A SUSTAINED INCREASE OF MICROSOMAL HEME OXYGENASE ACTIVITY FOLLOWING TREATMENT OF RATS WITH BACILLUS CALMETTE-GUERIN AND *CORYNEBACTERIUM PARVUM*: ITS POSSIBLE RELATION TO THE DECREASE OF CYTOCHROME P-450 CONTENT

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(Received January 10, 1985)

The alterations of various enzymes responsible for drug metabolism and heme metabolism were examined in the liver of female rats treated with *Bacillus Calmette-Guerin* (BCG) and *Corynebacterium parvum* (CP). Hepatic drug metabolizing enzyme activities and microsomal cytochrome P-450 and b_5 content were significantly decreased for up to 15 and 10 d by a single *i.v.* administration of BCG and CP, respectively. In contrast, microsomal heme oxygenase activity was markedly increased after BCG and CP treatment and the increased enzyme activity was sustained in parallel with the decrease of drug metabolizing enzymes.

Both BCG and CP also caused a significant decrease of δ -aminolevulinic acid synthetase activity shortly after their administrations. The decreased enzyme activity returned to normal levels by 12 h after the treatment of rats with BCG and CP. In addition, hepatosplenomegaly was observed in BCG and CP treated rats. Dose related changes of these microsomal enzymes were seen following the administration of BCG and CP. Additionally, there were sex differences in the effects of BCG and CP on the alteration of microsomal enzymes, female rats being more sensitive than male rats. These results suggest that the decrease of cytochrome P-450 and b_5 content and drug metabolizing enzyme activities by BCG and CP could be related, at least in part, to the prolonged increase of heme oxygenase activity, that may lead to the increased breakdown of heme available for the synthesis of these hemoproteins.

Keywords — *bacillus calmette-guerin*; *Corynebacterium parvum*; heme oxygenase; cytochrome P-450; δ -aminolevulinic acid synthetase

INTRODUCTION

Bacillus Calmette-Guerin (BCG) and *Corynebacterium parvum* (CP), which are potent non-specific immunostimulants, have been used experimentally and clinically as immunotherapeutic agents with malignant diseases.^{1,2)} It has been shown that hepatic microsomal cytochrome P-450 content and its-linked drug metabolizing enzyme activities are depressed for a prolonged time in the rat by immunoadjuvants treatment such as BCG,³⁾ CP,⁴⁻⁶⁾ interferon,⁷⁾ interferon inducing agents^{8,9)} and bacterial cell walls com-

ponents.¹⁰⁾

Azhary *et al.*¹¹⁾ have recently shown that polyribonucleic acid and tilorone also produce the changes in regulatory enzymes involved in heme biosynthetic and degradative pathway in the liver, such as the decrease of δ -aminolevulinic acid (ALA) synthetase and the induction of heme oxygenase. Furthermore, they reported that turnover rates of heme moiety of cytochrome P-450 is faster in the polyribonucleic acid or tilorone treated rats than the control.¹²⁾ It is also shown that some immunopotentiators,

such as OK-432, produce the increase of heme oxygenase¹³⁾ and the decrease of drug metabolizing enzyme activities.¹⁴⁾ These results suggest that hepatic microsomal cytochrome P-450 content and its-linked drug metabolism could be affected by the changes of heme metabolizing enzyme activities.

Considering these facts, this investigation was designed to examine the effects of BCG and CP on hepatic ALA synthetase, heme oxygenase activities as well as drug metabolizing enzyme activities.

MATERIALS AND METHODS

Chemicals and Immunopotentiators — Nicotinamide adenine dinucleotide phosphate (NADPH), nicotinamide adenine dinucleotide (NADH), hemin and pyridoxal phosphate were obtained from Sigma Chemical Co., glucose 6-phosphate and glucose 6-phosphate dehydrogenase were obtained from Oriental Yeast Co. All other chemicals used were of reagent grade available commercially.

Dried BCG vaccine was obtained from Japan BCG Laboratory. CP (*Corynebacterium parvum* C-7 012484) was kindly donated by Dr. T. Arai, Department of Microbiology, Showa College of Pharmaceutical Sciences. CP was cultivated in Brain Heart Infusion (DIFCO Laboratories) at 37 °C for 2 d by the method of Saino *et al.*¹⁵⁾ The cells were collected by centrifugation at 8000 rpm for 20 min. After washing twice with 0.9% NaCl solution, the cells were suspended in the 0.9% NaCl solution and then killed by heating at 70 °C for 30 min. After washing with 0.9% NaCl solution, the cells were collected by centrifugation at 8000 rpm for 20 min and lyophilized.

