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# Contrast media injection in the rat after multiple renal insults

## No evidence of additional nephrotoxicity

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**Abstract** Experiments were performed to determine whether water-soluble contrast media (CM) show nephrotoxic properties when injected into rats after multiple renal insults. The latter consisted of combinations of prostaglandin synthesis inhibition (with indomethacin) and/or salt depletion and/or uninephrectomy. Renal function was evaluated by standard clinical methods to measure parameters such as urinary output, urinary osmolality, urinary creatinine excretion and serum creatinine. CM injected after prostaglandin synthesis inhibition alone did not influence urinary creatinine excretion or serum creatinine. After a combination of renal insults a significant increase in median serum creatinine values from 61.88 μmol/l [interquartile range (IR) 17.68] [0.70 mg% (IR 0.20)] to 97.24 μmol/l (IR 79.56) [1.10 mg% (IR 0.90)] was observed but CM or sham injections did not prevent a normalization of serum creatinine. The pattern of recovery of serum creatinine was not influenced by previous kidney mass reduction. It is concluded that the nephrotoxic properties of CM cannot be detected with standard clinical methods in rats after multiple renal insults.

**Key words** Contrast media · Salt depletion
Indomethacin · Hydropenia · Uninephrectomy

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Renal toxic complications of water-soluble contrast media (CM) observed in patients are difficult to reproduce in experimental animal models [7]. Previous work from our laboratory was not able to demonstrate a negative effect on renal function after CM injection into either normal rats or animals that were subjected to renal ischaemia [11]. These negative results are in agreement with the clinical evidence that radiocontrast-induced renal failure is low in frequency, except in the presence of a combination of risk factors such as large doses of CM, volume depletion, pre-existing renal failure and diabetes [3].

Investigators [4, 7, 10] have suggested that minor but multiple renal injuries are able to make the kidney more vulnerable to CM. In order to test this hypothesis, we performed a series of experiments increasing stepwise the number of renal insults prior to CM administration. Simple and clinically used methods for evaluation of renal function parameters such as diuresis, urinary osmolality, urinary creatinine excretion and serum creatinine were used. Both a high- and a low-osmolar contrast agent were injected.

## Methods

All the animals were Sprague-Dawley rats approximately 3 months old. Normal values for diuresis, urinary osmolality, urinary creatinine excretion and serum creatinine were determined in 22 untreated rats and were reported in an earlier study [9]. The different protocols are illustrated in Fig. 1.

A first group of 20 male Sprague-Dawley rats were housed in metabolic cages. After the 48-h acquaintance period, urine collections were started. The urine was first sampled for 2 days to be used as control. Twenty-four hours prior to the i.v. injection of either CM or glucose, food and fluid intake were withdrawn. Two hours before the i.v. injection, indomethacin was administered intraperitoneally (i.p.) [5 mg/kg body weight (BW)]. The injections of CM and glucose in the left vena femoralis were performed with the animal under ether anaesthesia. The injected volume of 5% glucose in water was 1.5 ml ($n$=7). The CM administered were sodium ioxithalamate (Telebrix-12-sodium, Geurbet, Aulney-sous-Bois, France) with an osmolality of 610 mosm/kg ($n$=7) and meglumine diatrizoate, (Angiografine, Schering, Berlin, Germany) with an osmolality of 1530

