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Protection by a polyvalent vaccine against challenge infection and pyelonephritis

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Summary. The protective effect of immunization with a polyvalent vaccine (SolcoUrovac) was studied in the mouse and the rat. The i.m. immunization increased the resistance of mice to challenge infection with all homologous strains of bacteria. The LD_{50} values for *E. coli, Proteus mirabilis* and *Streptococcus* were 3.5–4.5 times and that of *Klebsiella* as much as 600 times that in nonimmunized mice. Protection against challenge with heterologous *E. coli* was also achieved and persisted for about 20 weeks. Immunization with the vaccine also provided marked protection against pyelonephritis in rats. Kidneys with abscesses were seen only one-third as often as in controls, and the size of the individual abscesses was substantially smaller in the vaccinated animals. Based on the quantity of bacteria in the kidneys it was postulated that the vaccination increased the clearance of bacteria.

Key words: Immunization – Experimental pyelonephritis – Protection against UTI – *E. coli* – *Klebsiella* – *Proteus* vaccine

In the treatment of human urinary tract infections (UTI) immunization with suitable bacteria offers an alternative to chemotherapy. In earlier papers positive vaccination results against haematogenous pyelonephritis were described [3, 7, 11, 16]. Believing that this route, in most cases, is not relevant to the pathogenesis of pyelonephritis in humans – since the majority of UTI in humans is due to ascending infection – other authors used retrograd pyelonephritis in rats as a model [5, 6, 8]. In these and other experiments [1, 2] vaccination with inactivated complete bacteria protected against ascending infection. In all reports the role of antibodies as protective agents against the infection was emphasized.

Recently, we immunized mice with a polyvalent vaccine, SolcoUrovac (Solco Basle, Birsfelden, Switzerland), and found an increase of antibodies not only in serum but also in urine [10]. Additionally, we observed a marked increase of the total amount of immunoglobulins which could not be explained by an increase of specific antibodies only. The same phenomenon was observed after immunization with a vaccine (Chol-Tab Berna, Berne, Switzerland) against cholera and *Salmonella* (unpublished data). These effects, we postulated, could be caused by nonspecific stimulation of the immune system. This stimulation can impart protection not only against infection by the strains of bacteria used for immunization, but also against other strains.

In this study we investigated the potency of the protective effect in mice of SolcoUrovac against a challenge with homologous strains of bacteria and also, against some antigenetically heterologous strains. The study of protection against retrograde pyelonephritis in rats after immunization with this vaccine was carried out in order to learn more about the efficacy of the vaccine and the protective role of antibodies in urine.

Materials and methods

The vaccine SolcoUrovac

SolcoUrovac is a vaccine containing whole heat-inactivated bacteria of the species *E. coli* (6 strains 7.5×10^8 bacteria), *Klebsiella pneumoniae* (1.5×10^8 bacteria), *Proteus vulgaris* and *P. morganii* (each species: 3.75×10^7 bacteria) and *Streptococcus faecalis* (2.5×10^7 bacteria).

The *E. coli* strains include haemolytic strains (4 out of 6) and express MS-pili (5 out of 6) as well as P-pili (4 out of 6), the latter mediating specific Gal-Gal binding (3 out of 6); they display the serotypes O: 1, 4, 6, 17, 75, (77), K: 1, 3, 5, 13, (95), H: 1, 5, 7, 33.

Immunization and challenge of mice

NMRI white female mice (Swiss outbred strain) weighing 22–24 g were vaccinated i.m. three times with 50 µl of SolcoUrovac vaccine at intervals of 7 days or 2 weeks (challenge with heterologous strains). The control group was given 50 µl aluminium phosphate, i.e. the vehicle of the lyophilized vaccine, only, also i.m. The group size was 20 or 30 animals. At 10 days or 2 weeks (challenge with heterologous strains) after the last injection the mice were challenged by i.p.

Table 1. Protection of mice vaccinated with SolcoUrovac against i.p. challenge infection with homologous strains of bacteria[a]

Bacteria used for challenge[b] (homologous strains)	Equivalent to LD_{50}				Relative increase of LD_{50} by vaccination (vaccinated/control group)
	Untreated animals		Vaccinated animals		
	OD_{600}	CFU injected bacteria	OD_{600}	CFU injected bacteria	
E. coli (455 UB)	1.8×10^{-2}	7.2×10^{6}	7×10^{-2}	2.8×10^{7}	3.9×
Klebsiella pneumoniae (16 B)	9×10^{-6}	2×10^{3}	5×10^{-3}	1.2×10^{6}	600×
Proteus mirabilis (63 B)	1.6×10^{-1}	7.4×10^{7}	5.6×10^{-3}	2.6×10^{8}	≈3.5×
Streptococcus faecalis (676)	4.6	1.0×10^{9}	21	4.5×10^{9}	≈4.5×