Animals and Treatment — Male and female Sprague-Dawley rats, weighing 150–160 g at the beginning of the experiments, were used in this study. They were fed a commercial diet *ad libitum*. BCG and CP were suspended in sterile 0.9% NaCl solution and administered to experimental rats through the tail vein at various doses in a volume of 0.2 ml. Control rats of BCG ex-

periments were always injected with a solution of 15% sodium glutamate in 0.9% NaCl solution, corresponding to the amount of the glutamate contained in the BCG vaccine.

Tissue Preparation — The rats were sacrificed by decapitation and their livers were removed. The removed livers were weighed immediately and were perfused with 0.9% NaCl solution. For the assay of ALA synthetase, a portion of non-perfused liver was homogenized in 3 vols. of 10 mM Tris-HCl buffer (pH 7.4) containing 0.9% NaCl and 0.5 mM ethylenediaminetetraacetic acid (EDTA). The remaining perfused liver was homogenized with 9 vols. of 0.25 M sucrose. The homogenate was centrifuged at 9000 *g* for 15 min and the resulting supernatant was centrifuged at 105000 *g* for 1 h. The pellet was suspended in 0.1 M phosphate buffer (pH 7.4) and used as enzyme sources for the determination of heme oxygenase, cytochrome P-450, cytochrome b₅ and drug metabolizing enzyme activities.

Enzyme Assays — Heme oxygenase activity was assayed by the method of Tenhunen *et al.*¹⁶⁾ by determining the formation of bilirubin in the presence of cytosolic biliverdin reductase with and without the addition of NADPH. ALA synthetase activity was assayed by the method of Marver *et al.*¹⁷⁾ using a total liver homogenate as enzyme source. The ALA produced was determined colorimetrically after condensation with acetylacetone and isolation of the pyrrole compound formed on the Dowex-1-acetate column.¹⁸⁾ Cytochrome P-450 and b₅ contents were determined by the method of Omura and Sato.¹⁹⁾ Aminopyrine demethylase activity was measured²⁰⁾ by determining the formaldehyde released according to the method of Nash.²¹⁾ Aniline hydroxylase activity was determined by the method of Imai *et al.*²²⁾ Protein content was determined by the method of Lowry *et al.*²³⁾ using bovine serum albumin as the standard.

RESULTS

Effects of BCG and CP on Liver Weight, Spleen Weight and Protein Content of Hepatic Microsomes

of Female Rats

As shown in Table I, administration of BCG (40 mg/kg) and CP (30 mg/m², 4.9 mg/kg) to female rats resulted in increase of liver and spleen weight. Liver weights of BCG and CP treated rats reached maximum of 117 and 114% of the control at 6 d after the treatments, respectively. Spleen weights of BCG and CP treated rats were significantly increased and reached maximum of 206 and 197% of the control at 10 d after the treatments, respectively. Levels of hepatic microsomal protein of BCG and CP treated rats were not significantly different from the control throughout the experimental periods.

*Time Course of the Effect of BCG on Microsomal Drug Metabolizing Enzyme Systems and Heme**metabolizing Enzymes*

Fig. 1 shows the time course of the effect of a single administration of BCG to female rats at a dose of 40 mg/kg ($6-7 \times 10^8$ organisms/m²) *i.v.* on hepatic microsomal aminopyrine demethylase and aniline hydroxylase activities and cytochrome P-450 and b₅ contents. The BCG dosage approximates that administered to rats by Farquhar *et al.*³⁾ and to man by scarification in the immunotherapy of malignant melanoma.²⁴⁾ Aminopyrine demethylase and aniline hydroxylase activities expressed in mg microsomal protein were significantly decreased shortly after the administration of BCG and reached minimum at 3 d. The depressed enzyme activities were sustained for 15 d after BCG administra-

TABLE I. *Effect of BCG and CP on Liver Weight, Spleen Weight and Protein Content of Hepatic Microsomes of Female Rats*