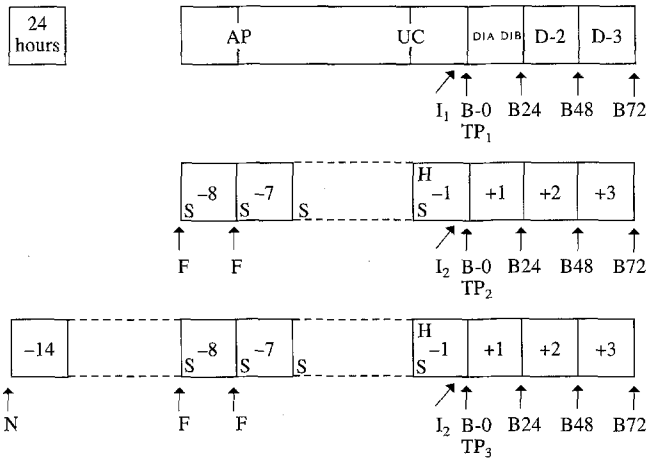


**Fig. 1** Schematic illustration of the protocols: *AP*, acquaintance period, *UC*, urine for control, *D-1*, first 24-h urine sample, *D1A*, first 16-h sample of *D-1*, *D1B*, last 8-h sample of *D-1*, *D-2*, second 24-h urine sample, *D-3*, third 24-h urine sample, *B*, blood sample, *B-O*, control, *B24*, after 24 h, *B48*, after 48 h, *B72*, after 72 h, *I*, indomethacin injection, $I_1$, 5 mg/kg BW i.p., $I_2$, 10 mg/kg BW i.v., *TP*, test product injection, $TP_1$, glucose (1.5 ml) or CM (700 mg I/kg BW), $TP_2$, glucose (3 ml) or CM (2800 mg I/kg BW), $TP_3$, glucose (6 ml) or CM (2800 mg I/kg BW), *F*, furosemide (50 mg/kg BW i.p.), *S*, salt-free diet, *H*, hydropenia, *N*, nephrectomy, – *number* days prior to test product injection, + *number* days after test product injection

mosm/kg ($n$=6), given at a dose of 700 mg iodine/kg BW. This represents volumes of 1.5–2.0 ml sodium ioxithalamate and approximately 0.75 ml meglumine diatrizoate.

The urine was sampled over 3 days after the test product injections. The 1st day was divided into two collection periods. Day 1A (D1A) covered the first 16 h and day 1B (D1B) the last 8 h. The following 2 days, the urine was collected over 24-h periods: day 2 (D-2) and day 3 (D-3). No food or fluid restriction was applied after the test product injections.

During the whole experiment four blood samples were taken: the first (B-O) as a control, was a venous sample just prior to the i.v. injection of the test products, i.e., 2 h after indomethacin administration; the following blood samples were taken 24, 48 and 72 h after CM or glucose injections (B24, B48 and B72).

A second group of 50 Sprague-Dawley rats were evaluated only by follow-up of the serum creatinine and were not kept in metabolic cages. This group was divided into 31 animals in which no kidney mass reduction was performed (CKM complete kidney mass) and 19 animals which were uninephrectomized (RKM reduced kidney mass) 2 weeks prior to the test product injections. Left uninephrectomy was performed through a median laparatomy with the animal under general anaesthesia with i.p. pentobarbital (60 mg/kg BW).

The animals in the second group were all salt depleted by i.p. injection of furosemide (50 mg/kg BW) the 8th and 7th days prior to the injections of the test products and by feeding them a salt-free diet with glucose 2.5% as drinking water during this period. Twenty-four hours prior to the injection of test product, a food and fluid restriction was also applied to these animals.

Two hours prior to the injections of test product, the animals were anaesthetized by i.p. injection of pentobarbital (60 mg/kg BW). The left vena jugularis was catheterized through a neck incision and indomethacin was injected (10 mg/kg BW). Two hours later a blood sample was collected and the i.v. test injection was performed. The jugular vein was then ligated, the skin incision was closed and the animals were allowed to recover. Free access to food and drinking water was allowed.

The injected test products were sodium ioxithalamate, meglumine diatrizoate, meglumine iothalamate (Contrix, Guerbet, Aulney-sous-Bois, France) with an osmolality of 1483 mosm/kg and 5% glucose in distilled water. The injected amount of CM was calculated in order to administer 2800 mg iodine/kg BW, corresponding to approximately 8 ml of sodium ioxithalamate and 3.5 ml of the two other CM. The sham injections were 3 ml 5% glucose in the CKM group and 6 ml in the RKM group. The number of animals in each test product group varied between 6 and 12 animals per group.

Twenty-four (B24), 48 (B48) and 72 (B72) h after the injection of the test products blood sampling was performed.

In a separate group of 40 animals, exactly the same protocols were followed for light macroscopic evaluation of the kidneys 24, 48 and 72 h after the injection of test product. After i.p. injection of pentobarbital (60 mg/kg BW), the kidneys were perfused-fixed with a 25% gluteraldehyde buffered solution and renal tissue was processed for light microscopy. The histological evaluation of the different tissue samples was blinded.

For statistical evaluation of the results, the rather small sample sizes required the use of non-parametric tests [1]. Therefore, descriptive parameters are expressed by the median value with the interquartile range (IR) (the latter always given in parentheses in the text and figures).

To compare the control values (CON and BO) with the normal data, a Mann-Whitney U-test was used. A Friedman analysis was used to compare the postinjection values with the control values. Analysis of variance (ANOVA) was used to compare the results of the sham series with those of the CM groups.

