BIOPHYSICS AND BIOCHEMISTRY

Effect of *Lactobacillus casei 114001* Probiotic on Bioactivity of Rutin

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Male Wistar rats were maintained for 2 weeks on a semisynthetic ration with 0.4% rutin or on the same ration with 0.4% rutin and *Lactobacillus casei 114001* suspension in physiological saline in a dose of 2×10^9 CFU per rat. Addition of *Lactobacillus casei 114001* potentiated biological activity of rutin. Its antioxidant efficiency increased due to more pronounced increase in antioxidant capacity of the plasma, decrease in plasma content of LPO products, and more pronounced increase in reducing activity and antioxidant capacity of the cytosol of the liver and intestinal mucosa. The probiotic sharply increased the capacity of rutin to suppress procarcinogenic activity of bacterial β-glucuronidase.

Key Words: *probiotics; rutin; liver; intestine; intestinal microflora*

Flavonoids are the largest class of bioactive food components with well-studied antioxidant properties, which probably determine reduced risk of some pathologies, including cardiovascular diseases and cancer, in individuals regularly feeding fruit and vegetables.

Biological activity of flavonoids largely depends on their bioavailability, because in plant substrates they are present in the form of glycosides. Most flavonoids are absorbed in the small intestine; enzymes of the intestine (glycoside hydrolases) and intestinal microflora participate in deglycosylation of flavonoids, the key stage in their metabolism. Intestinal UDP-glucuronosyltransferases (UDP-GT) and sulfotransferases conjugate the released aglycones with the formation of glucuronides and sulfoconjugates, which then enter the circulation [6,16]. Enzymes of the intestinal microflora play an important role in the metabolism of flavonoids. Most alimentary polyphenols (up to 95%) undergo metabolic transformations in the large intestine under the effect of bacterial enzymes.

Quercetin and its most prevalent natural glycoside rutin (quercetin-3-O-β-D-glucose-α-L-rhamnose) are typical flavonoids. Some *in vitro* studies showed that rutin and its aglycone quercetin exhibit high antiradical and antioxidant activities and produce antioxidant and cytoprotective effects under conditions of oxidative stress [3,14]. Rutin is not absorbed in the small intestine and is deglycosylated under the effect of bacterial β-glucosidases and α-rhamnosidase in the large intestine. Of rutin metabolites, only quercetin conjugates (primarily glucuronides) and its methylated derivatives are present in rat plasma [6,12,13]. Enzymes of intestinal bacteria not only deglycosylate rutin, but also further metabolize it with the break of quercetin heterocycle and formation of phenol acids: 3,4-dihydroxyphenylacetic, hydroxyphenylacetic, and 3-methoxy-4-hydroxyphenylacetic acids [10,16]. High activity of β-glucuronidase of the intestinal microbiota creates the possibility for enterohepatic recycling of quercetin formed from glucuronides en-

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tering the intestinal lumen with the bile or secreted from the intestine [6].

Published data on antioxidant properties of rutin are scanty and contradictory; however, the presence of antioxidant activity in some quercetin glucuronides (*e.g.* quercetin-3-O-β-D-glucuronide) and phenol acids was reported by some authorities [10].

Here we studied the effect of *Lactobacillus casei 114001* (*L. casei*) probiotic strain on biological activity of rutin evaluated by its antioxidant effects and on activity of some enzymes of the intestine and intestinal microflora involved in rutin metabolism. Our previous *in vitro* and *in vivo* studies showed that *L. casei* exhibits antioxidant activity, produces some glycosyl hydrolases (*e.g.* β-glucosidase), and can modulate enzyme activity of intestinal micrtobiota [2].

MATERIALS AND METHODS

The study was performed on 3 groups of male Wistar rats (8 rats per group). Rutin was added to semisynthetic ration of groups 1 and 2 rats for 14 days (to 0.4%). During this period, rats of the control and experimental group 1 intragastrically received 0.5 ml physiological saline, while experimental group 2 rats received *L. casei* suspension in physiological saline (2×10^9 CFU per rat). Taking into account the data on rutin pharmacokinetics [12], the rats were feed last time 12 h before the end of the experiment.

