Divergent Molecular Mechanisms Underlay CO- and CORM-2-Induced Relaxation of Corpora Cavernosa

Kelly Decaluwé, MSc, Bart Pauwels, MPharm, Charlotte Boydens, MSc, and Johan Van de Voorde, PhD

Department of Pharmacology, Ghent University, Ghent, Belgium

DOI: 10.1111/j.1743-6109.2012.02831.x

ABSTRACT

Introduction. Similar to nitric oxide (NO), the principal mediator of penile erection, carbon monoxide (CO) possesses vasodilator capacities. However, whether CO could be a therapeutic target for treating erectile dysfunction (ED) is unexplored. The danger associated with systemic administration of CO has led to the development of CO-releasing molecules (CORMs), releasing CO in a local, safe and controlled way. These CORMs have shown positive outcomes in cardiovascular studies. More knowledge on the (patho)physiological functions of CO in erectile function and the potential therapeutic role of CORMs is required.

Aim. The present study aims the assessment of the effect of CO and CO donor CORM-2 on the corporal tension and the underlying molecular mechanisms.

Methods. Organ bath studies were performed measuring isometric tension on isolated mice corpora cavernosa (CC) strips. Responses to CO (10–300 μmol/L) and CORM-2 (10–100 μmol/L) were measured in the presence/absence of activators/inhibitors of different molecular pathways.

Main Outcome Measures. CO and CORM-2 relax corporal strips concentration dependently, although the molecular mechanisms behind the corporal relaxation seem to differ completely.

Results. CO induces corporal relaxation by activating soluble guanylyl cyclase (sGC), increasing cyclic guanosine monophosphate (cGMP) concentrations. The molecular mechanism involved in CORM-2-induced corporal relaxation is not related to sGC activation and remains obscure.

Conclusions. Both CO and CORM-2 induce corporal relaxation, although the underlying molecular mechanisms show no resemblance. That CO induces corporal relaxation through a mechanism similar to that of NO could be of importance as it indirectly offers the possibility that endogenous CO might serve as a backup system for insufficient NO availability in cases of ED. Whether CORM-2 possesses the same capacity remains questionable and requires further research. **Decaluwé K, Pauwels B, Boydens C, and Van de Voorde J. Divergent molecular mechanisms underlay CO- and CORM-2-induced relaxation of corpora cavernosa. J Sex Med 2012;9:2284–2292.**

Key Words. Corpora Cavernosa; Carbon Monoxide; CO-Releasing Molecule-2; Targets to Pharmacologically Affect Penile Erectile Tissue

Introduction

At present erectile dysfunction (ED) is mainly treated using phosphodiesterase-5 inhibitors. However, a large percentage of patients is or becomes refractory to this treatment. Other therapeutic options are limited and less attractive as they are more invasive. This necessitates research for the development of new therapeutic targets [1,2].

An evolving potential new therapeutic target is the heme oxygenase/carbon monoxide (HO/CO) pathway [3]. Hedlund et al. detected immunoreactivity to both HO-1 and HO-2 in the endothelium of corpora cavernosa (CC) [4]. Research reports on CO in the field of erectile (dys)function are scarce. One study illustrated that HO-1 cDNA-liposome complex transfer augments cGMP concentrations in cavernosal tissue with subsequent sinusoidal relaxation. Moreover, HO-1 gene transfer

enhanced corporal relaxation in aged rats, suggesting that activating the HO/CO pathway may ameliorate the erectile function even in the elderly [5]. Furthermore, improved erectile function by the antioxidant α-tocopherol in hypertensive rats could be blocked with an HO-inhibitor, implying that HO-inducers may also ameliorate the erectile function in hypertensive rats [6]. Guo et al. illustrated that enhancing endogenous CO production using an HO-1 inducer had a relaxant effect on the cavernosal smooth muscle cells [7]. This observation further provides evidence that the HO/CO pathway possesses potential as a new therapeutic target for treating ED. This is further confirmed by the observation that high blood pressure and intracavernosal responses in spontaneous hypertensive rats are normalized after chronic administration of the HO-1 inducer hemin [8]. Finally, Abdel Aziz et al. reported that both the gene expression and enzymatic activity of HO-1 were strongly decreased in cavernosal tissue of diabetic rats, resulting in diminished erectile function. The use of an HO-1 inducer elevated expression and activity of HO-1 and significantly improved the erectile function of these rats [9]. This study suggested that the decline in erectile function in diabetic rats may be attributed to a downregulation of the HO/CO pathway and may indicate that stimulating this pathway is efficient to treat ED in diabetic patients. Despite these promising results, more research is required to assess the role of the HO/CO pathway in erectile (dys)function before clinical trials can be started targeting the HO/CO pathway.

Based on the increasing knowledge on the physiological importance of the HO/CO pathway [10], molecules have been developed releasing CO with controllable kinetics, the so-called CO-releasing molecules (CORMs) [11–14]. Development of these molecules has been a crucial step in pharmacological research of CO. One of these CORMs is CORM-2, which is being frequently used to evaluate the (patho)physiological properties of CO and its potential therapeutic applications [15].

Aim of the Study

The present study aims to evaluate relaxation responses induced by CO and CORM-2 in mice isolated CC and to compare the molecular mechanisms underlying the relaxation effects observed with CO and CORM-2.

Materials and Methods

Animals

Male mice (129SvEvS7) (10–14-week-old) were used. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Laboratory Animals published by the U.S. National Institute of Health (NIH Publication no. 85-23, revised 1996). The studies were approved by the local Ethical Committee for Animal Experiments, Faculty of Medicine and Health Sciences, Ghent University, Belgium. The animals were housed on a 12-hour light/dark cycle and fed a standard chow diet with water ad libitum. On the day of the experiment, the mice were sacrificed by cervical dislocation.

Preparation of Corporal Strips

Penile tissue was dissected free and the CC were separated from each other by cutting the fibrous septum between them and excised at the base. Following, CC were transferred into a petri dish containing ice-cold Krebs-Ringer bicarbonate (KRB) solution. Both corporal strips ($1 \times 1 \times 5$ mm) were then mounted horizontally for isometric tension recordings in 10 mL myograph chambers (made by own technical staff), containing KRB solution at 37°C (pH 7.4) and continuously gassed with a mixture of 95% O_2 – 5% CO_2. After 30 minutes resting time, the preparations were gradually stretched to a stable resting force of 0.45 g and allowed to equilibrate during 60 minutes. Changes in isometric force were recorded as one end was fixed to a force displacement transducer and the other to a micrometer. To verify the contractile activity of the preparations, CC were contracted three times with norepinephrine (NE) (5 μmol/L) at the end of the equilibration period and each time washed and allowed to relax to the basal tension. When reaching a stable resting tension, corporal strips were precontracted again with NE, and when the contraction was stabilized, the response to acetylcholine (1 μmol/L) was examined to evaluate the functionality of the endothelium within the corporal strips. Preparations that were not able to relax minimally 10% from the maximum tension were excluded from the study. Thereafter, the CC were once again washed. When reaching a stable resting tension the experimental protocols were started.

Experimental Protocols

Cumulative concentration–response curves to CO (10–300 μmol/L) and CORM-2 (10–100 μmol/L) were obtained in cavernosal strips contracted with