Methylprednisolone inhibits endotoxin-induced depression of contractile function in human arteries in vitro

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Summary
We have studied the effect of methylprednisolone on endotoxin-induced depression of contractile function in human gastroduodenal arteries. Endotoxin diminished the contractile response to noradrenaline in both the presence and absence of endothelium. This attenuation began after 4 h and reached a maximum after 10 h of endotoxin exposure. The cGMP content of endotoxin-treated rings was approximately seven-fold higher than in control rings. These endotoxin-mediated responses were blocked by L-NAME and methylene blue. These data indicate that the main cause of vascular hyposensitivity to noradrenaline was massive generation of nitric oxide. Pretreatment with methylprednisolone at concentrations (2.0–20.0 μg ml⁻¹) similar to those achieved in plasma after therapeutic administration dose-dependently inhibited these endotoxin-mediated responses. These data support the concept that pharmacological administration of methylprednisolone has the potential to prevent endotoxin-induced depression of the contractile response to noradrenaline seen in endotoxaemic shock. (Br. J. Anaesth. 1996; 76: 251–257)

Key words

Prolonged exposure of arterial tissue to endotoxin or to any of several cytokines has been shown to suppress its normal contractile response to catecholamines [1]. Recent reports suggest that α adrenergic desensitization in inflammatory conditions is not mediated by changes in the number of receptors or their affinity for agonists, but may be caused by excessive production of endogenous nitric oxide (NO) [2–4]. There is strong evidence that inhibition of NO production may reverse the vascular hyporeactivity to pressor agents seen in patients with septic shock [5, 6]. NO is synthesized from the amino acid L-arginine through activation of NO synthase (NOS). It increases cellular levels of guanosine 3′, 5′-cyclic monophosphate (cGMP) which then induces vasorelaxation [7]. Two distinct forms of NOS have been identified in vascular tissues; these have been designated “constitutive” and “inducible” forms of NOS. Constitutive NOS (cNOS) is present in endothelium and contributes to vasodilatation under normal conditions [7]. The inducible form (iNOS) is expressed mainly in smooth muscle cells several hours after stimulation by a variety of stimuli, such as endotoxin or cytokines, and induces the production of a large amount of NO [8–10]. This raises the possibility that this enzyme may play a major role in vascular desensitization to pressor agents, leading to profound hypotension during inflammatory conditions. For this reason, pharmacological manipulation of iNOS might prove clinically useful in mitigating the abnormal vascular reactions in such conditions.

Expression of iNOS was found to be inhibited by the protein synthesis inhibitor cycloheximide [9]. Aminoguanidine, which is a nucleophilic hydrazine, was found to inhibit the relaxation of arterial rings from endotoxin-treated rats [11]. Moreover, iNOS requires tetrahydrobiopterin as a co-factor and inhibition of tetrahydrobiopterin synthesis was found to abolish endotoxin-induced NOS production in rat aortic smooth muscle cells [12]. However, cycloheximide has several severe side effects and other potentially useful drugs are as yet unlicensed and have still to undergo formal toxicological studies.

Glucocorticoids have also been shown to inhibit the expression of iNOS in several species [9, 13], indicating that the use of glucocorticoids may be useful in suppressing iNOS activity in humans. Although it has been suggested that glucocorticoids may have the capacity to prevent or suppress the development of cardiovascular collapse [14], not enough is known about the usefulness of glucocorticoids as potential inhibitors of the overproduction of NO evoked by endotoxin in human arteries.

In the present study, we investigated the effect of methylprednisolone, at concentrations within the range of serum concentrations reached during i.v. infusion of methylprednisolone 30 mg kg⁻¹ in adult humans, on endotoxin-induced attenuation of noradrenaline-induced contraction in human gastroduodenal arteries in vitro. In addition, we measured cGMP concentrations in the same vascular tissues as an index of NOS activity.

