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Influence of hypotensive drugs on lipopolysaccharide (LPS)-induced serum concentrations of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6 in spontaneously hypertensive rats (SHR)

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Abstract:
A growing body of evidence suggests that some drugs used in cardiovascular diseases may modulate the level of proinflammatory cytokines. Therefore, we have investigated whether propranolol, doxazosin, amlodipine and indapamide can influence lipopolysaccharide (LPS)-induced serum concentrations of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-6 in spontaneously hypertensive rats (SHR). The animals were divided into five groups as follows: SHR + MET (control rats receiving 1% solution of methylcellulose), SHR + PROP (rats receiving propranolol – 40 mg/kg), SHR + DOX (rats receiving doxazosin – 10 mg/kg), SHR + AML (rats receiving amlodipine – 25 mg/kg) and SHR + IND (rats receiving indapamide – 1.5 mg/kg). Solution of methylcellulose (1%) and hypotensive drugs were administered by a gavage once a day for 21 days. Arterial blood pressure was measured in conscious rats, using the tail-cuff method. Serum TNF-α, IL-1β and IL-6 concentrations were measured with enzyme-linked immunosorbent assay kits. Additionally, total cholesterol and high density lipoproteins (HDL) cholesterol were evaluated. Propranolol and amlodipine significantly decreased LPS-stimulated TNF-α level after 21 days of administration in SHR. Three-week administration of propranolol, amlodipine and indapamide lowered IL-1β serum concentration after LPS stimulation. Doxazosin caused a significant increase of the IL-6 serum concentration in SHR. The results were accompanied by a statistically significant decrease in systolic, diastolic and medium blood pressure after 21 days of administration for propranolol and amlodipine. Indapamide lowered diastolic and medium blood pressure while doxazosin only diastolic blood pressure after 21 days. Hypotensive drugs showed no effect on lipid level. The present data indicate that hypotensive drugs possess additional properties, beyond their most commonly known mechanism of action. The elucidation of interactions between hypotensive drugs and cytokines could be of great importance in cardiovascular diseases (e.g. atherosclerosis).

Key words:
propranolol, doxazosin, amlodipine, indapamide, proinflammatory cytokines, LPS, SHR

Abbreviations: CRP – C-reactive protein, IL – interleukin, LPS – lipopolysaccharide, SHR – spontaneously hypertensive rats, TNF-α – tumor necrosis factor alpha

Introduction

A growing body of evidence suggests that inflammation may participate in the pathogenesis of cardiovascular diseases such as atherosclerosis [24]. Hypertension may be also considered as a low-grade inflammatory disease but direct pathways linking inflammation and hypertension remain to be elucidated [28]. Observations have been made linking the presence of high blood pressure, or hypertension, with increased proinflammatory cytokine level in patients. Bautista et al. [2] found elevated levels of tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6 in apparently healthy patients while Chae et al. [4] suggested similar results for intercellular adhesion molecule-1 (ICAM-1) and IL-6. Dalekos et al. [6] recorded a slight correlation between IL-1β concentration and mean blood pressure in hypertensive patients. However, the scarcity of cytokine-related data in hypertension is confusing and clear evidence is still lacking. Proinflammatory cytokine-modulating properties of hypotensive drugs could be beneficial to hypertension complications (i.e. atherosclerosis).

Accordingly, in this study, we have made an attempt to investigate the effect of a β-blocker (propranolol), α_1-blocker (doxazosin), calcium channel blocker (amlodipine) and thiazide-related diuretic drug (indapamide) on lipopolysaccharide (LPS)-induced serum concentrations of TNF-α, IL-1β and IL-6 in spontaneously hypertensive rats (SHR). The sympathetic nervous system and catecholamines can play a role in modulating the immune system functions [10]. Therefore, propranolol and doxazosin could also exert an impact of proinflammatory cytokine level. Literature data show that calcium channel blockers also have a potential modulating influence on cytokine concentrations. There are scarce data concerning the potential of indapamide to modulate cytokine concentration.