We used rutin from Sichuan Xieli Pharmaceutical Co. Ltd and *L. casei 114001* strain provided by Danone Research Center (France).

Antioxidant activity of blood plasma was evaluated by 3 parameters using the following methods [1]: total antioxidant capacity (AOC) was evaluated in the Hb–H_2O_2–luminol system, reducing activity was estimated in the FRAP test system (Ferric Reducing Antioxidant Power), and total antioxidant status (TAS) was assayed using RANDOX TAS kits (Randox Laboratories). The letter method is based on inhibition of the formation of stable colored radical $ABTS^+$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) in a mixture of ABTS-metmyoglobin-H_2O_2 in the presence of antioxidants. Blood content of MDA was also measured.

Reducing activity in the system of FRAP and AOC was measured in the cytosol fraction (105,000*g*) of the liver and intestinal mucosa (a 15-16-cm fragment of small intestine from the pylorus). We also measured activity of antioxidant enzymes catalase, SOD, glutathione peroxidase, and glutathione transferase using 1-chloro-2,4-dinitrobenzene as the substrate and quinone reductase as described previously [1].

In the cytosol of the intestinal mucosa, activities of rutin metabolism enzymes β-glucosidase and β-glucuronidase were measured using substrates 4-nitrophenyl-β-D-glucopyranoside and 4-nitrophenyl-β-D-glucuronide, respectively [7]. Activity of UDP-GT in mucosa homogenates was measured using *p*-nitrophenol as the substrate [1].

For measuring enzyme activity of intestinal microflora, the content of the cecum was *in situ* mixed 1:15 (w/v) with 0.1 M K-phosphate buffer (pH 6.5), homogenized in a Potter–Elvehjem homogenizer with Teflon pestle for 120 sec at 1200 rpm, and centrifuged at 12,000 rpm for 15 min (4°C). Activities of β-glucosidase and β-glucuronidase were measured in the supernatants [7].

The data were processed by ANOVA dispersion analysis using Statgraphics software. The differences were significant at $p<0.05$.

RESULTS

Analysis of integral parameters of the antioxidant status of rats (Fig. 1) showed that addition of rutin to the ration (experimental group 1) does not affect reducing activity of blood plasma, but significantly increased TAS (by 10%) and AOC (by 28%) and decreased MDA accumulation (by 20%). Reducing activity of the cytosol from the liver and small intestinal mucosa considerably increased by 36 and 10%, respectively, and AOC increased by 68 and 21%, respectively. In rats of experimental group 2, changes in the parameters of the antioxidant status were more pronounced than in group 1 rats. For instance, AOC of blood plasma increased to 145% compared to the control, while plasma content of MDA decreased by 35%. Reducing activity of the cytosol from the liver and small intestine attained 163 and 117% of the control, respectively, while AOC attained 196 и 143%, respectively.

Rutin had no effect on catalase activity in the liver, slightly inhibited SOD, and significantly increased activity of glutathione peroxidase (by 12%), glutathione transferase (by 13%), and quinone reductase (by 2.7 times, Table 1). Combined administration with *L. casei* (experimental group 2) had no effect on rutin-induced changes in activity of antioxidant enzymes of rat liver. In the small intestinal mucosa of experimental group 1 rats, catalase and SOD activities were considerably reduced compared to the control (by 53 and 13%, respectively), glutathione peroxidase activity did not differ from the control, while glutathione transferase and quinone reductase activities surpassed the control by 31 and 25%, respectively (Table 2). In animals of experimental group 2, activities of intestinal catalase and SOD remained low, activity of glutathione peroxidase and glutathione transferase were within the control range, while quinone reductase activity surpassed the corresponding parameter in the control and experimental group 1 by 65 and 32%, respectively.

