

PREVENTION OF GLYCEROL-INDUCED ACUTE RENAL FAILURE IN RABBITS BY THE THROMBOXANE-ANTAGONIST BM 13.505

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Summary

We compared the effects of the new thromboxane (TX)-antagonist BM 13.505 (BM) with that of the prostaglandin (PG)-synthase inhibitor indomethacin in glycerol-induced acute renal failure in rabbits. We estimated renal TXA₂-formation by calculating the excretion rate of TXB₂ and renal function by determining the serum creatinine concentration and examined kidney morphology after autopsy 72 hours after injection of glycerol. Glycerol injection stimulated renal formation of TXA₂. Indomethacin reduced the stimulated TX-excretion, whereas BM showed no effect. Indomethacin enhanced the severity of the acute renal failure produced by glycerol, while BM protected against the development of glycerol-induced acute renal failure. These results confirm that TXA₂ is an important pathogenic mediator in glycerol-induced acute renal failure in rabbits. Indomethacin enhancement of glycerol-induced acute renal failure may result from inhibition of the biosynthesis of PGI₂ and PGE₂ as vasodilating PGs that may play an important compensatory role in this model.

Introduction

Acute renal failure in humans remains a poorly understood response of the kidney to hypoperfusion, presumably mediated by an acute ischaemic insult [1]. There is no experimental model whose pathology resembles that of the common hypotensive form, in which vasoconstriction is believed to play a considerable role. One of the well established models used to study the pathogenic mechanism in acute renal failure is glycerol-induced acute renal failure in rabbits. A decreased renal blood flow and vasoconstriction of large- and medium-sized renal vessels have been demonstrated [2]. Furthermore, a direct correlation between the level of renal function as determined by serum creatinine and the metabolic potential of microsomal preparations from kidney for thromboxane (TX) biosynthesis have been reported [3]. This suggests that the

potent vasoconstrictor TXA₂ is generated by the kidney following glycerol administration and may mediate the renal failure in this model.

We have recently reported that a representative of a new class of sulpho-
namidophenylcarboxylic acids, BM 13.177, antagonizes TXA₂ and cyclic
prostaglandin (PG) endoperoxides at the TX receptor of platelets [4] and
arterial smooth muscle preparations [5]. More potent analogues of BM 13.177
with similar properties have been identified and one of them, BM 13.505,
4-[2-(4-chlorobenzenesulphonylamino)-ethyl] benzene acetic acid, was used
in this investigation.

In the present study, we compared the effect of the TX-antagonist BM
13.505 with that of the PG-synthase inhibitor indomethacin on the severity
of glycerol-induced acute renal failure in rabbits. It is well known that indo-
methacin enhances glycerol-induced acute renal failure in this species [6],
probably due to inhibition of the biosynthesis of all prostaglandins. A selective
TX-receptor antagonist provides an approach for evaluating the contribution
of TXA₂ to the pathophysiological events associated with glycerol-induced
acute renal failure.

Methods

The experimental protocol was similar to that described by Torres et al [6].
Male mixed breed rabbits (2 to 3.5kg), fed a standard laboratory diet and
water ad libitum, were divided into three groups (glycerol, glycerol + BM 13.505
and glycerol + indomethacin; n=7-8) and kept individually in cages especially
designed for urine collection. Twenty-four hours later blood was taken for
determinations of serum creatinine and electrolyte concentrations (control
period). Then, 5mg/kg BM 13.505 (glycerol + BM group) or 8mg/kg indomethacin
(glycerol + indomethacin group) were injected into the marginal vein of the
ear, the glycerol group received only isotonic saline (0.1ml/kg). Thirty minutes
later, all groups were given 50 per cent glycerol in isotonic saline by injection
into the loose subcutaneous tissue behind the neck (under light hexobarbitone
anaesthesia). Two and six hours after glycerol, BM or indomethacin (suspended
in maize oil) were injected subcutaneously into the rabbits of groups glycerol +
BM and glycerol + indomethacin respectively (same doses as IV, 0.1ml/kg).
Maize oil alone (0.1ml/kg) was injected into the rabbits of the glycerol group.
Blood samples were collected again 24, 48 and 72 hours after glycerol injection,
urine was collected in 24 hour fractions. Seventy-two hours after glycerol
injection the animals were killed and the kidneys fixed in Bouin's solution.

The serum creatinine concentration (picric acid method), Ca⁺⁺ (chromogen
test), both as test combinations (Boehringer Mannheim, Mannheim, FRG)
were determined by means of a COBAS BIO-Analyser (Hoffmann-La Roche,
Basel, Switzerland). All other electrolytes in serum and urine were determined
by means of ion-sensitive electrodes in a NOVAS-system (NOVAS, Darmstadt,
FRG). TXB₂ in urine was measured by radioimmunoassay utilizing antisera
from the Institute Pasteur, Paris. The urinary TXB₂ excretion rate was then
calculated.