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Materials and methods

PREPARATION

With the approval of the Ethics Committee of Kagoshima University School of Medicine, the omentum was resected from the stomach of each of 42 patients immediately after gastrectomy had been performed under general anaesthesia; each omentum was stored in oxygenated Krebs solution. The patients, who were all less than 70 yr old (mean 65.8 (range 38–69) yr) and who had suffered from gastric cancer, had no vascular disease or other complications. Furthermore, none had taken any anti-inflammatory drugs, such as aspirin or steroids, or vasoactive drugs before operation. Thirty minutes or less after the omentum had been resected, the gastroepiploic arteries were isolated in a dissecting chamber filled with Krebs solution, and fat and connective tissue removed carefully under a binocular microscope. Two vascular rings of length 2 mm were prepared from each artery for tension recording. One ring was carefully denuded of endothelium by inserting a small forceps into the lumen and gently rolling the ring backwards and forwards in the dissecting chamber. The lack of a functional endothelium was confirmed by the absence of endothelium-dependent relaxation of noradrenaline-induced contractions normally induced by acetylcholine 1 × 10⁻⁵ mol litre⁻¹.

ORGAN CHAMBER EXPERIMENTS

Mechanical activity in the human gastroepiploic arterial rings was assessed using a strain gauge (UL-100GR, Minebea, Tokyo) with the ring in a tissue bath filled with Krebs solution bubbled continuously with 95 % oxygen: 5 % carbon dioxide. The bath had a volume of 1.0 ml and the temperature of the solution was maintained at 37 °C. Krebs solution was infused continuously at a rate of 1 ml min⁻¹ by a pump (Perista pump SJ-1211, ATTO, Tokyo) from one end of the bath and aspirated simultaneously by a water pump from the other. Resting tension set was at a value (20 mN) shown by the length–tension relationship to allow maximal active tension to be induced by noradrenaline 1.0 μmol litre⁻¹. The contractile response was measured as the maximum amplitude of the phasic response read from the pen recorder, and the response at the beginning of the experiment was normalized as a value of 1.0 for each ring. After 2 h of equilibrium in Krebs solution, noradrenaline 1.0 μmol litre⁻¹ was applied to the vascular rings for 7 min every 30 min. After the amplitude of the contraction induced by noradrenaline 1.0 μmol litre⁻¹ had become constant, endotoxin 10 μg ml⁻¹ (lipopolysaccharide) from Funakoshi (Tokyo, Japan). The chemicals used were: noradrenaline bitartrate, acetylcholine chloride, endotoxin (lipopolysaccharide: E. coli 055: B5), methylprednisolone, methylene blue, l-arginine and indomethacin, all form Sigma Chemical Company (St Louis, MO, USA), and N⁵-nitro-l-arginine methyl ester (l-NAME) from Funakoshi (Tokyo, Japan).

SOLUTIONS

The composition of the Krebs solution was as follows (mmol litre⁻¹): Na⁺ 137.4, K⁺ 5.9, Mg²⁺ 1.2, Ca²⁺ 2.6, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 134.4 and glucose 11.5. All solutions contained indomethacin 10 μmol litre⁻¹ to prevent the effects of prostanooids and l-arginine 200 μmol litre⁻¹ as a substrate for NOS. They were bubbled with 95 % oxygen: 5 % carbon dioxide which adjusted the pH to 7.4. As contamination with endotoxin may be assumed to be substantial even in solutions made using distilled water, we used sterilized glassware and endotoxin-free water obtained from Ostuka Chemical Co. (Tokyo).

STATISTICAL ANALYSIS

Results are expressed as mean (SD). The amplitude of the contraction induced by noradrenaline at the start of the experiment was normalized as a relative tension of 1.0 for each ring. Differences between the endotoxin-treated and methylprednisolone-treated groups were evaluated by analysis of variance. If significant differences were detected, Student’s unpaired t test was used. To evaluate the dose-

was infused continuously at a rate of 1 ml min⁻¹ every 30 min. After the amplitude of the agents (tissue by adding it to the Krebs solution. Other agents (N⁵-nitro-l-arginine-methyl ester (l-NAME), methylene blue) were infused in Krebs solution so that the tissue was exposed to the dose indicated in the text. To determine the effect of glucocorticoid on endotoxin-induced attenuation of the evoked contractions, methylprednisolone was added in Krebs solution at a concentration of 20.0, 2.0 or 0.2 μg ml⁻¹. The arterial rings were immersed in medium containing methylprednisolone just after being prepared and throughout the experiment. To study the effect of glucocorticoids on ongoing endotoxin-induced attenuation, methylprednisolone 20.0 μg ml⁻¹ was added 6 h after the start of some experiments, when attenuation induced by endotoxin had already begun.