Materials and Methods

Animals

The study was conducted on male, spontaneously hypertensive rats (SHR), 12–14 weeks old, with initial body weight between 240–290 g (at the start of the study), which had free access to standard food and water. Their body weight was monitored during the experiments. The animals were housed in standard plastic cages, 10 animals per cage, at a constant temperature of 22°C and under a 12 h light-dark cycle. All experiments were conducted between 8 a.m. and 4 p.m. The rats had been familiarized with the environment and the equipment for three weeks before the study. Preliminary examinations were carried out in the second and third week in order to select the animals. The rats with high blood pressure fluctuations were excluded from the study. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Local Ethics Committee for the Experiments on Animals (no L/BD/206).

The following preparations were used in the experiments: propranolol (Polfa, Warszawa, Poland), doxazosin (KRKA, Poland), amlodipine (Adamed, Poland), indapamide (Servier, France), methylcellulose (Sigma, USA), LPS from *Escherichia coli* serotype 055:B5 (Sigma, USA).

Experimental design

Rats were divided into 5 experimental groups as follows:

1) SHR + MET (control hypertensive rats receiving 1% solution of methylcellulose),
2) SHR + PROP (hypertensive rats receiving propranolol – 40 mg/kg),
3) SHR + DOX (hypertensive rats receiving doxazosin – 10 mg/kg),
4) SHR + AML (hypertensive rats receiving amlodipine – 25 mg/kg),
5) SHR + IND (hypertensive rats receiving indapamide – 1.5 mg/kg)

Control rats received 1% solution of methylcellulose (1 ml/kg) by a gavage as a vehicle (SHR + MET). Propranolol, doxazosin, amlodipine and indapamide were suspended in 1% solution of methylcellulose and administered every day by a gavage in a 1 ml/kg volume at doses of 40 mg/kg, 10 mg/kg, 25 mg/kg and 1.5 mg/kg per day, respectively. All compounds were administered for 21 days. Control arterial blood pressure measurement was carried out after the first, the second and the third week of drug administration (+ measures for selection criteria and before the treatment).

Twenty four hours after the last administration of hypotensive drugs or 1% solution of methylcellulose, the rats received a small, single dose of LPS (*ip*; 0.1 mg/kg in a 1 ml/kg volume of saline). After 2 h, the rats were anesthetized with ether and the blood samples were collected by heart puncture. The blood was allowed to clot overnight at 4°C before centrifuging for 20 min at 2000 × g. The serum was removed and stored at –20°C until the assays (cytokine and lipid level). Preliminary studies showed no detectable values of cytokines in serum of SHR. Small dose of LPS was administered in order to achieve a measurable cytokine level. The time of blood sample collection after LPS administration was chosen according to Dredge et al. [8].

Blood pressure determination

Arterial blood pressure was measured in conscious rats by a manometer manufactured by LETICA (Panlab S.L., Spain), using tail-cuff method. Before the measurements, the animals were placed inside a warming chamber (about 34°C) for 30 min. The aim of the procedure was to calm the animals and dilate the tail blood vessels. Arterial blood pressure was measured at least three times for each animal (values were based on the mean of several successive measurements). Changes in pressure were expressed as the percentage of baseline values.

Lipid profile determination

Total cholesterol levels were determined with the cholesterol oxidase method using a commercially available kit (Cholesterol CHOD PAP, Biolabo, Maizy, France).

HDL cholesterol was measured with the cholesterol kit after low density lipoproteins, very low density lipoproteins and chylomicrons from the samples had been precipitated by phosphotungutic acid and magnesium chloride (HDL-cholesterol – PTA, Biolabo, Maizy, France).

Serum cytokine levels

Serum TNF-α, IL-1β and IL-6 concentrations were measured in duplicate with a commercially available enzyme-linked immunosorbent assay kit (Quantikine, R&D Systems, USA) according to the manufacturer's instructions.

