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Comparison of two different cell culture methods in evaluation of biocompatibility of latex urinary catheters

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Summary. International biocompatibility standards for urinary catheters have not yet been set. The used in vivo animal tests earlier have been shown to be insensitive and expensive. The present study compared the sensitivity and other properties of two in vitro cell culture methods, the reference method of the British Standards Institution (BSI) [2] and the thymidine incorporation method (DNA synthesis inhibition test, [7]. The cell culture toxicities of thirty-seven latex catheters were measured and 84.8% of the catheters tested passed the BSI reference test while 94.6% passed the thymidine incorporation test. The overall batch-to-batch correlation was poor ($p > 0.05$), but within brands the correlation was better. This is obviously to be explained by the differences the characteristics of the tests and/or the chemical composition of the catheters. The thymidine incorporation test is easier to perform, requires less manpower, and is therefore less liable to subjective interpretations than the BSI test. The biocompatibility toxicity limits for latex urinary catheters need to be tightened. Our experience in this study indicates that the thymidine incorporation test or tests similar to it, which can be highly automated can be recommended for biocompatibility screening in large series, and BSI reference test can be used additionally in unclear cases.

Key words: Urinary catheters – Biocompatibility testing – Cell culture – Toxicity

The harmful side effects of latex urinary catheters have been well known since publication of the first reports of unexpected urethral strictures after short-term catheterization with latex catheters [6, 8, 9, 11, 12]. Recently, special attention has been paid to the biocompatibility properties of urinary catheters. However, there are as yet no international standards laying down quality requirements for these catheters.

A new in vitro method for tissue toxicity testing of urinary catheters was introduced a few years ago, based on the inhibition of DNA synthesis in cell cultures [7]. The method has been shown to give results that correlate well with those from in vivo animal methods, and its sensitivity, like that of other cell culture methods has proved to be higher than that of the animal methods [5, 10].

The British Standards Institution (BSI) has prepared its own test for cell culture toxicity of urinary catheters [2]. In this method the cell death in cell cultures caused by catheter extracts is measured both macroscopically and microscopically after the cell cultures have been fixed and stained.

The aim of the present study was to compare the sensitivity and properties of the BSI reference method with those of the method introduced by Ruutu and her associates [7].

Materials and methods

Thirty-seven latex catheters were used as test material. The test specimens were each cut into two parts: one part was tested with the thymidine incorporation method [7] and the other part with the BSI reference method [2]. The catheter halves were not cut down further. The tips and balloons of the catheters were not included in the tests.

Thymidine incorporation test

The test specimens were eluted under sterile conditions in 50 ml RPM 1-1640 culture medium at 37°C for 2 days. The eluate was then supplemented with 10% fetal calf serum and added at 3%, 10%, 30%, 60% and 100% concentrations to cell cultures in microtitre plates. The culture medium without catheter eluate was used as a control. The Jurkat (T-cell leukaemia) cell line was used. After culturing for 48 h, cell proliferation was measured by adding 20μCi [^{3}H]tdr (tritiated thymidine) to each well 6 h before termination of the cultures. The CPM (counts per minute) value for each well was recorded. Determinations were done in triplicate and the medians used as representative values. The IC_{50} values for the test specimens were determined graphically (see Fig. 1).

Table 1. Scoring of toxicity values (the limits are based on test specifications)

BSI reference method	
Toxicity	Eluate concentration causing cell death
Toxic	<49%
Intermediate	50%–100%
Atoxic	No cell death noted at any eluate concentration
Thymidine incorporation test	
Toxicity	IC_{50} value
Toxic	<30%
Intermediate	31%–99%
Atoxic	>100%

BSI reference method

RPM 1-1640 was used as culture medium. The amount of culture medium was determined by the external diameter of each catheter so as to have a surface area not less than 0.5 cm^2 and a mass of not less than 0.1 g per millilitre of extractant volume. The eluting of test specimens and the adding of fetal calf serum were done as above.