ASSAY OF CGMP

After fat and connective tissue had been removed, gastroepiploic arterial rings (weight 30–50 mg) with the endothelium intact were allowed to equilibrate for 1 h in beakers containing Krebs solution bubbled with 95 % oxygen: 5 % carbon dioxide. One of the rings was then incubated with endotoxin 10 μg ml⁻¹ and the other with either endotoxin 10 μg ml⁻¹ with methylprednisolone 20 μg ml⁻¹, endotoxin 10 μg ml⁻¹ with l-NAME 1 mmol litre⁻¹ or endotoxin 10 μg ml⁻¹ with methylene blue 10 μmol litre⁻¹. Approximately 12 h later, the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX) 100 μmol litre⁻¹ was added to each preparation, 30 min before cGMP determination and then each ring was homogenized rapidly in a glass homogenizer. cGMP was extracted from the homogenate using trichloroacetic acid and the amount of cGMP in the extract measured using a cGMP enzyme immunoassay kit (Amersham International, Little Chalfont, UK).

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Results

EFFECT OF ACETYLCHELONE ON NORADRENALINE-INDUCED CONTRACTION

When a functional endothelium is present, but not when it is absent, noradrenaline-induced contractions can be relaxed by acetylcholine. In our rings with intact endothelium, acetylcholine $1 \times 10^{-5}$ mol litre$^{-1}$ relaxed the noradrenaline 1.0 $\mu$mol litre$^{-1}$-induced contraction to 58.8 (8.3)% ($P < 0.01$, Student’s unpaired $t$ test, $n = 42$; the amplitude of the noradrenaline 1.0 $\mu$mol litre$^{-1}$-induced maximal contraction being normalized as 100%). On the other hand, in our endothelium-denuded rings, acetylcholine-induced relaxation was not observed (95.0 (5.2)%,$n = 42$).

EFFECT OF ENDOTOXIN ON NORADRENALINE-INDUCED CONTRACTIONS

After 2 h equilibration of the tissues in Krebs solution, noradrenaline 1.0 $\mu$mol litre$^{-1}$ was applied for 7 min every 30 min. After the response to noradrenaline 1.0 $\mu$mol litre$^{-1}$ had become stable, endotoxin 10 $\mu$g ml$^{-1}$ was applied for 12 h. In the absence of endotoxin, the amplitude of contraction induced by noradrenaline 1.0 $\mu$mol litre$^{-1}$ in endothelium-intact rings remained stable for more than 12 h (after 12 h, 1.05 (0.17) times the initial maximal contraction, $n = 7$). Similarly, the amplitude of noradrenaline-induced contractions in endothelium-denuded rings was stable for 12 h (after 12 h, 1.16 (0.20), $n = 7$). Endotoxin 10 $\mu$g ml$^{-1}$ gradually attenuated the noradrenaline-induced contractions over a long time period, both in endothelium-denuded and endothelium-intact rings (fig. 1). Thus after 12 h treatment with endotoxin 10 $\mu$g ml$^{-1}$, the amplitude of contraction was 0.43 (0.07) in endothelium-intact rings ($n = 7$) and 0.53 (0.09) in endothelium-denuded rings ($n = 7$). Table 1 shows the effect of 12-h exposure to endotoxin on the contraction induced by noradrenaline 1.0 $\mu$mol litre$^{-1}$, and the effect of an inhibitor of NOS, $\text{L-NAME}$ 1.0 mmol litre$^{-1}$ and that of an inhibitor of guanylate cyclase, methylene blue 10 $\mu$mol litre$^{-1}$. Endotoxin-induced relaxation was abolished by administration each of these drugs.

dependency of the effect of methylprednisolone, regression analysis was conducted with replication and Spearman rank correlation coefficients. Probabilities less than 5% ($P < 0.05$) were considered significant.

Table 1  Attenuation of noradrenaline-induced contraction induced by 12-h exposure to endotoxin and its inhibition by $\text{L-NAME}$ or methylene blue (MB) (mean (SD)). The initial maximal contraction induced by noradrenaline is given the value 1.0. **$P < 0.01$ compared with value after 12 h of noradrenaline-induced contractions (Student’s t test)

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EFFECT OF METHYLPREDNISOLONE ON ENDOTOXIN-INDUCED ATTENTION OF CONTRACTION

Endotoxin-induced inhibition of noradrenaline-induced contraction was reduced or abolished by methylprednisolone in a dose-dependent manner. Figure 2 shows typical records of contractions induced by noradrenaline 1.0 μmol litre⁻¹ in endotoxin 10 μg ml⁻¹-treated rings and in rings treated with both endotoxin and methylprednisolone 20 μg ml⁻¹. After treatment with endotoxin had been in progress for 6 or 12 h, the noradrenaline-induced contraction was reduced significantly in both endothelium-intact and endothelium-denuded rings. This inhibition was prevented in both types of ring when methylprednisolone 20 μg ml⁻¹ was present in the medium throughout the experiment, the amplitude of contraction remaining constant for 12 h.