Treatment	Time (d)	Liver weight (g/100 g body weight)	Spleen weight (g/100 g body weight)	Hepatic microsomal protein (mg/g liver)
BCG ^{a)}				
Control	3	3.97 ± 0.13	0.317 ± 0.037	13.38 ± 1.04
Treated		4.26 ± 0.16	0.399 ± 0.061	13.49 ± 0.96
Control	6	3.86 ± 0.11	0.324 ± 0.013	13.69 ± 0.79
Treated		4.52 ± 0.18 ^{b)}	0.447 ± 0.025 ^{b)}	14.23 ± 1.08
Control	10	3.95 ± 0.15	0.350 ± 0.027	13.70 ± 1.66
Treated		4.39 ± 0.32	0.721 ± 0.091 ^{b)}	14.54 ± 1.25
Control	15	4.11 ± 0.14	0.374 ± 0.022	13.23 ± 1.20
Treated		4.21 ± 0.20	0.641 ± 0.097 ^{b)}	14.14 ± 1.73
CP ^{a)}				
Control	3	3.98 ± 0.18	0.295 ± 0.018	14.46 ± 1.26
Treated		4.11 ± 0.11	0.351 ± 0.032 ^{b)}	14.08 ± 1.37
Control	6	3.68 ± 0.07	0.283 ± 0.011	14.48 ± 0.62
Treated		4.19 ± 0.09 ^{b)}	0.361 ± 0.031 ^{b)}	14.29 ± 0.67
Control	10	4.02 ± 0.13	0.269 ± 0.020	14.83 ± 0.94
Treated		4.17 ± 0.16	0.528 ± 0.039 ^{b)}	15.49 ± 0.70
Control	15	3.99 ± 0.19	0.289 ± 0.041	13.20 ± 1.02
Treated		4.12 ± 0.18	0.449 ± 0.062 ^{b)}	13.60 ± 0.95

Each value is the mean ± S.E. of four rats. Average body weights of the control, BCG-treated and CP-treated rats on d 0 were 163 ± 6 g, 167 ± 4 g and 170 ± 4 g, respectively.

a) Female Sprague-Dawley rats were sacrificed at the time indicated after BCG (40 mg/kg) or CP (30 mg/m²) *i.v.* injection and determined liver weight, spleen weight and hepatic microsomal protein. b) Significantly different from the corresponding control, $p < 0.05$.

tion. Likewise, microsomal cytochrome P-450 and b_5 contents were decreased throughout the experimental period. When the enzyme activities and hemoprotein content were expressed in g wet weight of liver rather than mg microsomal protein, the extents of the decrease of these parameters were more profound. These results are well compatible to those reported by other investigators.³⁾

Under the same experimental conditions, hepatic heme oxygenase and ALA synthetase activities were also determined. As shown in Fig. 2,

heme oxygenase activity was significantly increased shortly after the administration of BCG and reached its maximum of 2.7-fold increase at 3 d. The increased heme oxygenase activity seems to be closely related to the decrease of microsomal cytochrome P-450 and b_5 contents and drug metabolizing enzyme activities throughout the experimental periods. On the other hand, mitochondrial ALA synthetase activity was decreased soon after BCG treatment and the decreased enzyme activity was sustained for up to 12 h, and thereafter the enzyme activity returned

FIG. 1. Time Course of the Effect of a Single Administration of BCG on Hepatic Microsomal Drug Metabolizing Enzyme Activities and Cytochrome Content

Female Sprague-Dawley rats were sacrificed at the time indicated after BCG at a dose of 40 mg/kg i.v. injection through the tail vein and determined aminopyrine demethylase, aniline hydroxylase, cytochrome P-450 and cytochrome b_5 . (○), enzyme activity in control rats ($n=5$); (●), enzyme activity in treated rats ($n=6$). A, enzyme activity expressed as microsomal protein; B, enzyme activity expressed as g wet weight of liver. a) Significantly ($p < 0.05$) different from the corresponding control.

to normal levels.

Time Course of the Effect of CP on Microsomal Drug Metabolizing Enzyme Systems and Heme Metabolizing Enzymes

Alterations of hepatic microsomal aminopyrine demethylase and aniline hydroxylase activities and cytochrome P-450 and b_5 contents were determined in the female rats by other immunoadjuvant, CP (30 mg/m², 4.9 mg/kg) *i.v.* treatment and the results are shown in Fig. 3.

The changed patterns of the microsomal hemoprotein contents and enzyme activities in CP-treated rats were almost similar to those of the BCG-treated rats. However, the duration of the depressing effect of CP on these hemoproteins and enzyme activities was rather shortened as compared to that of BCG. CP treatment also resulted in the increase of hepatic heme oxygenase activity. As shown in Fig. 4, heme oxygenase activity was increased rapidly after treatment of rats with CP and the increased enzyme

activity was sustained for 10 d. Additionally, CP showed a rapid and short-lived depressive effect on hepatic ALA synthetase, as shown in Fig. 4.

