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contents

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Each article is accompagnied with an English summary

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INTRACAVERNOUS APPLICATION OF SIN-I IN RABBIT AND MAN : FUNCTIONAL AND TOXICOLOGICAL RESULTS

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MEYER M.F., TAHER A., KRAH H., STAUBESAND J., BECKER A.J., KIRCHER M., MAYER B., JONAS U., FORSSMANN W.G., STIEF Ch.G. – Intracavernous application of SIN-I in rabbit and man : functional and toxicological results.

Ann Urol, 1993, **27**, n° 3, 179-182.

SUMMARY : The mode of action of the active metabolite SIN-I of the vasodilator prodrug molsidomine was studied *in vitro* and *in vivo* in corpus cavernosum of rabbit and man. SIN-I produces a dose-dependent relaxation of isolated human cavernous smooth muscle strips. In the rabbit, the intracavernous application of SIN-I increased the intracavernous pressure to a full erection (approximately 100 cm H_2O). This response wa shighly reproductible. SIN-I was also injected intracavernously 6 times in five rabbits over 2 weeks ; no inflammatory or fibrotic reactions were found on histology. SIN-I may be a reliable drug for the treatment of impotence without side-effects.

KEY-WORDS : SIN-I. – Relaxation of cavernous smooth muscle strips. – No inflammation and fibrosis.

MEYER M.F., TAHER A., KRAH H., STAUBESAND J., BECKER A.J., KIRCHER M., MAYER B., JONAS U., FORSSMANN W.G., STIEF Ch.G. – Injection intra-caverneuse de SIN-I chez le lapin et l'homme : résultats fonctionnels et toxicologiques. *(En Anglais).*
Ann Urol, 1993, **27**, n° 3, 179-182.

RÉSUMÉ : L'action du métabolite actif SIN-I du précurseur du vasodilatateur molsidomine a été étudiée *in vitro* et *in vivo* sur le corps caverneux du lapin et de l'homme. Le SIN-I cause une relaxation, dépendante de la dose, de bandelettes de corps caverneux humain isolées. Chez le lapin, l'injection intra-caverneuse de SIN-I augmente la pression intra-caverneuse jusqu'au niveau d'une érection complète (environ 100 cm H_2O). Cet effet est reproductible à un fort pourcentage. D'autre part, on a procédé à des injections intra-caverneuses de SIN-I 6 fois en deux semaines chez le lapin ; aucune réaction inflammatoire ou fibreuse n'a été constatée à l'examen histologique. Le SIN-I pourrait constituer un traitement de l'impuissance virile sans effets secondaires.

MOTS-CLÉS : SIN-I. – Relaxation de fibres musculaires lisses caverneuses. – Absence d'inflammation et de fibrose.

INTRODUCTION

To induce an erection, the penile arteries and sinusoids have to dilatate, thereby decreasing the resistance to penile blood flow [1]. However, the mechanism of penile smooth muscle relaxation has not been fully elucidated yet. Nitric oxide (NO), which is believed to accound for the biological actions of endothelium-derived relaxing factor [2, 3] was recently suggested to be of importance in the regulation of penile smooth muscle tone, both in the flaccid state [4] and during erection [2, 4]. SIN-I causes its effects by « non-enzymatical liberation of NO » [5].

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In view of the observations that NO is involved in mediating penile erection and that certain nitrovasodilators have been employed clinically to induce erection in impotent patients, the present study was initiated to assess the dose-dependent relation between the intracavernously applied SIN-I dose and the evoked penile erection in the rabbit model. As it was previously described the chronic application of the non-specific phosphodiesterase-inhibitor papaverine causes fibrosis and inflammation [6]. Therefore the present study was designed to evaluate whether the chronic short-term intracavernous application of 0,1 mg SIN-I (6x in 14 days) in the corpus cavernosum of the rabbit causes undesired side effects of any degeneration of penile tissue. Furthermore, *i vitro* and *in vivo* studies of the effect of SIN-I on cavernous smooth muscle relaxation were done.

MATERIALS AND METHODS

Organ bath experiments

Corpus cavernosum excised from human penis during penectomy for penile carcinoma or penile prosthesis implantations is dissected into small strips of 0,3 × 0,7 cm. The specimen are mounted under 0.5 gm, tension in organ chambers containing Krebs – bicarbonate solution at 37 °C gassed with 95 % O_2 5 % CO_2. Isometric contractions are measured using Statham transducers connected to Linseis polygraphs. The preparations are allowed to equilibrate for 60-90 min. After this period, norepinephrine or endothelin 10^{-6} are added to contract the strips ; after a stable tension is reached, SIN-1 is added in doses between 10^{-9}-10^{-4} M.