Statistical analysis

Results are expressed as the mean ± SD. The normality of distribution was checked by means of Kolmogorov-Smirnov test with Lilliefors test. The statistical evaluation was performed using analysis of variance (ANOVA) and *post-hoc* comparisons were performed by means of Least Significant Differences (LSD) test. If the data were not normally distributed, statistical evaluation was performed by using ANOVA (Kruskall-Wallis) and Mann-Whitney U test. Differences were considered significant when $p < 0.05$.

Results

Blood pressure

The SHR selected for the experiments had initial mean arterial blood pressure values as follows: systolic pressure 204.54 ± 14.93 mmHg, diastolic pressure 148.15 ± 12.82 mmHg, medium pressure 166.77 ± 11.35 mmHg. The SHR did not show changes in any values of pressure during administration of 1% solution of methylcellulose.

Propranolol at the dose of 40 mg/kg caused a significant decrease in systolic, diastolic and medium blood pressure in the third week of treatment in comparison with the control (SHR + MET). Doxazosin (10 mg/kg) administered for 21 days significantly decreased diastolic blood pressure. There was also a significant decrease in medium blood pressure in the second week of drug administration. Amlodipine at a dose of 25 mg/kg caused a significant decrease in systolic, diastolic and medium blood pressure in the third week of treatment in comparison with the control group. Indapamide at the dose of 1.5 mg/kg decreased the values of diastolic and medium blood pressure in the third week of administration in comparison with the control. The results are shown in Table 1.

Lipid profile

Propranolol, doxazosin, amlodipine and indapamide did not cause statistically significant changes in comparison with the control group in the examined lipid parameters: total cholesterol and HDL cholesterol. Mean total cholesterol and HDL cholesterol levels were as follows: SHR + MET – 63.38 ± 7.67 mg/dl,

Tab. 1. The influence of the repeated administration by a gavage of doxazosin (SHR + DOX), propranolol (SHR + PROP), amlodipine (SHR + AML) or indapamide (SHR + IND) on arterial blood pressure in SHR

Time after drug administration [week]	Mean changes in arterial blood pressure (% of initial values)														
	systolic					*diastolic*					*medium*				
	SHR + MET	SHR + DOX	SHR + PROP	SHR + AML	SHR + IND	SHR + MET	SHR + DOX	SHR + PROP	SHR + AML	SHR + IND	SHR + MET	SHR + DOX	SHR + PROP	SHR + AML	SHR + IND
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	97.13 ± 8.47	98.71 ± 8.01	98.51 ± 5.95	92.87 ± 6.07	97.04 ± 8.57	95.25 ± 12.44	88.08 ± 8.22	96.73 ± 8.20	88.86 ± 11.73	94.25 ± 13.64	95.76 ± 8.99	92.09 ± 6.55	96.57 ± 6.99	89.89 ± 7.48	95.34 ± 9.94
2	102.81 ± 13.96	94.22 ± 9.91	96.15 ± 8.65	98.89 ± 11.38	97.19 ± 8.32	101.06 ± 18.56	90.93 ± 12.19	96.06 ± 11.76	91.66 ± 11.42	93.36 ± 9.54	101.52 ± 14.76	91.99* ± 10.64	94.39 ± 9.34	93.89 ± 10.12	94.62 ± 6.53
3	105.45 ± 14.12	96.28 ± 14.78	91.93* ± 6.35	92.36* ± 9.58	96.68 ± 8.19	102.35 ± 19.49	88.82* ± 20.82	88.52* ± 9.14	85.57* ± 10.62	89.46* ± 13.19	103.86 ± 17.56	91.74 ± 18.59	89.63* ± 5.78	87.82* ± 8.64	92.12* ± 9.30

Each parameter is presented as the mean and standard deviation (SD). * $p < 0.05$ in comparison with SHR + MET (control group receiving 1% solution of methylcellulose)(n = 8–15)

29.43 ± 4.49 mg/dl; SHR + DOX – 58.7 ± 7.71 mg/dl, 30.38 ± 6.91 mg/dl; SHR + PROP – 65.34 ± 9.3 mg/dl, 30.41 ± 6.92 mg/dl; SHR + AML – 54.36 ± 9.28 mg/dl, 28.87 ± 10.57 mg/dl; SHR + IND – 62.33 ± 9.6 mg/dl, 34.84 ± 9.97 mg/dl, respectively.

