STUDY OF MORBIDITY IN ORTHOTOPIC SMALL INTESTINE TRANSPLANTATION WITH WISTAR RATS. Experimental study

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ABSTRACT – ***Background*** - Transplantation of the small intestine is a surgical procedure currently under investigation for its possible application in the treatment of patients with short bowel syndrome, aiming at the reintroduction of an oral diet. ***Aim*** - To define the morbidity and mortality of intestinal transplantation in small animals using microsurgery. Intra and postoperative morbidity and mortality were studied in Wistar rats submitted to orthotopic intestinal allotransplantation. ***Material and Method*** - The animals were divided into three groups: group A (37 donor animals), group B (37 recipient animals), and group C (10 control animals). Group B was divided into three subgroups according to survival time. Subgroup TI consisted of animals that died during surgery or due to causes directly related to surgical intervention, subgroup T2 consisted of animals that died between the 4th and 29th postoperative day, and subgroup T3 consisted of animals that survived after 30 days. Transplanted animals were evaluated in terms of surgical technique used (vascular and intestinal anastomosis), graft quality, surgical time, and clinical parameters. The animals that died by the 29th postoperative day were submitted to autopsy and the remaining ones were sacrificed after 30 days. ***Result*** - There was a high rate of complication of a surgical nature. Early mortality rate, i.e., mortality up to the third postoperative day, was 54% with vascular anastomosis being the major cause of death. Surgical time was evaluated in a restricted and homogeneous group and showed a strong prognostic value in terms of successful transplantation. Clinical parameters such as weight loss, reduction of ingestion, reduction of motor activity and diarrhea were directly correlated with acute rejection. ***Conclusion*** - The experimented intestinal transplant is a procedure companied by considerable morbidity and mortality due to surgical complications in postoperative period, vascular anastomosis and total surgical time.

HEADINGS – Intestine, small, transplantation. Morbidity. Mortality. Short bowel syndrome. Rats, Wistar.

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INTRODUCTION

In 1959, LILLEHEI et al.[30, 31, 32, 33, 34] described for the first time the technique for small intestine transplantation in dogs. Many experimental studies were conducted during the subsequent years, as well as studies involving clinical transplants. When LILLEHEI et al.[33] performed the first clinical transplant in 1967, the patient only survived the surgery for 12 hours. In 10 cases of transplantation reported in the literature from 1964 to 1970, survival ranged from 12 hours to 76 days. We wish to point out two patients submitted to this surgical treatment at the University Hospital of São Paulo Medical School, São Paulo, SP, Brazil, by OKUMURA et al.[42], one of whom survived 12 days in 1969. OKUMURA et al.[42] were the first to contribute to the description of the physiopathology of the transplanted intestine since the patient who survived for 12 days also permitted the study of histopathology of the orthotopically transplanted intestine, but with two Thiry-Vella stomata. This technique permitted the collection of serial biopsies for histopathology. However, the early enthusiasm about intestinal transplantation declined in view of rejection and sepsis, which became recurrent due to the difficulty in solving immunological problems and which were usually more intense compared to other organs. The introduction of total parenteral nutrition in the 1970's, which permitted satisfactory treatment of patients with major intestinal resections, created great hopes for the possibility of a long survival. More recently, the advent of home parenteral nutrition favored even more the quality of life of the patients with short bowel syndrome and the resumption of their regular activities, as an alternative to the proposal of intestinal transplantation.

The development of potent immunosuppressive agents in the 1980 and 1990 decades, such as cyclosporin A4, followed by FK 506[12, 23, 54] and rapamycin[4, 51], led to renewed interest in transplantation of the small intestine. Small intestine transplantation may be the solution for the definitive treatment of short bowel syndrome[62] and also for several other clinical entities that lead to chronic intestinal malabsorption, such as necrotizing enterocolitis, intestinal atresia, Crohn disease and trauma.

