the aggregative stability of the system. It will thus be easily understood that factors contributing to lengthening of the nucleation time of bile play an important role in the treatment of the physicochemical stage of gallstones and in the prevention of their formation.

EXPERIMENTAL METHOD

We studied the effect of HNL on the physicochemical properties of bile at 20 ± 1°C. We studied cystic bile obtained by duodenal catheterization from 25 patients with gallstones and 10 clinically healthy subjects. Laser radiation was applied to the bile by means of a monofiber light guide, in special tubes in which contact with air was prevented.

The sources of laser radiation were the LG-38, LG-75-1, and LGN-104 lasers, generating at a wavelength of 0.63 μm. The power of the laser radiation at the end of the light guide corresponded to safe therapeutic parameters (5, 10, and 15 mW) and the duration of exposure was 5, 10, or 20 min. The pH of the bile was measured on a type EV-74 universal ionometer, and surface tension was measured by the method of greatest pressure of bubbles on the Rebinder instrument, viscosity was determined in a capillary viscosimeter by the method of the flow of liquid through a capillary tube, and the nucleation time was determined visually and microscopically.

EXPERIMENTAL RESULTS

The results are shown graphically in Figs. 1 and 2. Results showing changes in pH of stable and destabilized bile and their dependence on nucleation time are given in Fig. 1a. Healthy human bile is stable in its physicochemical properties, i.e., colloidally stable, whereas bile from patients with gallstones is destabilized, i.e., lithogenic. Changes in pH and nucleation time of destabilized bile after irradiation by HNL are shown graphically in Fig. 1b.

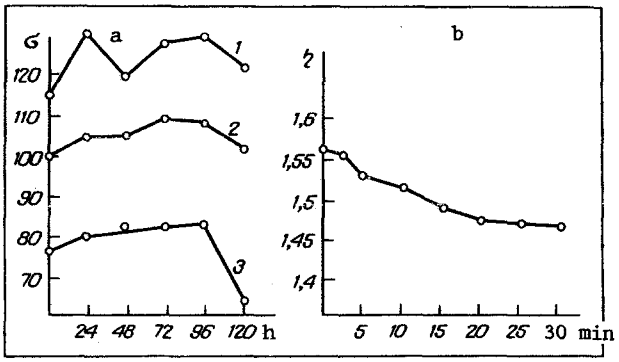

Fig. 2. Dependence of surface tension of bile on nucleation time (a) and viscosity of destabilized bile on duration of irradiation by HNL (b). 1) Destabilized bile before irradiation, 2) after irradiation, 3) stabilized bile.

As Fig. 1a shows, the process of sedimentation is initiated in destabilized bile after standing for 18-24 h, which leads to a sharp change in its pH. For healthy human bile before the settling process, there was little change in pH. After irradiation of destabilized bile by HNL (Fig. 1b) the pH fell from 7.8 to 7.4, and these changes depended directly on the duration of irradiation. HNL irradiation for 5 and 10 min was most effective.

The investigations into the effect of HNL radiation on pH thus indicate that the hydrogen concentration is corrected to some degree after HNL irradiation.

Changes in the surface tension of bile depending on nucleation time, before and after irradiation, and changes in the viscosity of destabilized bile depending on the duration of irradiation are given in Fig. 2.

It will be clear from Fig. 2a that the surface tension for stable bile changes very little depending on its nucleation time, whereas for destabilized bile these changes were 1.5-2.0 times greater than the values for stable bile. A different picture was observed after irradiation. The surface tension of the destabilized bile fell from 118-120 to 100-103 erg $\cdot$ cm^2. This form of surface tension is evidence of the beneficial effect of laser radiation on the physicochemical properties of bile. Similar positive shifts were found in relation to viscosity of the destabilized bile (Fig. 2b). After irradiation by HNL the viscosity of the destabilized bile showed a moderate decrease, and these changes were observed after exposures of 6, 10, and 15 min. At longer intervals after HNL irradiation viscosity was unchanged and remained virtually constant. During HNL irradiation relative stabilization of the bile took place (Figs. 1 and 2). The value of pH for unirradiated destabilized bile, depending on the time it was allowed to stand, gradually decreased, to reach 7.3 after the 1st day. Changes in pH during the 1st day were accompanied by changes in the physical state of the bile, i.e., changes in its color, with the appearance of turbidity and floccules. Aggregation in the bile took place with a fall of pH, i.e., during sedimentation, uptake of bile cations took place, and this was probably the cause of the fall of pH. On the 2nd day the pH gradually rose, and later the process was repeated. A different picture was observed in irradiated bile. The value of pH changed only a little in the course of the 1st day, and only after standing for 48 h was the pH of the bile appreciably lowered, and its color changed; on the 3rd day the fall of pH coincided with floccule formation. These investigations show that definite stabilization of bile takes place during irradiation by HNL.