Figure 2. Typical examples of the effect of methylprednisolone 20 μg ml⁻¹ on endotoxin 10 μg ml⁻¹-induced attenuation of contraction in endothelium-intact and endothelium-denuded rings from human gastroepiploic arteries. Noradrenaline (NA) 1.0 μmol litre⁻¹ was applied to the vascular rings for 7 min every 30 min over a 12-h period. A: Effect of endotoxin 10 μg ml⁻¹ (begun at time 0) on contractions induced by NA 1.0 μmol litre⁻¹. Endotoxin-treatment was continued for 12 h and the L-NAME 1.0 mmol litre⁻¹ was applied. B: Effect of methylprednisolone 20 μg ml⁻¹ on the attenuation of contraction induced by endotoxin 10 μg ml⁻¹. Methylprednisolone was present throughout the 12-h incubation.
Effect of methylprednisolone on endotoxin-induced reaction in human artery

Changes in cGMP content of vascular tissues

We chose to measure the cGMP content of rings with intact endothelium as their status was probably closer to that of normal blood vessels. Endotoxin greatly increased the amount of cGMP in rings with intact endothelium cut from human gastroepiploic arteries. The measured amounts of cGMP were: before exposure to endotoxin, 223.2 (78.6) fmol/mg protein and after 12 h application of endotoxin, 1628.6 (790.0) fmol/mg protein (P < 0.05, n = 6). This endotoxin-induced increase in cGMP content was blocked by methylene blue 10 μmol litre\(^{-1}\) (cGMP content 52.9 (74.1) fmol/mg protein, P > 0.05, n = 6) and by L-NAME mmol litre\(^{-1}\) (cGMP content 256.3 (66.8) fmol/mg protein, P > 0.05, n = 6). The increase in cGMP content in endotoxin-treated rings was also prevented by continuous application of methylprednisolone 20 μg ml\(^{-1}\) (cGMP content 339.7 (197.5) fmol/mg protein, P > 0.05, n = 6) (fig. 4).

Discussion

These results demonstrate that pretreatment of isolated human vascular tissues with methylprednisolone inhibits, in a concentration-dependent manner, endotoxin-evoked depression of the contractile response to noradrenaline. This inhibitory activity of methylprednisolone was seen at concentrations as low as those achieved in plasma after therapeutic administration.

In the present study, contractions of arteries elicited by noradrenaline remained more or less constant throughout each experiment in the absence of endotoxin, suggesting that the anaesthetics and neuromuscular blocking drugs used did not alter vasoreactivity under the experimental conditions. On the other hand, in arterial rings exposed to endotoxin 10 μg ml\(^{-1}\), noradrenaline-induced contractions showed a gradual decrease in tension both in the presence and absence of endothelium for several hours after the initial exposure to endotoxin. These results indicate that noradrenaline-induced contraction, which occurs via stimulation of α-adrenergic receptors, was depressed easily by endotoxin. Moreover, this effect of endotoxin was...
reversed by the NO inhibitor, l-NAME, and by the guanylate cyclase inhibitor, methylene blue. Consequently, we conclude that endotoxin evoked massive production of NO, and thus diminished the contractile response to noradrenaline. A similar conclusion was reached after experiments on rat aortic rings incubated with endotoxin [15]. Indeed, it was demonstrated that the reduced sensitivity to catecholamines in endotoxaemic rats was caused by overproduction of endogenous NO [2, 3].