The eluate was then added at 1%, 3%, 10%, 30%, 50%, 70% and 100% concentrations to cell cultures in microtitre plates. The culture medium without catheter eluate was used as a control. The American Type Cell Collection CCL 81 Vero [kidney, African Green Monkey (*Cercopithecus aethiops*)] cell line was used. After culturing for 48 h the cultures were fixed with methanol and stained with Giemsa stain. Each culture was then examined both macroscopically and microscopically to determine cytotoxicity. Macroscopic examination was to detect absence of stain due to sloughing of dead cells. Microscopic examination was performed using an inverted microscope for evidence of cell death unaccompanied by sloughing.

In the thymidine incorporation test the catheters with IC_{50} values below 30% were regarded as toxic [10]. In the BSI reference method the corresponding toxicity limit is 44% (the lowest eluate concentration at which cell death is noted), but since we only had 30% and 50% eluate concentrations we chose 50% as the limit in the BSI method.

For further analysis the toxicity values were scored in three groups as presented in Table 1. The limits were based on the test specifications given above. Catheters that were so atoxic that the IC_{50} values could not be determined (or no cell death was observable at the concentration of 100%) were analysed as a separate group.

Table 3. Toxicity values with both methods according to manufacturer (thymidine incorporation test/BSI reference method)

Manufacturer	Toxic	Intermediate	Atoxic	Total
A	1/0	5/2	3/7	9
B	0/0	2/2	0/0	2
C	0/0	1/2	4/3	5
D	0/2	2/0	1/1	3
E	0/3	0/3	8/2	8
F	0/1	1/2	3/1	4
G	1/0	2/5	3/1	6
Total	2/6	13/16	22/15	37

For statistical analysis the Kappa measurement reliability and Spearman rank correlation tests were used, with $p < 0.05$ taken as the criterian of significance.

The effect of the cell culturing period on test reliability had been analysed earlier with both methods in a pilot study. This employed eight batches of catheters and the test methods were similar to those described above. Cytotoxicity was recorded after 24 h, 48 h and 72 h, and cytotoxicity proved to increase after 24 h but not further after 48 h. Therefore, 48 h was chosen as the culturing period, as in the earlier protocols [2, 7].

Results

In the overall analysis, the correlation between the results of the two tests was nonsignificant (Table 2); the disagreement between the test results was most prominent for brands D and E (Table 3). However, the correlation was better between different batches of catheters from the same manufacturer. In the intrabrand analysis, for each catheter brand one method was more sensitive than the other, making the overall correlation nonsignificant. There were not significant differences in toxicity between the brands from different manufacturers (Table 3). Six catheters showed cytotoxicity in the BSI reference test; in the thymidine incorporation test two of these catheters showed intermediate toxicity and four of them appeared totally atoxic. Fifteen catheters were shown to be atoxic by the BSI reference test; in the thymidine incorporation test ten of these were atoxic, while five showed intermedi-

Table 2. Results in both test groups: correlation nonsignificant ($p > 0.05$)

		Thymidine incorporation test			Total
		Toxic	Intermediate	Atoxic	
BSI reference method	Toxic	0.0%	5.4%	10.8%	16.2%
	Intermediate	5.4%	16.2%	21.6%	43.2%
	Atoxic	0.0%	13.5%	27.0%	40.5%
	Total	5.4%	35.1%	59.5%	100.0%

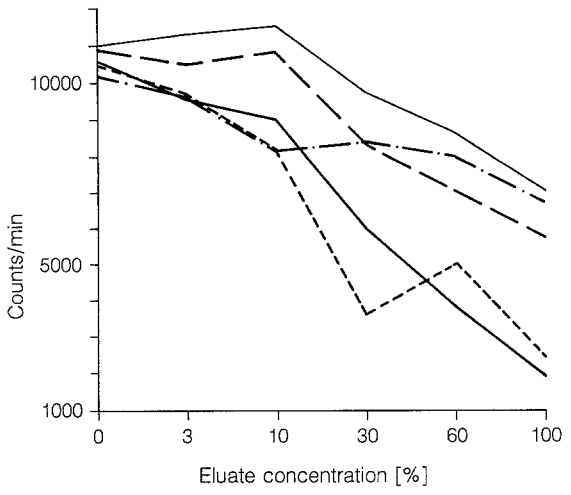

Fig. 1. Toxicity curves of five catheters from manufacturer A in thymidine incorporation test

ate toxicity. Sixteen catheters showed toxicity at eluate concentrations of 49%–100% (intermediate toxicity) in the BSI reference test; in the thymidine incorporation test eight of these were atoxic, while six showed intermediate and two marked toxicity ($IC_{50} < 30\%$) (Table 2).