Cytokine level

Changes in serum concentration of TNF-α after 21 days of drug administration are shown in Figure 1. Propranolol and amlodipine caused a statistically significant decrease, whereas doxazosin and indapamide did not significantly influence the cytokine level when compared to SHR control group. The following concentrations were found: SHR + MET (2895.74 ± 1194.81 pg/ml), SHR + DOX (2061.09 ± 947.723 pg/ml), SHR + PROP (459.41 ± 442.32 pg/ml), SHR + AML (248.60 ± 294.96 pg/ml), SHR + IND (3043.84 ± 1953.99 pg/ml).

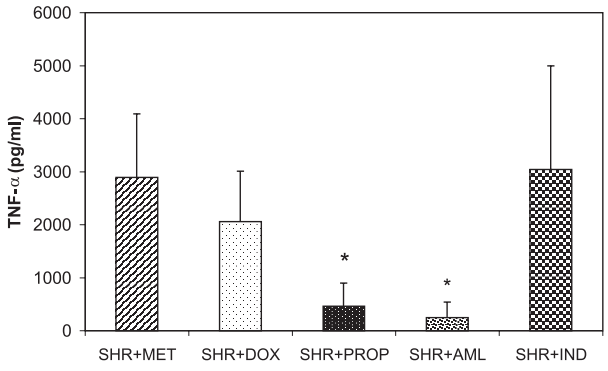

Fig. 1. The influence of the repeated administration (21 days) of doxazosin (SHR + DOX), propranolol (SHR + PROP), amlodipine (SHR + AML) or indapamide (SHR + IND) on serum concentration of TNF-α in comparison with the control group (SHR + MET) (n = 8–13). Each parameter is presented as the mean and standard deviation (SD). * $p < 0.05$ in comparison with SHR + MET

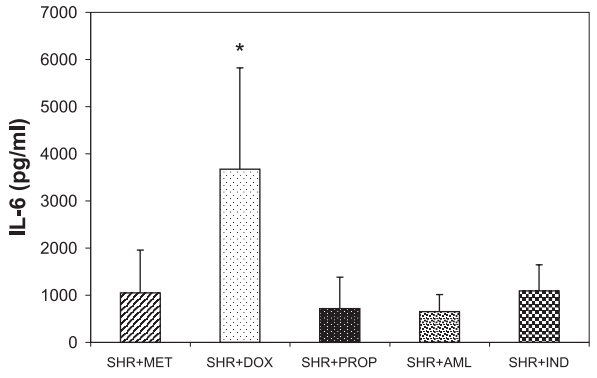

Fig. 2. The influence of the repeated administration (21 days) of doxazosin (SHR + DOX), propranolol (SHR + PROP), amlodipine (SHR + AML) or indapamide (SHR + IND) on serum concentration of IL-6 in comparison with the control group (SHR + MET) (n = 8–15). Each parameter is presented as the mean and standard deviation (SD). * $p < 0.05$ in comparison with SHR + MET

In the present study (Fig. 2), doxazosin caused a statistically significant increase in IL-6 concentration as compared to SHR control group (3675.10 ± 2145.99 pg/ml *vs.* 1048.56 ± 910.1 pg/ml). Three-week administration of propranolol, amlodipine and indapamide did not significantly influence IL-6 level (713.08 ± 671.13 pg/ml; 649.12 ± 362.47 pg/ml and 1090.86 ± 551.78 pg/ml, respectively) (Fig. 2).