Endotoxin also increased the intracellular concentration of cGMP in our arterial tissues, the content of cGMP in rings treated with endotoxin for 12 h being about seven-fold greater than that in control rings. This effect of endotoxin was blocked also by both methylene blue and l-NAME. These studies with inhibitors indicate that the increase in cGMP evoked by endotoxin may result from activation of a soluble form of guanylate cyclase. Previous reports by other authors have also indicated that release of NO from vascular walls activates guanylate cyclase, producing cGMP [2–4]. The mechanisms by which cGMP evokes smooth muscle relaxation have been studied [16–18] and the conclusion reached that increased smooth muscle cGMP content is associated with a reduced intracellular calcium level. Thus, these results may explain the endotoxin-induced reduction in sensitivity to noradrenaline, as the response of vascular smooth muscle to α adrenergic agents is dependent mainly on the concentration of free Ca$^{2+}$ in the myoplasm [19].

The interaction of glucocorticoids with cardiovascular tissue has been shown to play an important role in cardiovascular function. It is clear that they potentiate the constrictor response of vascular smooth muscle to catecholamines after several days’ exposure, although there is still no consensus on the exact mechanisms involved [20–22]. Interestingly, recent data have indicated that glucocorticoids inhibit induction of iNOS in vascular tissue and reduced the sustained hypotension induced by endotoxin in animal experiments [23–25]. In our experiment, the glucocorticoid methylprednisolone, partially or completely prevented, in a dose-dependent manner, endotoxin-induced diminution of the contractile response to noradrenaline. Additionally, accumulation of cGMP in endotoxin-treated human arterial rings was blocked by the presence of methylprednisolone 20.0 μg ml$^{-1}$. These results suggest that methylprednisolone suppressed endotoxin-induced expression of iNOS and thus prevented endotoxin-induced depression of sensitivity to noradrenaline in human arteries.

These preventative effects of methylprednisolone in our in vitro study support the concept that the drug could be used as a therapeutic agent for reducing or preventing the vascular hyporeactivity mediated by endotoxin. The concentration of methylprednisolone in human plasma has been reported to reach a maximum of 20.0 μg ml$^{-1}$ just after i.v. administration of 30 mg kg$^{-1}$ in the adult human. There was a rapid decrease for approximately 12 h (12 h after injection, the concentration was about 2.0 μg ml$^{-1}$) and then a slower decline over the next 12 h [26]. In our experiments, methylprednisolone 20.0 μg ml$^{-1}$ completely prevented the attenuation of contraction induced by endotoxin and methylprednisolone 2.0 μg ml$^{-1}$ reduced it slightly but significantly, while 0.2 μg ml$^{-1}$ had no effect. The effective concentration of methylprednisolone used here correlates well with the serum concentrations found during the first 12 h after i.v. infusion of methylprednisolone 30 mg kg$^{-1}$ in the adult human. Therefore, i.v. infusion of 30 mg kg$^{-1}$ should have the potential to prevent or reduce the desensitization to catecholamines induced by endotoxin in human arteries.

However, in our study, when methylprednisolone 20 μg ml$^{-1}$ was given after the attenuation of contraction induced by endotoxin had started, it failed to elicit inhibition. A recent report by Marumo and colleagues [27] has suggested that, in rat cultured smooth muscle cells, glucocorticoids act by directly inhibiting expression of the iNOS gene, rather than by reducing its enzymatic activity (which persists for several days) [9]. These results suggest that when iNOS has been induced sufficiently by endotoxin, methylprednisolone might be unable to exert a preventative effect on the generation of NO, which would already have been raised by the increase in iNOS. Therefore, methylprednisolone might need to be given before, or shortly after, exposure to the bacterial pathogens that produce endotoxin.

Glucocorticoids have powerful immunosuppressive and anti-inflammatory actions and are pharmacologically useful in the treatment of various diseases. However, there is still considerable controversy on their use in the treatment of severe sepsis or septic shock [28, 29]. A recent report has warned of the possible adverse effects of methylprednisolone in the treatment of critically ill patients [30]. On the other hand, a massive dose of methylprednisolone 30 mg kg$^{-1}$ repeated once after 4 h if the patient remains unstable has been reported to reduce early mortality and circulatory failure in septic shock [28]. Our results support the concept of a beneficial effect of methylprednisolone on the vascular hyporeactivity associated with immunologically released NO in humans. Moreover, as recent data provide strong evidence of an important role for mediators such as cytokines or phagocytic cells in the pathogenesis of septic shock [31], the desirable immunosuppressive effects of glucocorticoids may include a direct cytotoxic action. Thus the efficacy of glucocorticoids in septic conditions may depend on the balance between their protective and deleterious roles.

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References