Intracavernous application of SIN 1

To evaluate the effect of SIN-1 *in vivo,* the rabbit model was chosen ; we did not want to present human data since a patients response to any intracavernously applied drug is modified by his etiology of the erectile dysfunction he is suffering from.

After sedation with i.m. ketamine (10 mg), 9 animals (3.5 to 5 kg BW) were anaesthetized with i.v. pentobarbital (15 mg kg^{-1}) through a needle placed in an ear vein. The rabbits (New Zeeland white rabbits) are placed in a supine position on a thermoregulated operating table (model 11A, Hugo Sachs Elektronik, Germany. Additional heat is provided with a heating lamp. The penile skin is removed by blunt dissection and a 21-gauge needle is inserted into the left corpus cavernosum for pressure recording. The needle is connected to a fluid line via a threeway stopcock which allows for the intracavernosal application of SIN 1. To prevent clotting, 50 I.U. heparin is given through this route every 2-3 hours. This dose of heparin is well below the dosis needed to induce changes in penile hemodynamics [7]. After the pressure returns to baseline, 1 ml saline is given intracavernosally in order to flush the drug away and to avoid clotting.

To study the effects of SIN-I the drug is administered intracavernously at dosages of 0,01, 0,02, 0,05 and 0,1 mg through the 21-gauge needle until in every animal a dose - response - curve is achieved.

Histopathological study

SIN-I of 0,1 mg is injected intracavernously 6 times in 5 rabbits in between 14 days. Then the rabbits are sacrified. A long seghment (3 cm) of the penis including the injected site is collected for light-microscopic examination, fixed in 10 % neutral formalin solution and stained with HE and Masson - trichrome. For immunohistofluorescence the penile tissue is fixed by immersion in 4 % formaldehyde at 4 °C overnight. Five minutes sections are cut on a Reichert-Jung microtome and incubated with antisera diluted 1 : 250 (NO-synthetase-antibody against neuronal NO-synthase, no staining of endothelial NO-synthase ; raised in rabbit). Bound antiserum is visualized with fluorescin-conjugated pig anti-rabbit IgG diluted 1 : 40.

Drugs

The following drugs are used : ketamine, pentobarbital, heparin, SIN-I, a gift from Dr Heenning and Dr Grewe, Cassella, Germany) NO-synthetase antiserum (Dr Meyer, Universität Graz), fluorescin conjugated pig anti-rabbit IgG.

RESULTS

Organ bath study

Concentration response relationships of the effect of SIN-I on isolated human cavernous tissue are depicted in figure 1. The dose-response-curves for the relaxing effect of SIN-I on isolated human cavernous tissue precontracted with norepinephrine and as well as the effect of papaverin, a non-specific phosphodiesterase-inhibitor show that concentrations of 10^{-9} and 10^{-7} M SIN-I causes dose-dependent relaxations which are close to their maximum at 10^{-6} M. Papaverine showed the most potent effect at concentrations of 10^{-5} M and 10^{-4} M in a very small therapeutical width, shown by the step increase of the dose effect curve.

Intracavernous application of SIN-I

The intracavernous application of SIN-I induces a dosage - dépendent erectile response. A injection of 0,02 mg SIN-I evoked a full penile erection for 10 minutes. 0,05 mg induced a full erection for 16 minutes whereas the intracavernos application of 0,1 mg leads to a long-lasting response of full penile erection and persisted for about 84 minutes with severe side – effects concerning the systemic blood pressure (the blood pressure drops for about 45-50 cm H_2O in the first 4 minutes).