In our experiments, we observed a decrease in IL-1β values in propranolol-, amlodipine- and indapamide-treated SHR in comparison with the control group (72.48 ± 60.68 pg/ml; 94.56 ± 72.57 pg/ml; 126.25 ± 118.50 *vs.* 264.60 ± 133.73 pg/ml, respectively). Doxazosin did not significantly change this proinflammatory cytokine concentration (241.95 ± 217.98 pg/ml) (Fig. 3).

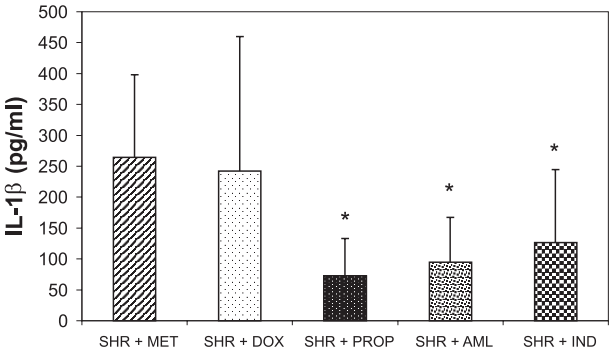

Fig. 3. The influence of the repeated administration (21 days) of doxazosin (SHR + DOX), propranolol (SHR + PROP), amlodipine (SHR + AML) or indapamide (SHR + IND) on serum concentration of IL-1β in comparison with the control group (SHR + MET) (n = 10–15). Each parameter is presented as the mean and standard deviation (SD). * $p < 0.05$ in comparison with SHR + MET

Discussion

The currently available literature data indicate the involvement of inflammatory processes, including the role of proinflammatory cytokines, in the pathogenesis of cardiovascular diseases, especially atherosclerosis [24]. Proinflammatory cytokines may also participate in cardiovascular disorders observed in obesity and metabolic syndrome [7, 37]. The reports concerning the increased TNF-α, IL-1β and IL-6 levels in subjects with elevated blood pressure or in patients with arterial hypertension also seem interesting [2, 4, 6]. However, the lack of unequivocal data in the literature results in problems associated with interpretations of the role of these inflammatory mediators in pathogenesis and/or progression of arterial hypertension. Research results indicate that drugs used in the treatment of cardiovascular diseases may modulate cytokine concentrations. We have decided to study the influence of several hypotensive drugs on LPS-stimulated concentration of proinflammatory cytokines in an animal model of essential hypertension (SHR). A recent study by Sun et al. [30] showed that SHR had a higher inflammatory status than Wistar-Kyoto rats (WKY) in different tissues. The authors concluded that inflammation might play a potential role in the pathogenesis of hypertension and secondary organ complications. Similar findings were reported by Sanz-Rosa et al. [27]. Hypertension was accompanied by increased IL-1β and IL-6, but not TNF-α plasma levels in SHR in comparison with normotensive Wistar-Kyoto rats. Elevated concentrations of proinflammatory cytokines found in hypertensive animals are also reflected by clinical observations. Jastrzębski et al. [15] demonstrated the increased levels of TNF-α and fibrinogen in hypertensive subjects with target organ damage.

In our studies, propranolol significantly decreased LPS-induced TNF-α concentration in SHR. The fact of functional relations between the autonomic nervous system and the immune system is well known [10]. The effect of activation or blocking of β-adrenergic receptors on immune system cells has also been demonstrated. Literature reports indicate various effects of cAMP, an increase in its level reduces TNF-α, or IL-2 concentrations, whereas, as a rule, it causes an increase in IL-5, IL-6 or IL-10 concentrations. Generally, no significant effect on IL-1 levels is observed [40]. Mastronardi et al. [19], in *in vivo* studies on rats, demonstrated that propranolol increased the plasma concentration of TNF-α three times and enhanced the reaction to LPS, whereas a β-agonist – isoproterenol lowered LPS-induced TNF-α secretion. The above data suggest the ability of the sympathetic nervous system to inhibit the release of this cytokine by stimulation of β-adrenergic receptors. This was also confirmed in other acute *in vitro* and *in vivo* studies with propranolol and β-adrenergic receptor agonists [21, 39]. Thus, the decrease in TNF-α concentration by propranolol observed in the present study is different from the results obtained by other authors. However, in our study the drug blocking β-adrenergic receptors