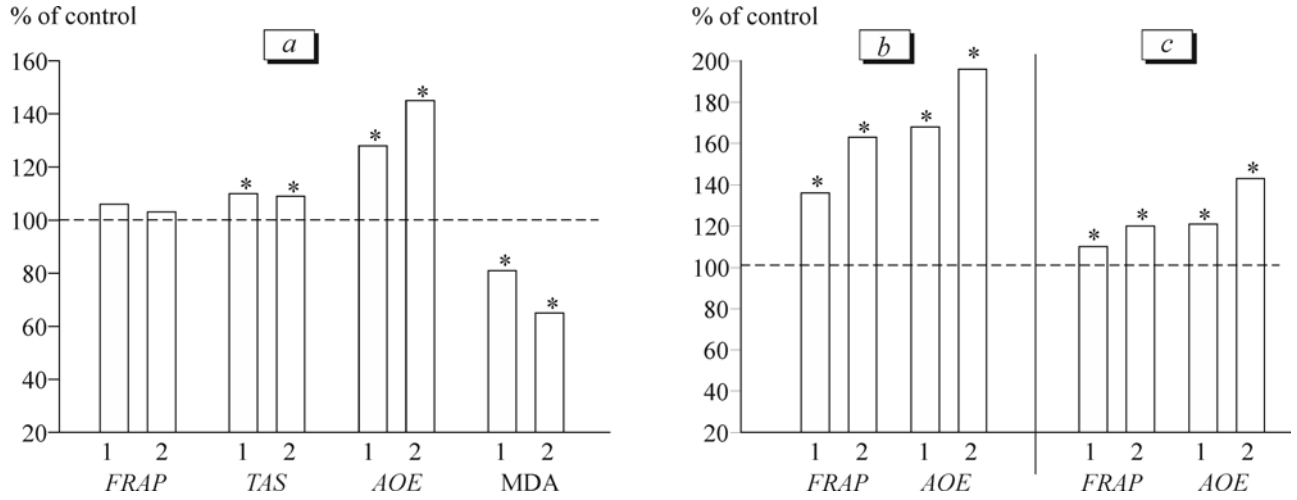

Fig. 1. Changes in the parameters of antioxidant status in the plasma (*a*), liver (*b*), and intestinal mucosa (*c*) of rats receiving rutin (*1*) or rutin with *L. casei* (*2*). Here and on Fig. 2: dashed line corresponds to the control. *$p<0.05$ compared to the control.

Evaluation of activity of enzymes involved in rutin metabolism showed that administration of rutin for 14 days (experimental group 1) had no effect on activity of intestinal β-glucosidase and β-glucuronidase, but elevated UDP-GT activity to 176% of the control (Fig. 2, *a*). Combined administration of rutin and *L. casei* (experimental group 2) led to considerable decrease in intestinal β-glucosidase activity (by 26%) and simultaneously 2-fold decreased UDP-GT activity compared to the control.

Activity of β-glucosidase of cecum microbiota did not appreciably differ from the control in rats of experimental group 1 receiving rutin alone, but decreased (by 20%) in rats receiving combined treatment with rutin and *L. casei* (experimental group 2, Fig 2, *b*). Activity of bacterial β-glucuronidase in animals receiving rutin alone and in combination with *L.casei* decreased by 42 and 83%, respectively.

Thus, addition of 0.4% rutin to ration considerably modulated parameters of the antioxidant status in rats. This manifested in a significant increase in total antioxidant status and AOC of blood plasma and a decrease in the content of LPO products. Reducing activity and AOC in the liver also considerably increased. Similar changes in these parameters were revealed in the small intestinal mucosa. Taking into account the fact that AOC of the liver and intestine is primarily determined by activity of antioxidant enzymes, we can hypothesize that the observed increase in liver AOC in rats receiving rutin is associated with the increase in glutathione peroxidase, glutathione transferase, and quinone reductase activities.

Published data suggest that rutin and its aglycone quercetin are potent antioxidants superior to vitamins C, E, and β-carotene in *in vitro* experiments [3,16]. At the same time, ambiguous effects of quercetin and rutin administered *per os* on parameters of antioxidant status should be noted. For instance, considerable increase in liver cytosol TAS was observed in rats receiving a ration with high rutin content (1%) [9].