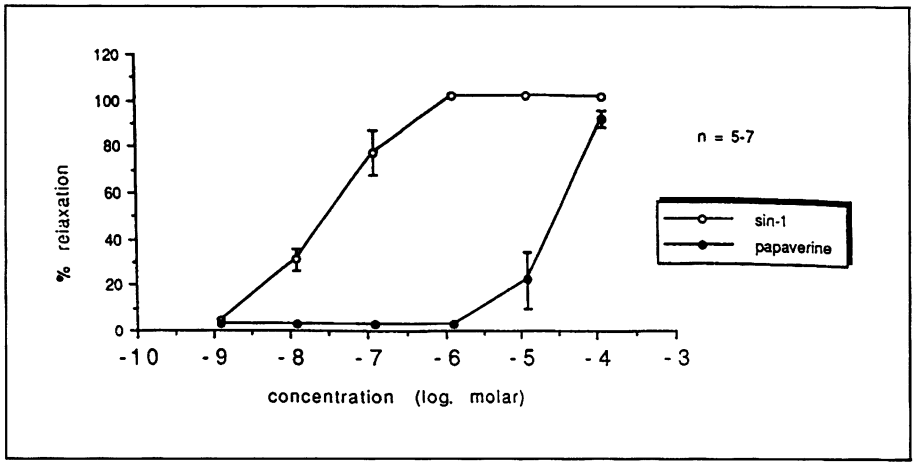
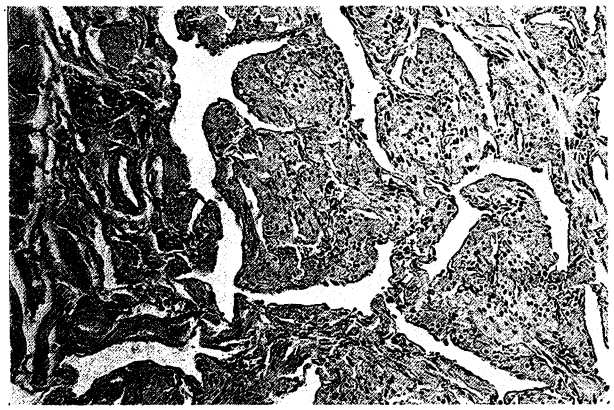


Fig. 1. — Cavernous tissue of the SIN-I treated rabbits. A u. B) Massen-Goldner (× 400, × 100); FITC-method with NO-synthase-antibody in man.

Fig. 1. — Tissu caverneux des lapins traités par SIN-I A- × 400 ; B × 100.



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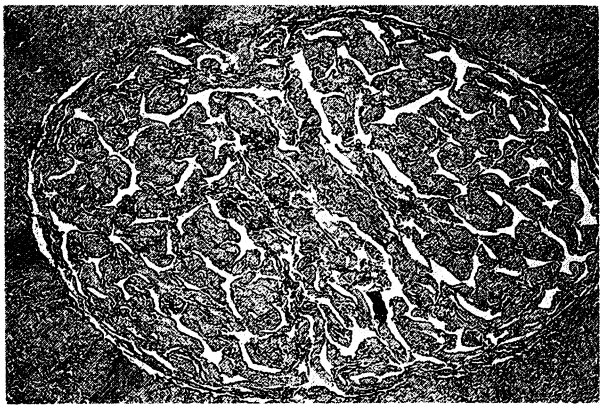

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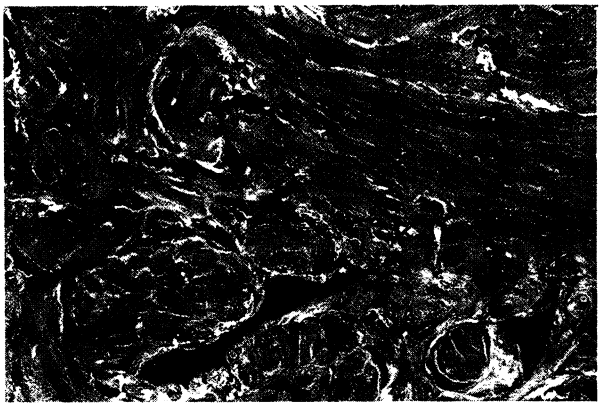

c

Fig. 2. — Relaxation of cavernosal smooth muscle to SIN-I (a, b) and papaverine (c). Tone was induced by norepinephrine. Data represent mean responses to each concentration of SIN-I. Vertical bars represent standard error of mean. Concentrations of the drug are indicated as logarithm of molarity (log M).

Fig. 2. — Relaxation du muscle lisse caverneux traité par SIN-I (a, b) et papavérine (c). Contraction induite par norepinéphrine. Ces tracés représentent les réponses moyennes à chaque concentration de SIN-I. Barre verticale : erreur standard moyenne. Les concentrations des agents chimiques sont indiquées par le logarithme de sa molarité (log M).

Histopathological study

It was shown by HE and Masson - Goldner stain that the caernous tissue from the rabbits consists of loose sinusoid spaces separated by connective tissue trabecula containing bundles of smooth muscle cells, nerve fibers and arterioles. In the corpus cavernosum of the rabbits treated by repeated injections of SIN-I, nor local fibrosis neither any sign of inflammation was seen. The intracavernous tissue does not exhibit any hyperplasia including fibrous tissue and smooth muscle mass (fig. 2 a et b).