was administered for 3 weeks. It is conceivable that long-term administration of propranolol might have led to changes (an increase) in β-adrenergic receptor sensitivity. This would explain the different response of TNF-α concentration to stimulation with LPS. On the other hand, a change in proinflammatory cytokine concentration may be associated with improved hemodynamic conditions, because the systolic, diastolic and medium blood pressure was significantly lower after 21 days of treatment. In our study, we did not observe significant changes in IL-6 concentration after propranolol administration, whereas LPS-induced IL-1β concentration decreased. In most cases, stimulation of β-adrenergic receptors led to an increase in IL-6 concentration [40]. Reduction of proinflammatory cytokine concentrations by propranolol was observed locally in the myocardium. In a study of viral myocarditis in mice, Wang et al. [36] observed that propranolol suppressed TNF-α, IL-6 and IL-10 gene expression.

In the present study, we also investigated the effect of α1-adrenergic receptor blockade by doxazosin on LPS-induced secretion of proinflammatory cytokines. Doxazosin caused a significant increase in IL-6 concentration, did not influence IL-1β level, and insignificantly decreased concentration of TNF-α. The scientific literature published to date contains little information concerning the modulatory effect of doxazosin on the levels of proinflammatory cytokines. Only a few papers describe studies of the presence of α1-adrenergic receptors on immune system cells [16]. Some papers suggest that the stimulation of these receptors may be associated with activation of NF-κB and increased concentration of cytokines, e.g. TNF-α [3]. Fukuzawa et al. [13] demonstrated that doxazosin suppressed serum TNF-α activity in Balb/c mice after LPS stimulation (*iv*) at doses more than 10 times higher those used clinically. At the same time, no significant that changes in TNF-α, IL-6 and IL-1β were observed in *in vitro* studies of human peripheral blood mononuclear cells. The authors suggest that the effect of doxazosin may be associated with increased plasma noradrenaline levels observed in clinical studies. The decrease in TNF-α level observed in the present investigations was, however, statistically insignificant. The increase in IL-6 concentrations could potentially be related to „target change" in NA effect on β-adrenergic receptors, although this effect should be accompanied by a decrease in TNF-α concentration.

Studies in hypertensive patients with obesity, treated with doxazosin, revealed in the course of treatment a significant decrease in total cholesterol and insulin resistance, accompanied by a decrease in TNF-α and leptin levels [33].

In the present study, full hypotensive effect of doxazosin was not observed, which can be of importance for the modulating influence on proinflammatory cytokines. The treatment resulted in a significant decrease in diastolic blood pressure after 21 days and medium blood pressure after 14 days.

We decided to investigate also the effect of amlodipine at a dose of 25 mg/kg. In our previous study, amlodipine at a dose of 15 mg/kg decreased TNF-α level, increased IL-6 and did not influence IL-1β concentration despite the lack of hypotensive effect [1]. In this study, the changes in cytokine concentrations were accompanied by a marked hypotensive effect of the drug, and the decrease in systolic, diastolic and medium pressure after 21 days of treatment. Amlodipine significantly decreased LPS-induced TNF-α and IL-1β level in SHR. No significant effect of the drug on the concentration of IL-6 was observed, although there was a slight tendency towards a decrease in this cytokine level. Thus, the effect was opposite to that observed in our previous study for 15 mg/kg dose. Similar effects were described by Fukuzawa et al. [13] in mice, where amlodipine decreased TNF-α concentration after LPS stimulation. In an *in vivo* LPS (10 mg/kg, *iv*) stimulation experiment on Sprague-Dawley rats, Chou et al. [5] demonstrated that *iv* amlodipine (50 μg/kg) suppressed TNF-α and IL-1β levels. During *in vitro* studies, amlodipine dose-dependently attenuated production of these cytokines in LPS/IFN-γ-treated rat aortic smooth muscle cells. In a study of rats with induced myocardial ischemia, amlodipine significantly reduced TNF-α level in the myocardium [29]. The data obtained in animal experiments confirm the results of studies on human cells and clinical studies. The studies of human peripheral blood mononuclear cells demonstrated that amlodipine inhibited TNF-α secretion [17], as well as production of IL-1α, IL-1β and IL-6, induced by ouabain [20]. On the other hand, Fukuzawa et al. [13] observed an increased concentration of IL-1β, no changes in TNF-α and decreased IL-6 concentration after administration of amlodipine.