TABLE 1. Activity of Antioxidant Defense Enzymes in the Liver of Rats Receiving Rutin (Experimental Group 1) or Rutin with *L. casei* (Experimental Group 2) (*M*±*m*)

Enzyme	Group		
	control	experimental group 1	experimental group 2
Catalase, mmol/min×mg protein	0.81±0.05	0.81±0.03	0.76±0.03
SOD, U/min×mg protein	223±6	204±6*	197±3*
Glutathione peroxidase, μmol/min×mg protein	0.34.±0.01	0.38±0.01*	0.40±0.02*
Glutathione transferase, μmol/min×mg protein	0.84±0.04	0.95±0.01*	0.95±0.03*
Quinone reductase, μmol/min×mg protein	0.13±0.01	0.35±0.07*	0.33±0.07*

Note. *$p<0.05$ compared to the control.

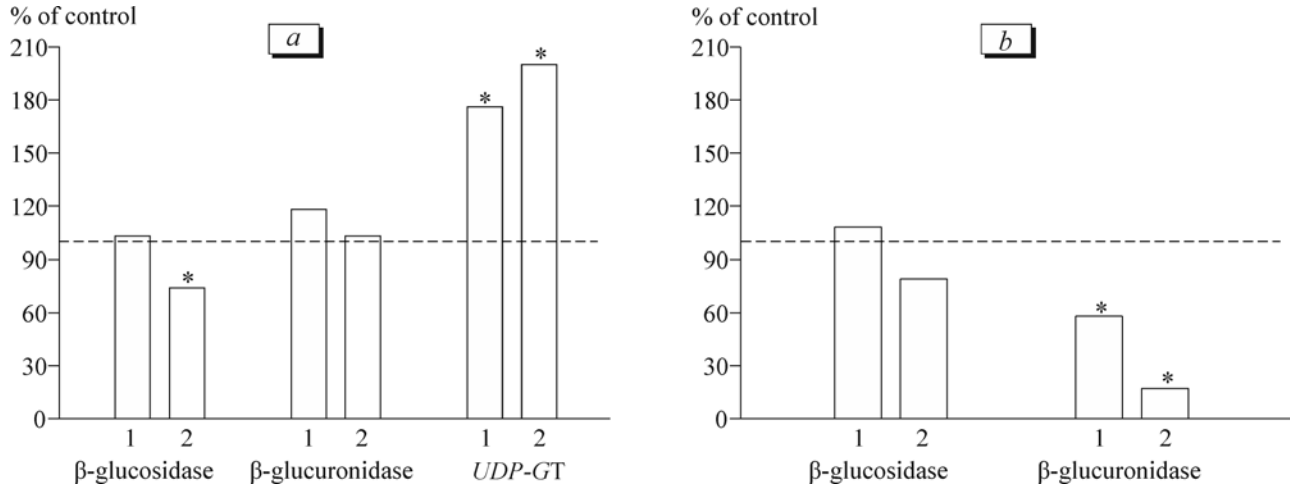

Fig. 2. Changes in activity of antioxidant enzymes of intestinal mucosa (*a*) and microbiota of the cecum (*b*) in rats receiving rutin (*1*) or rutin with *L. casei* (*2*).

However, no changes in AOC and reducing activity of the plasma were revealed in volunteers receiving 500 mg rutin per day for 6 weeks or various doses of quercetin (to 1000 mg) for 12 weeks, despite dose-dependent increase in blood quercetin content in all cases [4,15].

Inhibition of catalase, one of the major antioxidant enzymes, in the liver and especially in the intestine by rutin observed in our experiments was previously reported by other researchers. This is assumed to be related to a certain extent to chelating properties of rutin/quercetin. For instance, quercetin suppressed catalase activity in cultured HepG2 cells, despite increased expression of the corresponding gene [3]. Activity of catalase in the liver of rats receiving a ration with 1% rutin decreased 2-fold; this was accompanied by a trace element deficiency (Fe, Zn, Cu), which was supposed to be the cause of reduced activity of metal-containing enzymes catalase and SOD [9].