Dose Response of the Effects of BCG and CP on Microsomal Enzymes

Dose response of the effects of these two immunopotentiators on hepatic microsomal enzymes was examined, and the results are shown in Table II. Aminopyrine demethylase activity and cytochrome P-450 content tended to decrease and heme oxygenase activity tended to increase with the increasing doses of both BCG and CP in the female rats. An excellent inverse correlation also existed between heme oxygenase activity and cytochrome P-450 content (correlation coefficient, $r = -0.94$).

Sex Differences of the Effects of BCG and CP on Microsomal Enzymes

There is evidence that male and female rats respond differently to BCG and CP with respect to the decrease of drug metabolizing enzymes;

FIG. 2. Time Course of the Effect of a Single Administration of BCG on Hepatic Heme Oxygenase and ALA Synthetase Activities

Female Sprague-Dawley rats were sacrificed at the time indicated after BCG at a dose of 40 mg/kg *i.v.* injection through the tail vein and determined heme oxygenase and ALA synthetase. (○), enzyme activity in control rats ($n=5$); (●), enzyme activity in treated rats ($n=6$). Heme oxygenase activity was expressed as microsomal protein and g wet weight of liver. *a*) Significantly ($p < 0.05$) different from the corresponding control.

female rats being more sensitive than male rats.³⁾ Considering these facts, experiments were carried out to examine whether there are any differences in the effects of BCG and CP on heme oxygenase as well as drug metabolizing enzymes.

As shown in Table III, the extent of the decrease of cytochrome P-450 content and aminopyrine demethylase activity following either BCG or CP treatment were more pronounced in female rats as compared to male rats. The results well coincided with those of the previous findings.³⁾ In accordance with this fact, the increased

heme oxygenase activity after treatment with BCG or CP was only seen in female rats, but not or less extensive in male rats. The results indicate that both BCG and CP produce the decrease of cytochrome P-450 and aminopyrine demethylase activity accompanying with the increase of heme oxygenase activity.

DISCUSSION

The present investigation has confirmed that a single administration of BCG and CP to female rats resulted in the depression of hepatic

FIG. 3. Time Course of the Effect of a Single Administration of CP on Hepatic Microsomal Drug Metabolizing Enzyme Activities and Cytochrome Content

Female Sprague-Dawley rats were sacrificed at the time indicated after CP at a dose of 30 mg/m² i.v. injection through the tail vein and determined aminopyrine demethylase, aniline hydroxylase, cytochrome P-450 and cytochrome b₅. (○), enzyme activity in control rats (n=5); (●), enzyme activity in treated rats (n=6). A, enzyme activity expressed as microsomal protein; B, enzyme activity expressed as g wet weight of liver. a) Significantly (p < 0.05) different from the corresponding control.

microsomal cytochrome P-450 and b_5 content and drug metabolizing enzyme activities. The depression of microsomal enzyme activities in female rats sustained for more than 15 and 10 d in BCG-treated and CP-treated rats, respectively. Such a prolonged depression of microsomal enzyme activities observed after a single administration of BCG and CP to rats is a very unique phenomenon, usually not seen by the chemicals administration. The present results are well compatible to those reported by other investigators.³⁻⁶ Farquhar *et al.*³ have pointed out that the depression of the microsomal enzyme activities by BCG and CP is not simply due to the systemic toxicity of the agents and adjuvant-induced arthritogenic disease.

This study has also revealed that both BCG and CP produce a significant increase of heme oxygenase activity. Additionally, both BCG and CP temporary reduced hepatic ALA synthetase (Fig. 2, 4) activity. These facts indicate that both BCG and CP would perturb hepatic heme

metabolism.