In obese hypertensive type 2 diabetic patients, amlodipine administered for 12 weeks at a dose of 5–10 mg significantly decreased TNF-α concentration in com-

parison with baseline values [11]. On the other hand, Salomon et al. [26] observed that TNF-α and IL-6 levels significantly decreased after 30-day treatment with amlodipine in patients with congestive heart failure (NYHA II and III). Amlodipine was added to their existing treatment schedules with angiotensin-converting enzyme inhibitors, supplemented, if necessary, with diuretics. The ability of amlodipine to decrease TNF-α concentrations may be associated, according to Fukuzawa et al. [13] with its properties of a free radical scavenger [18] and vascular prostacyclin production [22]. Some drugs may cause a decrease in TNF-α levels in this way [9]. On the other hand, the interaction between Ca^{2+} and TNF-α should also be remembered, as well as a decrease in production of that cytokine by calcium channel blockers [10].

The literature contains also contradicting reports concerning the induction of synthesis of some cytokines (including IL-6) by calcium channel blockers [23, 25, 35].

We included also indapamide in the present study. It caused a significant decrease in diastolic and medium arterial blood pressure in SHR. It did not affect significantly the concentrations of TNF-α and IL-6, whereas it lowered the concentration of IL-1β after LPS stimulation. These are the first data concerning the influence of this drug on proinflammatory cytokines in the experimental model used by us. Despite the reports indicating that indapamide exhibits free radical scavenging properties [31, 34], or influences the production of PGI2 [32], in our study we did not observe any modulation of TNF-α concentration. Other authors also observed small immunomodulating effect of this drug. Xie et al. [38] noted an insignificant, slight decrease in MCP-1, MIP-1α and sP-selectin levels in hypertensive patients treated with indapamide for 4 weeks.

The present study also intended to assess the effect of hypotensive drugs on total cholesterol concentration and HDL cholesterol. Increased levels of lipid fractions in SHR might affect the function of the immune system, including monocytes. Aortic tissue from rabbits fed with 0.3% and 0.9% cholesterol diets showed significantly enhanced LPS-evoked levels of TNF-α mRNA [12]. Similarly, in LDLr–/– (low density lipoprotein receptor-deficient) mice, maintained on high cholesterol diet, administration of LPS was associated with higher plasma levels of TNF-α in comparison with wild-type mice [14]. No significant effect of the tested hypotensive drugs on the investigated lipid parameters was found in the present study.

Conclusions

Propranolol, doxazosin, amlodipine and indapamide modulated the LPS-induced serum concentrations of TNF-α, IL-1β and IL-6 in SHR. Propranolol and amlodipine decreased TNF-α and IL-1β levels and did not influence IL-6. Doxazosin significantly increased IL-6 concentration without influencing the TNF-α and IL-1β in SHR. Indapamide caused a significant decrease in IL-1β level and did not change the TNF-α and IL-6 concentration. The cytokine-modulating properties of propranolol, amlodipine and indapamide may be beneficial to hypertension complications (i.e. atherosclerosis). The identification of additional properties of hypotensive drugs (influence on proinflammatory cytokines) may be important for better understanding of their mechanism of action.

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