Creatinine in blood and urine was assayed with a Creatinine Analyser II (Beckman Clinical Instruments Division, Fullerton, USA), according to Jaffé's principle [5]. The urinary osmolality was measured with an Advanced Digimatic Osmometer, Model 3 DII (Needham Heights, Mass., USA) [8].

## Results

The weight of all the animals was approximately 300 g [median 296 g (IR 36)]. The normal values, obtained in an earlier protocol [11] in 22 unmanipulated rats and housed under the same conditions, were as follows: urinary output 2.96 µl/min 100 g BW (IR 0.95), urinary osmolality 2075 mosm/kg (IR 706), urinary creatinine excretion 0.0259 µmol/min 100 g BW (IR 0.0034) and serum creatinine 51.9 µmol/l (IR 15.0) [0.59 mg% (IR 0.17)].

### Indomethacin i.p. 2 h before the test product injections

*Urinary output* (Table 1)

The control values for urinary output were significantly lower ($P< 0.01$) than the normal values. This can be explained by the 24-h-long fluid restriction that these animals were subjected to. After i.p. indomethacin and i.v. glucose administration the urinary output remained low for 24 h [1.26 µl/min 100 g BW) (IR 1.22)]. After the CM injections, diuresis increased but not significantly. After injections of diatrizoate, the urinary output during D-2 and D-3 was significantly higher, not only compared with the control values but also when compared by ANOVA with the results obtained in the sham glucose-injected group. This is due to the extreme increase in diuresis during the last 2 days of the experiments in this group.

*Urinary osmolality* (Table 2)

The fluid restriction was also responsible for a significantly more concentrated urine ($P< 0.01$) during the control period, when the control values were compared with the normal median osmolality of 2075 mosm/kg. In the glucose and ioxithalamate group, the control osmolality was higher than all the subsequent values, while in the diatrizoate group the decrease in osmolality was not statistically significant. ANOVA between the glucose group and the CM groups showed no difference in the evolution of the urinary osmolalities.

**Table 1** Urinary output (µl/min 100 g BW) ([a] significantly higher than preinjection and day 1A value, [b] significantly higher than preinjection value)

| | Glucose ($n$=7) | Ioxithalamate ($n$=7) | Diatrizoate ($n$=6) |
|---|---|---|---|
| Before test product injection (TPI) | 1.32 (0.81) | 1.53 (0.28) | 1.80 (0.29) |
| After TPI | | | |
| Day 1A (0–18 h) | 1.26 (1.22) | 2.09 (1.76) | 2.07 (0.64) |
| Day 1B (18–24 h) | 1.93 (1.88) | 2.14 (2.60) | 2.06 (1.06) |
| Day 2 | 2.41 (1.72)[a] | 2.41 (1.64)[b] | 3.56 (0.89)[a] |
| Day 3 | 2.35 (1.23)[a] | 2.19 (1.72) | 3.64 (1.20)[a] |
| | $P$<0.05 | $P$<0.05 | $P$<0.01 |

**Table 2** Urinary osmolality (mosm/kg) ([a] significantly higher than all other values, [b] significantly lower than day 1A, [c] significantly higher than day 2 and day 3)

| | Glucose ($n$=7) | Ioxithalamate ($n$=7) | Diatrizoate ($n$=6) |
|---|---|---|---|
| Before test product injection (TPI) | 2950 (935)[a] | 2860 (1370)[a] | 2705 (590)[c] |
| After TPI | | | |
| Day 1A (0–18 h) | 1844 (531) | 2410 (1120) | 2034 (279) |
| Day 1B (18–24 h) | 1760 (606) | 1951 (1411) | 1921 (265) |
| Day 2 | 2004 (730) | 2000 (1304)[b] | 1665 (575) |
| Day 3 | 1749 (947) | 1873 (807)[b] | 1760 (424) |
| | $P$<0.005 | $P$<0.005 | $P$<0.01 |

*Absolute urinary creatinine excretion* (Table 3)

Comparison between the normal value of 0.0259 µmol/min 100 g BW (IR 0.0034) and the control values for glucose and ioxithalamate showed a significant difference ($P< 0.01$) while this was not the case with diatrizoate. Urinary creatinine excretion showed a trend toward a decrease after the injection of indomathacin and glucose, but this was not shown to be statistical significant. The same trend was present in the CM groups. ANOVA between the latter and the sham group was not significant.