In our experiments, rutin treatment increased activities of glutathione peroxidase, glutathione transferase, and quinone reductase, *i.e.* enzymes with common pathways of synthesis regulation. Expression of genes for these enzymes is regulated by transcription factor Nrf2, and quercetin is a natural activator of Nrf2 [11]. Cell culture experiments showed that quercetin induces activity and expression of glutathione peroxidase, quinone reductase, some glutathione transferase isoforms, and other antioxidant enzymes, which was associated with translocation of Nrf2 into the nucleus [11].

In animals receiving combined treatment with rutin and *L. casei*, the antioxidant effects were more potent compared to rats receiving rutin alone. This primarily concerns the increase in AOC and content of LPO products in blood plasma and potentiation of reducing activity and AOC of the liver and intestinal cytosol and was not related to changes in enzyme activities. Our previous studies showed that *L. casei* in

TABLE 2. Activity of Antioxidant Defense Enzymes in the Intestinal Mucosa of Rats Receiving Rutin (Experimental Group 1) or Rutin with *L. casei* (Experimental Group 2) (*M*±*m*)

Enzyme	Group		
	control	experimental group 1	experimental group 2
Catalase, μmol/min×mg protein	3.86±0.34	1.81±0.25*	2.98±0.34
SOD, U/min×mg protein	13.05±0.41	11.29±0.39*	8.48±0.47+
Glutathione peroxidase, nmol/min×mg protein	25.9±0.8	27.4±1.2	27.1±1.2
Glutathione transferase, μmol/min×mg protein	0.140±0.008	0.183±0.020*	0.135±0.003
Quinone reductase, nmol/min×mg protein	78.9±4.7	98.7±7.1*	129.8±19.1*

Note. $p<0.05$ compared to: *control, +group 1.

various *in vitro* model systems and in experiments on rats exhibited pronounced antioxidant activity [1,2]. An increase in AOC and considerable accumulation of MDA in blood plasma and an increase in reducing activity and AOC in the liver and small intestine were noted. This suggests that the observed increase in antioxidant effects in rats receiving rutin and *L. casei* is a result of superposition of their antioxidant effects.

We found no published reports on the effects of rutin on activity of flavonoid metabolism enzymes. The effect of lactic acid bacteria on activity of intestinal enzymes are little studied, despite their modulating effects on qualitative and quantitative composition of intestinal microflora and its enzyme activity were demonstrated in numerous studies.

We also revealed no considerable effects of rutin on activities of intestinal β-glucosidase and β-glucuronidase, while activity of UDP-GT playing an important role in providing bioavailability of rutin/quercetin sharply increased. Combined treatment with rutin and *L. casei* potentiated the inducing effect of rutin on activity of intestinal UDP-GT. It can be hypothesized that the effect of rutin on activity of this enzyme is related to quercetin activation of transcription factors Nrf2 and AhR followed by enhanced expression of genes for some UDP-GT isoforms and other antioxidant enzymes of the small intestine [5].

Of particular importance is the inhibitory effect of rutin on activity of bacterial β-glucuronidase observed is our experiment and the fact that in rats receiving rutin with *L. casei* only 17% activity of this enzyme was found in microbiota compared to its control level. This agrees with the fact that highly active β-glucuronidase produced by intestinal microflora, along with azoreductase and nitroreductase, plays an important role in the development of colorectal cancer [8]. Bacterial β-glucuronidase hydrolyzes glucuronides of various endogenous and exogenous toxic compounds and plays an important role in their enterohepatic recycling and accumulation of carcinogenic substances in the large intestine. Some experiments demonstrated a direct correlation of β-glucuronidase activity of intestinal microbiota with the incidence of chemically induced tumors [8]

Our results suggest that *Lactobacillus casei 114001* probiotic strain potentiates biological activity of flavonoid rutin, its antioxidant efficiency, and the capacity to inhibit procarcinogenic activity of intestinal microflora.

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