Although transplantation has been successful in some centers[18, 56], it has not been established as a definitive form of treatment. The method continues to be of limited use, presenting in most cases insufficiently encouraging results[10, 16, 17]. Despite several years of experience with both clinical and experimental intestinal transplantation, the difference in the results obtained by transplantation of this organ continues to be great compared to solid organs[38, 39]. We know that to make progress in this area we must improve the understanding of the immunological phenomena and of the physiopathology of the transplanted graft, as well as its rejection. The small intestine is very rich in lymphoid tissue with a high antigenic potential[34, 64], a fact favoring the study of immunologic mechanisms of rejection in experimental models.

The rat has been more frequently employed for multivisceral and small intestine transplantation[38] compared to other experimental animals because of some advantages such as easy manipulation, relative resistance to sepsis and the possibility of developing strains with immunologic purity[23, 29, 68], an important fact for immunologic studies. Thus, there is great interest in the development of an adequate technique for intestinal transplantation in these animals, representing a model to be reproduced on a large-scale basis with low mortality. The disadvantage is that, since the rat is a small animal, microsurgical techniques are required; however, despite its complexity, the technique can be dominated by surgeons after appropriate training, as long as some microsurgery norms are followed[20, 50].

The objective of the present investigation was to study the intra- and postoperative morbidity and mortality of allogenic orthotopic intestinal transplantation in Wistar rats using the technique standardized by ZHONG et al.[67], and ZHONG and GRANT[68] for these animals.

MATERIAL AND METHOD

1. Material

The major surgical items to be used are listed below:

- Microscope for microsurgery (D.F. Vasconcelos, type MC, M-5) with 6x, 10x and 16x magnification
- Instruments for microsurgery
- Anesthetics: ketamine (Parke-Davis) at the dose of 144 mg/kg administered intraperitoneally and complemented with sulfuric ether (Ibiza Química) applied by inhalation when necessary.
- Material for intestinal perfusion and preservation, crushed ice and lactated Ringer solution (B. Brown), liquemine (5000 U/mL, Roche).
- Metabolic cage standardized at LIM-37 (FMUSP), Medical Investigation Laboratory-37 (São Paulo University Medical School).

2. Experimental animals

A total of 84 male Wistar rats weighing 204 to 384 g reared in the animal house of the University Hospital of São Paulo Medical School were used. The animals were divided at random into three groups without any previous selection or analysis of clinical or laboratory parameters:

Group A - consisted of 37 donor animals

Group B - consisted of 37 recipient and transplanted animals who were divided into three subgroups for the study of morbidity and mortality of the intestinal transplant:

T1 - 20 animals that survived until the 3rd postoperative day
T2 - 10 animals that survived from the 4th to the 29th postoperative day
T3 - 7 animals that survived beyond the 30th day.

Group C - consisted of 10 control animals.

The research project was carried out sequentially, i.e., all the intestinal transplants were first performed and then the animals of group C were operated upon. The rats were chosen at random, also with respect to their assignment to groups A and B.

3. Surgical procedure

In order to study morbidity and mortality of the intestinal transplant, the 37 recipient rats were submitted to orthotopic intestinal transplantation according to the technique of ZHONG et al.[67], and ZHONG and GRANT[68] using an allograft and no type of immunosuppression. The control group was submitted to opening of the abdominal wall with total section of jejunum and distal ileum followed by primary anastomosis of the segments and closing of the wall.

4. Postoperative period

The animals received water ad libitum during the immediate postoperative period, and food was introduced after the first postoperative day.

The following parameters were evaluated:

4.1 Surgical time
Surgical time was subdivided into cold ischemia time (time of immersion of the intestinal graft in lactated Ringer solution at 4 ^{o}C; time of warm ischemia (time needed for the execution of the vascular anastomoses); total surgical time (total time spent to operate on the donor and recipient animals).

4.2 The following clinical parameters were evaluated: daily body weight, weight of the ingested diet, urinary volume, motor activity, feces consistency, presence of piloerection, hair loss, presence of tachypnea, conjunctival and nasal bleeding, and hyperemia of paws, ears and nose.