After paraffin embedding of fixed renal tissue, sections were stained with

TABLE I. Serum creatinine concentrations in control period and at 24, 48 and 72 hours after administration of glycerol with or without BM 13.505 or indomethacin (n=6-8); median (minimum-maximum)

Treatment	Before glycerol administration	Serum creatinine concentration ($\mu\text{mol/L}$)		
		24	48	72
Glycerol	105 (73-125)	177 (93-417)*	177 (75-506)	170 (89-434)***
Glycerol + BM 13.505	94 (65-119)	111 (82-179)	100 (71-159)	123 (89-186)*
Glycerol + indomethacin	109 (99-228)	348 (162-621)***	221 (120-897)***	189 (121-1049)*

*p=0.05; **p<0.05; ***p<0.01 p values refer to differences between the values in the columns and those before glycerol administration

TABLE II. Twenty-four hour urine excretion rates of TXB₂ before and after administration of glycerol with or without BM 13.505 or indomethacin (n=6-8); median (minimum-maximum)

Treatment	Before glycerol administration	Excretion rates of TXB ₂ (ng/24hr)		
		0-24hr	24-48hr	48-72hr
Glycerol	401 (142-645)	1256 (399-2057)**	269 (94-2826)**	132 (33-696)
Glycerol + BM 13.505	374 (253-630)	1174 (287-2555)*	168 (96-234)***	153 (20-201)***
Glycerol + indomethacin	282 (182-548)	728 (307-2345)***	75 (17-185)***	66 (20-124)***

*p=0.05; **p<0.05; ***p<0.01; p values refer to differences between the values in the columns and those before glycerol administration

HE, PAS and Goldner stains. The sections were examined on a blind basis and graded according to the degree of changes on a scale from 0 to +++.

Statistical analysis

Statistical analysis was performed using Wilcoxon's rank sum test. All values given represent medians (minimum-maximum).

Results

Twenty-four hours after glycerol injection the most prominent change in serum parameters was an increase in serum creatinine concentration in glycerol and glycerol + indomethacin groups compared with the values in the control period (Table I). The serum creatinine concentrations were significantly higher in the glycerol + indomethacin group than in the group injected with glycerol alone. Serum creatinine concentrations in the group treated with glycerol alone at 48 and 72 hours were equivalent to those observed at 24 hours, while in the glycerol + indomethacin treated animals serum creatinine values at 48 and 72 hours were lower than those at 24 hours. In contrast, a moderate increase of serum creatinine concentration was observed in only one animal of the glycerol + BM group, whereas in all other animals no change occurred.

Renal excretion of TXB_2 increased four-fold within 24 hours after glycerol injection compared with the control period (Table II). Almost the same increase was observed in the glycerol + BM group. TXB_2 excretion rate was found to be reduced in the glycerol + indomethacin group. On the following days, the renal excretion rate of TXB_2 by all groups was lower than before glycerol administration.

Mean urinary outputs did not change significantly after administration of glycerol. No significant differences between serum electrolyte concentrations before and after glycerol administrations were found.

The histopathology of the kidney at 72 hours after the administrations of glycerol showed coagulative necrosis and vacuolar hydropic degeneration of the proximal convoluted tubules; casts, especially in the distal tubules; dilatation of the tubules and oedema of the interstitium, partly with mononuclear cell infiltrations. These changes were similar to those described by Torres et al [6]. In individual animals from the glycerol and glycerol + indomethacin groups we found a good correlation between the increase of serum creatinine concentrations and the severity of the pathological findings, which were mainly dilatations and casts. These morphological changes were more pronounced in some of the animals treated with glycerol + indomethacin than in those treated with glycerol alone. Furthermore, in those animals which had very high serum creatinine concentrations during the whole experimental period necrosis were the prominent finding. In contrast only minimal changes were seen in some of the animals treated with glycerol + BM.

Discussion

The present results indicate that TXA₂ plays a key role in the pathogenesis of glycerol-induced acute renal failure in rabbits. A direct correlation between the serum creatinine and the metabolic potential of microsomal preparations for TXA₂ biosynthesis has been reported in this animal model [3]. This study showed that glycerol injection stimulated the renal excretion rate of TXB₂, the stable metabolite of TXA₂, and presented evidence that the potent vasoconstrictor TXA₂ is generated by the kidney following glycerol administration and may mediate the renal failure. Following glycerol administration renal impairment was demonstrated by increased concentrations of serum creatinine indicating a reduced glomerular filtration rate and by histological examination of the kidneys which showed pathological changes similar to those described by other authors [6].

Co-administration of glycerol and BM, prevented the increase of serum creatinine and only minimal morphological changes of the kidneys were observed, although it had no inhibitory effect on stimulated TXA₂ generation. This is consistent with the ability of BM 13.505 to antagonize TXA₂ at the TXA₂ receptor level and demonstrates the key role of TXA₂ in the pathogenesis of acute renal failure in this model.

We also confirmed the observation [6] that co-administration of glycerol + indomethacin enhanced the severity of the acute renal failure although TXB₂ excretion rate within the first twenty-four hours after glycerol administration was reduced. The moderate inhibitory effect of indomethacin on TXB₂ excretion rate within the first twenty-four hours may be an indication that stimulation of TXA₂ generation is only partially suppressed or that the dosage regimen used was not sufficient to block synthesis over the total collection period. The role of prostaglandins and thromboxanes in the aggravation of glycerol-induced acute renal failure by indomethacin is still not fully understood. The results of our study support a role for the prostaglandins E₂ and I₂ in protecting the kidney against the glycerol-induced acute renal failure. This is consistent with previous observations [6]. The excellent effect of BM 13.505 is in accordance with its ability to block the receptors of TXA₂ without inhibiting PGE₂ and PGI₂ synthesis.

References

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