The study of the effect of HNL on the physicochemical state of the bile thus showed that under the influence of laser irradiation the hydrogen ion concentration is corrected, the surface tension lowered, and the nucleation time of the bile increased, evidence of its beneficial therapeutic effect. This result requires further study of the problem, with the aim of working out optimal parameters of laser radiation for this common disease of gallstones.

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CHANGES IN THE FATTY ACID COMPOSITION AND LIPID PEROXIDATION ACTIVITY OF LIVER MICROSOMAL MEMBRANES IN EXPERIMENTAL MYOCARDIAL INFARCTION

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The writers showed previously in acute myocardial infarction there is a long-lasting (for 3 weeks) decrease in hydroxylating activity and the quantitative content of enzymes of the liver mono-oxygenase system [5]. The mechanisms of inhibition of microsomal liver mono-oxygenation in myocardial infarction have not been studied.

The aim of this investigation was to study changes in the fatty-acid composition, hydrophobic properties, and activity of lipid peroxidation (LPO) of liver microsomal membranes in the course of acute coronary-occlusive myocardial infarction.

EXPERIMENTAL METHOD

Experiments were carried out on 100 male Wistar rats weighing 180-220 g. In the experiments of series I, after left-sided thoracotomy and under ether anesthesia the left descending coronary artery of the animals was ligated. In the experiments of series II thoracotomy was performed but without ligation of the coronary artery (mock operation). Intact rats, kept under standard animal house conditions, served as the control. The animals were decapitated, 1, 3, 7, 14, and 21 days after the operation and the microsomal fraction of the liver was isolated [1]. Microsomal lipids were extracted by the method in [8]. The concentration of fatty acids in the lipid fraction were determined by gas chromatography on the "Chrom-4" instrument (Czechoslovakia), as described by the writers previously [7]. The resistance of the microsomal membranes to injury in vitro in NADPH-dependent LPO reactions was studied on the 6th, 14th, and 21st days of myocardial infarction [7]. The original parameters and their values after 10 min of induction of NADPH-dependent LPO were estimated: the rate of malonic dialdehyde (MDA) formation [2] and the cytochrome *P*-450 concentration [11]. Changes in hydrophobicity of the microsomal membranes were studied with the aid of the fluorescent probe 1-anilinenaphthalene-8-sulfonate (1,8-ANS$^-$) at the above-mentioned periods of infarction. The binding constant (K_b) for 1,8-ANS$^-$ was calculated by the method of double reciprocals [3, 7]. The probe was prepared in 40 mM Tris-HCl buffer (pH 7.4). The membranes (concentration 0.5 mg/ml) were titrated with the probe in the concentration range 5-40 μM. Fluorescence of the bound 1,8-ANS$^-$ was recorded on an MPF-4 fluorescence spectrophotometer (Hitachi, Japan). The wavelength of excitation was 360 nm and of fluorescence 480 nm. Spectral constants of interaction of a type II substrate (aniline) with cytochrome *P*-450 also were studied [4]. The spectral dissociation constant (K_s) for aniline and the maximal binding of aniline with cytochrome *P*-450 (ΔA_{max}) were determined. The concentration of cytochrome *P*-450 and the differential spectra of binding of aniline with cytochrome were recorded on a Hitachi-356 spectrophotometer (Japan). The pro-

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