[a] Mice were vaccinated i.m. with 50 µl SolcoUrovac given three times at intervals of 7 days
[b] 10 days after last vaccination the mice were challenged i.p. with 0.5 ml bacterial suspension of varying density

Table 2. SolcoUrovac protection of mice against i.p. challenge with heterologous strains of *E. coli*[a]

E. coli strain used for challenge	OD_{600} of challenge dose[b]	Survivors/total	Protection[c]
ATCC 23500, O2A, 2B:K5(1):H4	0.020	18/20	55%
Control	0.020	7/20	-
ATCC 23513, O18A, 18C:K77(B21):H7	0.003	9/20	25%
Control	0.003	4/20	-
ATCC 23504, O8:K8(L):H4	0.190	14/20	10%
Control	0.190	12/20	-
NCTC 9018, O18a, 18b:K76(B):H14	0.78	14/20	25%
Control	0.78	9/20	-

[a] Mice were vaccinated i.m. with 50 µl SolcoUrovac given three times at intervals of 2 weeks
[b] 2 weeks after the last vaccination the mice were injected i.p. with 0.5 ml living bacteria at densities indicated
[c] Protection was calculated from the percentage of surviving mice in the test group after subtraction of survivors in the corresponding control

injection of 0.5 ml bacterial suspension of tested strains of bacteria at various concentrations.

E. coli, *Klebsiella* and *Proteus* bacteria were cultivated for 24 h, subcultivated for 4 h and diluted with Veal Infusion Broth to the required concentrations. *Streptococci* were cultivated on blood agar plates for 24 h, scraped off and resuspended in the same broth. After the challenge the number of animals surviving for 5 days was recorded.

Immunization of rats

Female Sprague-Dawley white rats at 7 weeks of age (ca. 180 g) were injected i.p. with 0.5 ml (1 ampoule) of SolcoUrovac. A booster injection containing the same amount of vaccine was given after 3 weeks. The control groups were injected with 0.5 ml of 0.2% aluminium phosphate with 0.1‰ Thiomersal in 0.9% NaCl (vehicle of the vaccine).

Induction of pyelonephritis

Experimental pyelonephritis was induced in accordance with Brooks' model [4] and with some aspects of Kakar's model in mice [9].

At 14 days after the second vaccination the rats were anaesthetized with sodium pentobarbital, and the left ureter as well as the bladder were exposed by paramedian incision of the abdominal wall. The bladder was emptied by urethral catheterization and the warm (37°C) bacterial suspension of 10^6 living *E. coli* (one of six of SolcoUrovac *E. coli* strains, serotype 06:K13:H1) in 0.35 ml Veal Infusion Broth was slowly injected into the bladder. Then the left ureter was partially ligated by the following method: a 2-cm-long steel wire 1.1 mm in diameter was kept along the side of the ureter and tied to it with a silk ligature at its mid-level; then the wire was gently taken out. Finally, the muscles and the skin were stitched in separate layers.

To exclude the possibility that the pyelonephritis was caused by haematogenous infection an additional experiment was performed in six nonimmunized rats. At 30 min after the intravesical challenge blood samples (0.5 ml) were taken by cardiac puncture and cultivated for bacterial growth on MacConkey agar plates (Difco, Detroit, Mich.).

Bacteriological studies

Rats were sacrificed 5 days after intravesical challenge with the bacteria. Left and right kidneys were separately removed aseptically and homogenized in 10 ml sterile PBS in a tissue grinder. Serial 10-fold dilutions were prepared, and 0.2 ml of each dilution was plated

Table 3. Duration of protection against challenge with heterologous *E. coli* strain (ATCC 23500, O2A, 2B, K5(1):H4) after immunization with SolcoUrovac

Period after the last vaccination	Challenge dose (OD_{600})	Survivors/total		Protection[b]
		Test group	Control	
2 weeks	0.020	18/20	7/20	55%
5 weeks	0.020	15/20	6/20	45%
10 weeks	0.020	9/20	5/20	20%
20 weeks	0.020	8/20	8/20	0%

[a] Mice were immunized and challenged as before (see Table 2)
[b] Protection was calculated as before (see Table 2)

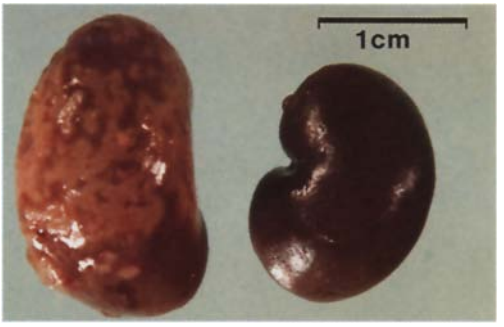

Fig. 1. Left kidney of a nonvaccinated rat, with abscesses occupying about 90% of surface, and for comparison the normal right kidney of the same animal

on MacConkey agar plates. The plates were incubated aerobically at 37°C overnight and the number of CFU (colony-forming units) was counted in each plate.