4.3 Necropsy

5. Correlation between surgical time and morbidity, late mortality and survival rates

5.1. Subgroups T2 and T3 were compared in terms of all time parameters considered.

5.2. Intragroup analysis was also performed in subgroup T2 and the Pearson correlation coefficient was calculated for the analysis of morbidity and mortality according to surgical time and its various components.

5.3. Step by step multivariate regression was used to determine the predictive value of total surgical time and survival time in subgroup T2.

6. Analysis of clinical parameters in subgroups T2 and T3 and in group C was based on:

6.1. Daily body weight: the median and percent variation in weight was studied for a period of 29 days in subgroup T2 and for a period of 30 days in subgroup T3 and group C.

6.2. Weight of the ingested diet: the weight was corrected as g/100 g animal body weight and is represented by percent ingestion over a period of 30 days in subgroups T2 and T3 and group C.

6.3. Urinary volume: urinary volume was corrected as mL/100 g animal body weight and is reported as percent urinary volume over a period of 30 days in subgroups T2 and T3 and in group C.

Clinical signs and symptoms: the following clinical parameters were analyzed as mean daily alterations: motor activity, feces consistency, piloerection, hair formation, tachypnea, conjunctival and/or nasal bleeding, and hyperemia of paws, nose and ears. These data were obtained over a period of 30 days for subgroup T3 and group C. We used a quantitative scale to report qualitative evaluation, in such a manner that the final result was inversely proportional to the clinical staging of the animal. The arithmetic mean of the data obtained every three days was calculated, yielding a total of 10 numerical results (Table 1).

TABLE 1 – Staging of clinical parameters according to the worsening

CLINICAL PARAMETER	STAGING	DIARY GRADUATION
Motor activity	High	1
	Medium	2
	Low	3
Feces consistency	Solid	1
	Pasty	2
	Liquid	3
Piloerection	Yes	1
	No	0
Hair formation	Yes	1
	No	0
Tachypnea	Yes	1
	No	0
Conjunctival and/	Yes	1
or nose bleeding	No	0
Hyperemia of paws,	Yes	1
nose and ears	No	0

$$\bar{\chi} = \frac{\Sigma \chi 1, \chi 2, \chi 3}{n}$$

7. Survival curve

The survival curve was analyzed only in subgroup T2 according to the method of Kaplan-Meier, i.e., as percentage over the period from the 4th to the 29th postoperative day.

8. Statistical analysis

The analysis of variable was applied to compare group B from group C by statistical study:

8.1 Kaplan Meier curve to analysis the survival
8.2 Multivariation regression to calculate the predictive values
8.3 Kruskal-Wallis test with Miller-Dunn test to compare three or more independent group with no parametric values
8.4 Friedman test with student Newman-Keuls test to evaluate same parameters analysed along the time with no parametric values
8.5 ANOVA analysis to study no repeated measurements with Student-Newman test and Keuls to compare three or more independent groups with parametric values
8.6 Pearson correlation and linear regression to compare two variable in same group.
8.7 The *t* Student test with Welch correction to compare two independent group with parametric values.
8.8 Mann-Whitney test to compare two independent group with no parametric values
8.9 The level of significance was set at *P* 0.05

RESULTS

Mortality was analyzed considering separately what occurred during surgery and after surgery, i.e., up to the third postoperative day (T1 animals) and what occurred during the postoperative period from the 4th to the 29th day (T2 animals) (Table 2).

Early operative mortality (subgroup T1)

The deaths that occurred during surgery or immediately after surgery were due to complications of the vascular anastomosis (15%) and anesthetic (5%). Necropsy revealed that 80% of the deaths were also due to vascular complications and 5% to a jejuno-jejunal fistula with peritonitis up to the 3rd postoperative day (Table 3).