Immunofluorescence

The staining of the NO-synthetase-containing axons supplying penile smooth muscle is substantiated by immunocytochemistry as seen in figure 2c.

DISCUSSION

The present study shows that the NO-donor SIN-I induces a relaxation of the cavernous smooth muscle *in vitro* and *in vivo*. Previous studies showed that NO which is involved in the mechanism of penil smooth

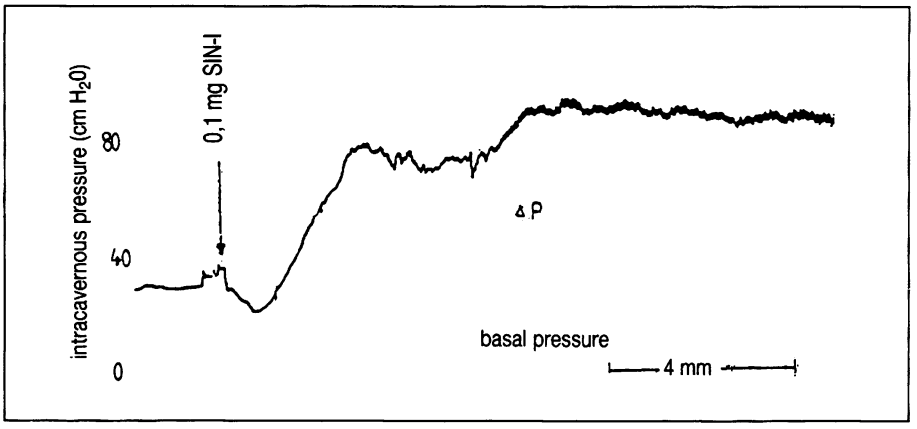

Fig. 3. – Tracing showing the increase in intracavernous pressure induced by intracavernous injection of 0.1 mg SIN-I. Then rise in pressure was described in terms of δP, which was defined as the maximal pressure obtained by SIN-I-injection minus the basal pressure before the injection. The low pressure increase directly after the SIN'I injection as well as the steady pressure below the final maximum in the first 3-4 minutes is due to the drop in systemic blood pressure.

Fig. 3. – Tracé montrant l'augmentation de la pression intra-caverneuse induite par injection intra-caverneuse de 0,1 mg de SIN-I. L'élévation est exprimée en terme de delta P ; c'est la pression maximale obtenue par injection de SIN-I diminuée de la pression de base avant injection. La pression basse s'élève après injection de SIN-I. La baisse de pression en fin d'expérience est due à la baisse de pression systémique.

muscle tone is directly released from nerves. Furthermore it was shown that inhibition of NO-synthesis *in vivo* inhibited nerve-induced penile erection [8]. This is supported by the finding that we could stain NO-synthetase containing axons supplying penile smooth muscle cells. This also shows that the relaxing effect of SIN-I is specific and physiological.

The dose-response-curve of SIN-I is in agreement with a reliable control using the drug in therapeutical dose range. Due to the fask kinetics of decomposition [9] in the cavernous tissue SIN-I should not provoke a prolonged erection. In contrast to these findings, the non-specific phosphodiesterase-inhibitor papaverin has a very narrow therapeutical with and the drug also implies the risk of a priapism [10-11].

Our *in vitro* findings are supported by the *in vivo* experiments. The intracavernous injection sinduces a dose-dependent erectile response, but even high doses of SIN-I regarding the penile volume of the rabbit compared to the human, dot not induce a prolonged erection.

After repetitive intracavernous injections of SIN-I in the rabbit no signs of fibrosis and/or inflammation are detected. These results may attribute to the anti-inflammatory effects as well as to the good tissue tolerability of the nitric oxide donor SIN-I. So when only a relatively low number of papaverine injections (n = 6) are performed, the low dose injections induc a marked cavernous inflammation and fibrosis [12]. So, intracavernous injection of SIN-I seems, at least, to be much better tolerated than papaverine. Further studies with more injections and different species have to be carried out before a final conclusion may be drawn.

Finally, we may emphasize that SIN-I seems to be an adequate therapeutical agent in the treatment of erectile dysfunction in terms of its therapeutical with and the nontoxic short-term effects concerning local degenerations after repeated applications. Future long-term studies in various species are necessary for the judgement of local toxicity.

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