Results

Protection of mice

The protection afforded to mice vaccinated with SolcoUrovac against infection with homologous strains (the same bacteria as in the vaccine) is shown in Table 1. LD_{50} of nonimmunized mice, presented in two units (optical density at 600 nm wavelength and colony-forming units, CFU) varied greatly and was dependent on the kind of bacteria used for challenge. The values for LD_{50} in immunized mice, also presented in the two units, were calculated from the results obtained after challenge using the same volume with increased numbers of bacteria.

Immunization i.m. with SolcoUrovac conferred protection against challenge with all homologous strains. In the case of *E. coli*, *Proteus mirabilis* and *Streptococcus* it was necessary to inject 3.5–4.5 times more living bacteria, and in the case of *Klebsiella* as much as 600 times more, to obtain the same mortality as in nonimmunized mice.

The vaccination with SolcoUrovac also afforded protection against infection with heterologous strains of *E. coli* (Table 2). The four *E. coli* strains used belonged to completely different serotypes (all three main antigens were different from any antigen in SolcoUrovac *E. coli* strains). In spite of these differences the protection amounted to 10–55%.

In order to explain the duration of the protection against heterologous strains the mice were challenged with one of the strains at various times after immunization (Table 3). The effect of vaccination in the given test decreased with time, but even 10 weeks after the last booster injection 20% of the protection remained. The protective effect did not disappear completely until 20 weeks after the last injection.

Pyelonephritits in rats

In pilot experiments with experimental retrograde pyelonephritis in rats (partially obstructed ureter), abscesses of the kidneys were produced in about 85% cases, with good reproducibility. In addition none of the cultures of blood samples taken from six rats 30 min after injection of *E. coli* bacteria into the bladder showed bacterial growth.

In this study, 12 rats were immunized and 12 were used as controls. The incidence of abscesses and their extension on the surface of the kidneys and the number of CFU obtained from each kidney are presented in Table 4. Among the immunized animals abscesses were found in only three kidneys (25%). In one kidney they covered 80%, in another, 15%, and in the third, 40% of the total kidney surface. In the nonimmunized group the incidence and also the size of the abscesses were considerably greater: abscesses were seen in 10 of 12 cases, and in 5 kidneys they occupied 90% or more of the surface. Only in 2 cases was the area occupied by abscesses less than 50%.

All kidneys with abscesses were markedly enlarged, and their size was 150–200% of normal. The same was observed in some left kidneys without abscesses, but in these cases they were not enlarged by more than about a half.

In none of the right kidneys in either group of rats, i.e. on the side without partial obstruction, were any abscesses found. Figure 1 presents a left kidney of a nonommunized rat with acute suppurative pyelonephritis and, for comparison, the normal right kidney of the same animal.

There was a good correlation between the area occupied by abscesses and the bacterial counts in the kidneys (Table 4). The highest bacterial colony counts (from about 5×10^8 to 10^{10}) were found in kidneys with 80% and more of the surfaces covered by abscesses. Kidneys with smaller lesions also had smaller counts of bacteria. In the left kidneys of the immunized groups was found on average one-tenth as many bacteria as in the nonimmunized group.

The bacterial counts in the right kidneys, i.e. on the nonligated side, correlated with the counts in the left kidneys of the same animals, and thus also with the intensity of abscesses. However, the number of bacteria was only a fraction of that in the left kidney, and in the majority of animals with suppurative pyelonephritis there were fewer bacteria ($\approx 10^3$) in the right kidneys than in the

Table 4. Effect of immunization with SolcoUrovac on the incidence of abscesses and on bacterial counts of rat kidneys in experimental pyelonephritis

Immunized group				Control group			
Rat no.	Abscesses (% of kidney surface)	Bacteria per kidney		Rat no.	Abscesses (% of kidney[a] surface)	Bacteria per kidney	
		Left	Right			Left	Right
1	None	7.5×10^2	1.0×10^2	1	90%	7.6×10^9	1.2×10^8
2	None	1.2×10^3	<50	2	95%	1.1×10^{10}	4.0×10^6
3	None	1.6×10^3	1.0×10^2	3	90%	1.2×10^{10}	7.25×10^6
4	80%	4.45×10^9	4.0×10^6	4	90%	9.8×10^9	4.4×10^7
5	None[a]	6.6×10^4	2.3×10^4	5	80%	5.8×10^8	1.7×10^4
6	None[a]	2.4×10^4	2.2×10^4	6	none[a]	2.55×10^4	1.05×10^4
7	None	1.7×10^3	<50	7	25%	3.85×10^8	4.5×10^4
8	15%	3.75×10^7	8.5×10^3	8	75%	2.3×10^8	1.8×10^5
9	None	2.7×10^3	1.9×10^3	9	95%	1.35×10^9	9.2×10^6
10	None[a]	2.8×10^4	1.95×10^3	10	50%	2.25×10^8	1.8×10^7
11	40%	8.3×10^7	2.6×10^4	11	none[a]	4.1×10^4	7.9×10^3
12	None[a]	1.1×10^4	7.5×10^2	12	40%	6.4×10^7	1.5×10^4
	Mean	3.8×10^8	3.4×10^5			3.6×10^9	1.7×10^7