It should be pointed out that there is an inverse relationship between the decrease of cytochrome P-450 content and the increase of heme oxygenase activity following BCG and CP treatment. Such as inverse relationship between the decrease of cytochrome P-450 content and the increase of heme oxygenase activity has been shown by the treatment of animals with several agents including heavy metals,²⁵ and postulated to be a possible mechanism for the decrease of cytochrome P-450. We have also found this relation between cytochrome P-450 and heme oxygenase in thioacetamide-treated rat liver,²⁶ in tumor-bearing rat liver²⁷ and in regenerating rat liver.²⁸ Some immunopotentiators¹³ and interferon inducers¹¹ have been also demonstrated to increase heme oxygenase in experimental animals. It has been shown that the turnover of cytochrome P-450 heme moiety is markedly enhanced under the experimental conditions where heme oxygenase activity is induced by

FIG. 4. Time Course of the Effect of a Single Administration of CP on Hepatic Heme Oxygenase and ALA Synthetase Activities

Female Sprague-Dawley rats were sacrificed at the time indicated after CP at a dose of 30 mg/m^2 i.v. injection through the tail vein and determined heme oxygenase and ALA synthetase. (○), enzyme activity in control rats ($n=5$); (●), enzyme activity in treated rats ($n=6$). Heme oxygenase activity was expressed as microsomal protein and g wet weight of liver. a) Significantly ($p < 0.05$) different from the corresponding control.

Cd²⁹⁾ or interferon-inducing agents.¹²⁾ Thus, such an increased breakdown of heme during the induction of heme could lead, if not all, to the decrease of cytochrome P-450 content, thereby decreasing heme concentration available for the formation of the hemoprotein. The extents of the increase of heme oxygenase and the decrease of cytochrome P-450 seen on the administrations of both BCG and CP to female rats were dose dependent. In addition, male rats did not respond to both BCG and CP with respect to the increase of heme oxygenase and the decrease of cytochrome P-450 content. The results also suggest that the increase of heme oxygenase ac-

tivity owing to the treatment with both BCG and CP could be related to the decrease of cytochrome P-450 content.

However, it can not be ruled out that both BCG and CP can directly affect on cytochrome P-450 synthesis.

In this respect, further detailed studies will be required.

Because of the importance of immunotherapy for malignant diseases, the present findings would provide further information on the effects of immunostimulators on hepatic heme metabolizing enzymes in relation to the decrease of cytochrome P-450 content and the related

TABLE II. *Dose Response of BCG and CP on Hepatic Microsomal Aminopyrine Demethylase, Cytochrome P-450 and Heme Oxygenase*

Treatment	Aminopyrine demethylase (nmol/mg protein/min)	Cytochrome P-450 (nmol/mg protein)	Heme oxygenase (nmol/mg protein/h)
BCG^{a)}			
Control	4.627 ± 0.191 (49.640 ± 0.908)	0.560 ± 0.010 (7.984 ± 0.132)	0.973 ± 0.127 (12.880 ± 1.049)
10 mg/kg	3.645 ± 0.309 ^{b)} (42.830 ± 5.562)	0.526 ± 0.020 ^{b)} (7.050 ± 0.433) ^{b)}	1.681 ± 0.205 ^{b)} (25.365 ± 3.141) ^{b)}
20 mg/kg	3.300 ± 0.230 ^{b)} (32.822 ± 4.293) ^{b)}	0.466 ± 0.043 ^{b)} (4.963 ± 0.433) ^{b)}	2.069 ± 0.264 ^{b)} (29.893 ± 4.540) ^{b)}
40 mg/kg	1.793 ± 0.114 ^{b)} (26.141 ± 8.160) ^{b)}	0.350 ± 0.036 ^{b)} (4.493 ± 0.030) ^{b)}	2.148 ± 0.306 ^{b)} (32.029 ± 5.299) ^{b)}
60 mg/kg	1.454 ± 0.136 ^{b)} (23.016 ± 2.458) ^{b)}	0.340 ± 0.008 ^{b)} (3.979 ± 0.669) ^{b)}	2.386 ± 0.359 ^{b)} (36.096 ± 0.495) ^{b)}
CP^{a)}			
Control	4.273 ± 0.006 (48.598 ± 0.099)	0.571 ± 0.011 (7.077 ± 0.083)	0.971 ± 0.008 (12.118 ± 1.163)
10 mg/m ²	3.543 ± 0.379 ^{b)} (44.728 ± 6.578)	0.518 ± 0.066 (6.861 ± 1.036)	1.356 ± 0.061 ^{b)} (17.383 ± 0.875) ^{b)}
20 mg/m ²	3.282 ± 0.059 ^{b)} (41.300 ± 1.255) ^{b)}	0.491 ± 0.022 ^{b)} (6.468 ± 0.244) ^{b)}	1.522 ± 0.026 ^{b)} (20.199 ± 0.718) ^{b)}
30 mg/m ²	2.911 ± 0.198 ^{b)} (35.827 ± 0.403) ^{b)}	0.384 ± 0.033 ^{b)} (5.082 ± 0.322) ^{b)}	2.145 ± 0.300 ^{b)} (25.179 ± 3.483) ^{b)}
40 mg/m ²	1.245 ± 0.123 ^{b)} (23.753 ± 1.367) ^{b)}	0.295 ± 0.025 ^{b)} (3.752 ± 0.274) ^{b)}	2.569 ± 0.167 ^{b)} (32.958 ± 2.002) ^{b)}