*Serum creatinine* (Table 4)

The control value for serum creatinine in the glucose group was significantly higher than the normal value of 51.9 µmol/l (IR 15.00). In the CM groups, the median serum creatinine values were identical (61.88 µm/l) with an IR of 17.68 for ioxithalamate and 26.52 for diatrizoate. The B24 value taken after the glucose injection showed an increase in serum creatinine which was significant ($P< 0.05$). After injection of ioxithalamate, this increase was more pronounced and this value was statistically different from all other values. On the other hand, the B72 value was significantly lower than all other values in this group. After diatrizoate injection no statistically significant differences were observed.

Despite some statistically significant differences in the evolution of the serum creatinine values, mainly after injection of ioxithalamate, ANOVA was unable to show a difference in the pattern of evolution of the serum creatinine between the sham-injected and the CM injected animals.

Indomethacin i.v. 2 h before the test product injections

*Complete kidney mass group* (Table 5)

The serum creatinine control value showed a significant increase over the normal value ($P< 0.01$) (Mann-Whitney U-test). With ioxithalamate, however, the control value was also higher but when the interquartile range taken into account the difference was not significant. Twenty-four hours after glucose or CM injections the serum creatinine

**Table 3** Urinary creatinine output (µmol/min 100 g BW). In each group the Friedman analysis did not show significant (NS) changes

| | Glucose ($n$=7) | Ioxithalamate ($n$=7) | Diatrizoate ($n$=6) |
|---|---|---|---|
| Before test product injection (TPI) | 0.0222 (0.0072) | 0.0235 (0.0068) | 0.0247 (0.0040) |
| After TPI | | | |
| Day 1A (0–18 h) | 0.0194 (0.0074) | 0.0238 (0.0057) | 0.0218 (0.0083) |
| Day 1B (18–24 h) | 0.0220 (0.0065) | 0.0201 (0.0138) | 0.0216 (0.0089) |
| Day 2 | 0.0256 (0.0061) | 0.0202 (0.0060) | 0.0275 (0.0049) |
| Day 3 | 0.0214 (0.0050) | 0.0202 (0.0044) | 0.0270 (0.0041) |
| | NS | NS | NS |

values decreased; at the end of the experiment the B72 values were in the range of the normal values. ANOVA showed no significant differences between glucose and ioxithalamate, and between glucose and diatrizoate. Between glucose and iothalamate, however, the difference was significant ($P< 0.005$), suggesting a more rapid return to normal values after iothalamate injection.

*Reduced kidney mass group* (Table 6)

Two hours after i.v. indomethacin administration, serum creatinine in all groups was significantly higher than the normal value (Mann-Whitney U-test, $P< 0.05$). After glucose and iothalamate injections, no significant changes in serum creatinine occurred. In the ioxithalamate group, however, the control value was significantly higher than the B72 and B24 values ($P< 0.01$). ANOVA between glucose and ioxithalamate showed no significant diffence. As in the previous experiments, there was a statistically significant difference ($P< 0.01$) between glucose and iothalamate, but in contrast with the previous experiments this suggested a slower recovery to normal values after iothalamate injection.

The histological examinations of the kidneys revealed no pathological changes, regardless of whether the animals were challenged with CM or glucose.

## Discussion

In normal rats, CM are known to produce an increase in urinary output due to their osmotic load [6]. On the other hand, indomethacin has an antidiuretic effect [2]. The latter combined with fluid restriction is responsible for the decrease in diuresis observed during the first 24 h. The CM, injected 2 h after the i.p. administration of indomethacin, were unable to influence significantly this lower urinary output. ANOVA showed no differences between the glucose group and the group injected with the low osmolar ioxithalamate. The differences were significant between glucose and diatrizoate, a high osmolar contrast agent, but the question arises as to whether the CM administration alone can be responsible for the major increase in urinary output observed 24 h after the injection.

Prostaglandin synthesis inhibition is associated with a decrease in the urinary osmolality [12], a decrease which was observed in all animals regardless of whether they were challenged with CM or glucose. Also none of the changes in urinary creatinine excretion were statistically significant between the different groups.

Despite the significant increase in serum creatinine in the ioxithalamate group, ANOVA between this group and the diatrizoate group showed no significant difference, and in all groups the same pattern in evolution of serum creatinine was observed.