[a] Kidney enlarged

corresponding left kidneys. In the right kidneys from animals without pyelonephritis and without kidney enlargement – and these animals were present only in the immunized group – the bacterial counts did not exceed 2000, and in two cases the number was below 50 i.e., below the detection capacity of the method used, suggesting a possible absence of bacteria. The mean number of bacteria in the right kidneys of the vaccinated group was 1/50th that in the nonvaccinated group.

Discussion

In our experiments with animal infection models we were able to show the protective effect of immunization by SolcoUrovac a polyvalent bacterial vaccine against UTI in man. The i.m. immunization route was used throughout, since it directly reflects the situation in humans.

The protection test in mice against i.p. challenge infection is far from the natural situation in UTI, but it is an accepted model for testing of the efficacy of vaccines in general. The protective effect of immunization is demonstrated either by a lower mortality of mice at a given concentration of challenge bacteria or by an increase in the LD_{50} of challenge bacteria in the immunized, as gainst the nonimmunized, groups. Immunization conferred protection against challenge infection with homologous *E. coli* and, to a lesser extent, with heterologous *E. coli* strains, i.e. bacteria that share no O, K, or H antigens with any of the vaccine strains.

The rat model of obstructive and ascending *E. coli* pyelonephritis was chosen because this model resembles human pyelonephirits more closely than the status following haematogenous delivery of bacteria of after direct inoculation of bacteria into the kidney [12]. The reproducibility and the percentage of infected kidneys in this model were, in our hands, better and higher, respectively, than in the unmodified Brook's model [4], in which the ligature drew the ureter to one side. The volume of the *E. coli* inoculum introduced into the bladder did not exceed the required size. The inoculum entered the pelvis but not the blood stream, as confirmed by the fact that the blood was sterile 30 min after injection of the living bacteria into the bladder.

The results of the experiments showed that vaccination with SolcoUrovac prevents acute ascending *E. coli* pyelonephritis in rats. The differences between the group of immunized rats and the controls were clearcut. In the latter, kidneys with abscesses were seen more than three times as often as in the vaccinated group. In addition the size of the abscesses was much smaller in the immunized animals. This protection is assumed to be due to antibody to the *E. coli* used for challenge infection. In a previous paper [10] we found a high antibody titer in serum as well as in urine of mice vaccinated with SolcoUrovac. The antibody in the urine can act directly, and the antibody in serum can leave the circulation through the inflamed renal vessels into the outer medulla. Both can interrupt the infection by bactericidal and opsonic activity. Support for the decisive role of antibodies derives from an experiment

in rats using intravesical passive immunization, which protected the animals against pyelonephritis [9].

It is difficult to reach a conclusion based on the numbers of bacteria in kidneys, since the *E. coli* bacteria found in the kidneys could originate not only from the parenchyma of the organ but also from the blood, especially in the case of abscesses where bacteraemia may have been present. Nevertheless, the marked differences between the two groups in the numbers of bacteria in the kidneys allows the assumption that the clearance of bacteria was much greater after immunization than in nonimmunized animals.

The same conclusion can be made if we consider only the right (non-obstructed) kidneys in rats without abscesses. In two right kidneys of vaccinated animals the bacteria were eradicated – this was seen after immunization only – and the average number of bacteria was half that in corresponding kidneys of nonvaccinated rats.

Based on the above observations, we consider parenteral active immunization with inactivated bacteria of SolcoUrovac to provide effective protection against UTI.

The experiments described in this paper do not really allow us to speculate on the mechanism of the protection effect mediated by the vaccine. In an earlier publication, we described the appearance of specific IgG and IgA antibodies in blood and urine of mice in response to the vaccine [10], and an increase of sIgA in the urine of humans has also been demonstrated after vaccination [13, 14]. Specific antibodies presumably bind to bacteria, facilitate phagocytosis (opsonization) and decrease or neutralize the capacity of bacteria to adhere to the epithelial cell wall. These antibody-mediated effects may be responsible for the long-term prophylactic effect of the vaccine.

The factors determining natural and induced resistance to UTI have been discussed in some detail in the literature (for review see [15]).

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