Twenty-two of the catheters were so atoxic that no IC_{50} value could be determined in the thymidine incorporation test. The mean IC_{50} value of the remaining 15 catheters was 54%. In the BSI test 15 catheters were found to be totally atoxic and the mean eluate concentration (the lowest eluate concentration at which cell death was noted) for the remaining 22 catheters was 60%.

Discussion

The correlation between the results given by the two methods was poor. This unexpected finding may be partly explained by one basic difference between the tests: the thymidine incorporation test detects the cytotoxicity in cell cultures during the last 6 h at the test only, whereas the BSI reference method detects cytotoxicity during the whole culturing period, thus giving valuable information about the chronicity of cytotoxicity. This explanation is also supported by the higher intrabrand correlation.

Due to the low number of catheters tested, it was not possible to carry out intrabrand correlation. For each brand of catheter one method was slightly more sensitive than the other. These differences in sensitivity could, at least, partly be explained by the difference between the test methods mentioned above. Another explanation might be the different chemicals extracted from different catheters. The chemicals affect different targets in cells: the functions of the cell membrane, cellular energy production, DNA synthesis, protein synthesis, etc.

The thymidine incorporation test is technically easy to perform and allows the simultaneous testing of many catheters. It is therefore particularly suitable as a screening method for large series. It is far less time-consuming than the BSI reference method and requires little manpower since all the monitoring is done by the machines. This also makes the assessment of results less open to subjective interpretation and makes the test less expensive. The Jurkat cell line grows in suspensions and is therefore very easy to administer in the microtitre plate. It has remarkably stable growing properties. The Vero cell line used in the BSI method does not grow in suspensions and is therefore more difficult to administer in the microtitre plate: in order to grow properly, the monolayer has to attach itself to the surface of the microtitre plate, which makes it prone to artificial defects caused by unevenness of the plate surface. The monolayer is also subject to drying, which may produce large defects in the monolayer, thus mimicking the effect of toxic components in the culture medium.

In the thymidine incorporation test, the differences in the capacity of cell membranes to incorporate thymidine might be a source of artefacts. On the other hand, it is possible that toxic components in the eluate tested may damage the cell membranes and thus reduce thymidine intake. As mentioned above, the thymidine incorporation test measures cytotoxicity during the last 6 h of cell culturing. It would be better to alter the test procedure so that it becomes possible to measure cytotoxicity cumulatively.

In the BSI reference method the assessment of cytotoxicity is based on cell death as noted macroscopically or microscopically in the cell monolayer. However, a cell may seem perfectly normal both macroscopically and microscopically when DNA synthesis has in fact already been inhibited and the cell has ceased to grow.

In the present study the correlation between the test results in two cell culture methods was relatively poor: the catheters that showed marked cytotoxicity in one test passes the other and vice versa. Thus it seems that the cell culture toxicity testing methods themselves are vulnerable, and we therefore recommend that the toxicity limits should be raised in order to ensure the availability of urinary catheters of good quality. Our recommendation is that the catheter eluate as such (i.e. 100% eluate concentration) produced as above should be proved totally atoxic in a cell culture test, which would mean tightening the present toxicity limits two- or threefold. In our test material, 40.5% of the catheters would have passed the BSI reference test and 59.5% the thymidine incorporation test with this tightened toxicity limit. Figure 1 shows the toxicity curves of five catheters from manufacturer A in the thymidine incorporation test. Although three of these catheters were so atoxic that no IC_{50} value could be determined, a clear decline in the curves towards higher concentrations can be seen, indicating some degree of toxicity. We know by experience that latex products are potentially toxic [1, 3, 4, 10]. On the other hand, the quality of urinary catheters has markedly improved during the 1980s. We therefore believe that the kind of tightening of toxicity limits suggested above will be possible to carry out in the future. It would also remarkably reduce the costs of biocompatibility testing, since the use of different dilutions of the same catheter eluate could be abandoned.

In the present study the BSI reference method proved slightly more sensitive than the thymidine incorporation test. However, we think that the latter is more suitable as a screening test for large series. The BSI reference method could then be used in unclear cases where additional information about cytotoxicity is required.

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