**Table 4** Serum creatinine (μmol/l) ([a] significantly higher than B72, [b] significantly higher than all other values, [c] significantly lower than all other values, *NS*, Friedman analysis not significant)

| | Glucose ($n$=7) | Ioxithalamate ($n$=7) | Diatrizoate ($n$=6) |
|---|---|---|---|
| Before test product injection (TPI) | 66.30 (17.68) | 61.88 (17.68) | 61.88 (26.52) |
| After TPI | | | |
| B24 | 92.82 (26.52)[a] | 97.24 (79.56)[b] | 70.72 (26.52) |
| B48 | 66.30 (17.68) | 53.04 (17.68) | 53.04 (17.68) |
| B72 | 57.46 (26.52) | 44.20 (8.84)[c] | 53.04 (26.52) |
| | $P$<0.05 | $P$<0.005 | NS |

**Table 6** Serum creatinine (μmol/l) RKM ([a] significantly higher than B48 and B72, [b] *NS*, Friedman analysis not significant)

| | Glucose ($n$=6) | Ioxithalamate ($n$=6) | Diatrizoate ($n$=7) |
|---|---|---|---|
| Before test product injection (TPI) | 66.30 (8.84) | 97.24 (8.84)[a] | 88.40 (17.68) |
| After TPI | | | |
| B24 | 70.72 (26.52) | 75.14 (26.52) | 79.56 (17.68) |
| B48 | 61.88 (17.86) | 70.72 (8.84) | 70.72 (8.84) |
| B72 | 53.04 (8.84) | 61.88 (8.84) | 61.88 (17.86) |
| | NS | $P$<0.01 | NS |

**Table 5** Serum creatinine (μmol/l) CKM ([a] significantly higher than B72, [b] significantly higher than B72 and B48, [c] significantly lower than B24, NS Friedman analysis not significant)

| | Glucose ($n$=7) | Ioxithalamate ($n$=6) | Diatrizoate ($n$=6) | Iothalamate ($n$=12) |
|---|---|---|---|---|
| Before test product injection (TPI) | 88.40 (8.84)[a] | 83.98 (61.88) | 88.40 (8.84)[b] | 75.14 (35.36)[b] |
| After TPI | | | | |
| B24 | 70.72 (44.20) | 57.46 (8.84) | 57.46 (17.86) | 66.30 (8.84) |
| B48 | 70.72 (26.52) | 48.62 (17.86) | 53.04 (17.68) | 53.04 (8.84)[c] |
| B72 | 61.88 (26.52) | 44.20 (17.86) | 39.78 (8.84)[c] | 53.04 (8.84) |
| | $P$<0.05 | NS | $P$<0.005 | $P$<0.005 |

In summary, this first series of experiments clearly showed the changes in urinary output and urinary osmolality as noted after indomethacin injection in untreated animals. The additional challenge with CM did not influence these changes. More specifically, the absence of significant changes in urinary creatinine excretion and in serum creatinine allows the conclusion that the i.v. injection of CM in prostaglandin depleted rats does not provoke additional adverse effects on renal function. These negative results are difficult to explain on the basis of an insufficiently high dose of CM. Indeed, if 300 mg iodine/kg BW is the contrast dosage considered optimal in clinical uroradiology [9], the present experiments challenged the laboratory animals with doses as high as 2–9 times this clinically accepted dose in order to enhance any possible toxic effect of the CM.

When a combination of renal insults was applied to the non-nephrectomized animals, significantly higher serum creatinine values than the normal values were observed, and before the test product injections. However, after further challenge with either CM or glucose, serum creatinine normalized in a similar way in both groups. A previous uninephrectomy did not influence the pattern of recovery of renal function.

Using simple clinical parameters, we were unable to find data to support the fact that CM cause additional nephrotoxicity after multiple renal injuries. It could be argued that before the administration of CM the animals showed only a mild, but statistically significant, increase in serum creatinine compared with control animals. Although this renal impairment was rather recently induced when CM were given, Heyman et al. [7], using a comparable protocol, found a significant enhancement of the renal insufficiency 24 h after the CM administration. These results were not confirmed in the present study. However, this does not exclude the possibility that CM can produce an additional nephrotoxic effect, measurable by standard clinical parameters, when given in a situation of a more pronounced or longer existing renal impairment.

In conclusion, the present study using relatively simple renal functional parameters was able to show changes in diuresis and urinary osmolality, which were mainly due to the indomethacin injection, and not the administration of CM. The results obtained after multiple renal insults, including administration of CM, did not provide additional functional evidence for nephrotoxicity by contrast media. In addition, no consistent differences between high- and low-osmolar CM products could be demonstrated. A simple animal model for the nephrotoxic evaluation of contrast media is as yet not available.

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