Each value is the mean ± S.E. of four rats. Figures in parentheses represent activity of g wet weight of liver.

a) Female Sprague-Dawley rats were injected i.v. at a various dose levels of BCG or CP and sacrificed at 3 d after the treatment. b) Significantly ($p < 0.05$) different from the corresponding control.

enzyme activities.

Because, decrease of cytochrome P-450 containing drug metabolising enzymes in BCG- and CP-treated animals could lead to a modification of metabolism of chemotherapeutic drugs concurrently administered and alteration of their pharmacological ability. Based on these findings, further detailed studies will be necessary to examine whether such an inverse relationship between the increase of heme oxygenase activity and the decrease of cytochrome P-450 content is a generalized phenomenon seen in non-specific immunostimulators administration.

Acknowledgement We would like to thank Dr. K. Koyama of National Cancer Center Research Institute for the kind service in providing a convenient supply of *Corynebacterium parvum*.

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TABLE III. *Differential Effect of BCG and CP on Hepatic Microsomal Aminopyrine Demethylase, Cytochrome P-450 and Heme Oxygenase Activity in Male and Female Rats*

Treatment	Time (d)	Aminopyrine demethylase (nmol/mg protein/min)	Cytochrome P-450 (nmol/mg protein)	Heme oxygenase (nmol/mg protein/h)
BCG^{a)}				
♂ Control	3	6.300 ± 0.204	0.821 ± 0.003	0.606 ± 0.031
♂ Treated		5.270 ± 0.085 ^{b)} (84)	0.733 ± 0.005 ^{b)} (89)	1.173 ± 0.077 ^{b)} (194)
♀ Control	3	4.719 ± 0.136	0.541 ± 0.003	0.704 ± 0.004
♀ Treated		2.632 ± 0.219 ^{b)} (56)	0.391 ± 0.001 ^{b)} (72)	2.291 ± 0.089 ^{b)} (325)
♂ Control	6	6.045 ± 0.056	0.852 ± 0.002	0.609 ± 0.042
♂ Treated		3.995 ± 0.259 ^{b)} (66)	0.651 ± 0.036 ^{b)} (76)	1.121 ± 0.029 ^{b)} (184)
♀ Control	6	4.668 ± 0.266	0.579 ± 0.003	0.731 ± 0.071
♀ Treated		2.236 ± 0.303 ^{b)} (48)	0.347 ± 0.014 ^{b)} (60)	2.089 ± 0.293 ^{b)} (286)
CP^{a)}				
♂ Control	3	6.518 ± 0.006	0.827 ± 0.025	0.675 ± 0.002
♂ Treated		5.945 ± 0.054 ^{b)} (91)	0.715 ± 0.142 ^{b)} (86)	0.922 ± 0.101 ^{b)} (137)
♀ Control	3	4.273 ± 0.020	0.609 ± 0.011	0.758 ± 0.017
♀ Treated		2.418 ± 0.034 ^{b)} (57)	0.427 ± 0.016 ^{b)} (70)	2.017 ± 0.200 ^{b)} (266)
♂ Control	6	6.373 ± 0.027	0.843 ± 0.003	0.871 ± 0.087
♂ Treated		5.855 ± 0.003 ^{b)} (92)	0.676 ± 0.001 ^{b)} (80)	1.183 ± 0.067 ^{b)} (136)
♀ Control	6	4.018 ± 0.029	0.647 ± 0.001	0.817 ± 0.008
♀ Treated		3.045 ± 0.034 ^{b)} (76)	0.504 ± 0.011 ^{b)} (78)	1.586 ± 0.158 ^{b)} (194)

Each value is the mean ± S.E. of four rats. Figures in parentheses represent difference (%).

a) Male and female Sprague-Dawley rats, weighing 156 ± 3 g, were injected i.v. at a dose of 40 mg/kg BCG or 30 mg/m² CP and sacrificed at 3 or 6 d after the treatment. b) Significantly ($p < 0.05$) different from the corresponding control.

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