TABLE 2 – Causes of operative mortality in Group B

Causes	Mortality	
	n	%
Surgical	19	51,3%
Anesthetic	1	2,7%
TOTAL	20	54,0%

TABLE 3 – This table presenting in absolute and percentage complications of intraoperative and postoperative period until thirty day after surgery that cause death in subgroup T1

SUBGROUP T1			
Period of death	**N**	**%**	**Surgical complications**
Intraoperatory	1	5	Arterial anastomosis bleeding
	1	5	Venous anastomosis thrombosis
	1	5	Venous anastomosis stenosis
	1	5	Anesthetic
Postoperatory	6	30	Arterial anastomosis bleeding
	2	10	Venous anastomosis bleeding
	2	10	Venous anastomosis thrombosis
	2	10	Venous anastomosis twist
	2	10	Graft and haematoma bleeding
	1	5	Graft segmentar necrosis
	1	5	Fistula of jejunum and peritonitis
Total	20	100%	

Late mortality (subgroup T2)

Necropsy revealed that late mortality in subgroup T2 was due to peritonitis caused by perforation located in the intestinal graft, possibly due to rejection, in five animals (50%). The remaining five animals died of peritonitis caused by a jejuno-jejunal fistula (three animals), peritonitis and graft necrosis due to venous thrombosis (one animal), and obstruction due to volvulus and segmental necrosis of the intestinal graft (one animal).

Necropsy data for subgroup T3

The seven animals in subgroup T3 were sacrificed after 30 days of survival, i.e., between the 30th and 144th day. At autopsy, signs of intestinal adhesions with a slight thickening of the mesentery were observed, together with mild dilatation of the intestinal graft loops (Table 4).

TABLE 4 – Necropsy findings in subgroup T3

Necropsy findings	%	N
Peritonitis caused by perforation located in the intestinal graft with mesentery thickened with intestinal adherence	50	5
Peritonitis caused by jejunum fistula	30	3
Peritonitis with graft necrosis due to venous thrombosis	10	1
Peritonitis with the graft necrosis due to intestinal volvulus	10	1
TOTAL	100	10

Necropsy data for group C

The 10 animals in group C were submitted to autopsy on the 30th postoperative day. Only a large number of loose intestinal adhesions were found and two cases of infection of the incision in the abdominal wall occurred.

Surgical time

In the analysis of surgical time we did not consider the animals that died by the 3rd postoperative day, i.e., T1 animals. Thus, we only studied the animals that did not show operative complications related to the surgical technique (subgroups T2 and T3).

In the intergroup analysis we compared the results of T2 and T3. Evaluation of time of cold ischemia, time of warm ischemia and total surgical time showed a tendency to statistical significance ($P = 0.088$) for time of warm ischemia.

Linear regression analysis was applied to the data for time of cold ischemia, time of warm ischemia and total surgical time in relation to survival using the regression method of Pearson in subgroup T2. It can be seen that there was an inverse linear correlation between survival and total surgical time. Thus, total surgical time is of predictive value in terms of survival, at $P = 0.028$ (univariate regression).

Clinical parameters

Body weight

Mean weight gain was similar for group C and subgroup T3. In subgroup T2, however, there was a weight loss, with a significant difference ($P < 0.05$) compared to the other two groups.

Weight of the ingested diet

When subgroups T2 and T3 were compared to group C in the analysis of mean weight of the ingested diet (g/100 g body weight) T3 and C showed similar results, whereas T2 animals ingested significantly less food than the other two groups ($P < 0.05$).

Urinary volume (diuresis)

Comparative analysis of urinary volume (mL) corrected for 100 g body weight showed no significant difference between T2, T3 and C ($P = 0.55$).

Clinical signs and symptoms

Comparative analysis of the worsening of clinical parameters between group C and subgroup T3 showed that motor activity ($P = 0.055$) and feces consistency ($P = 0.042$) were significantly modified in T3, whereas the remaining five parameters showed no significant differences between groups.

Kaplan Meier survival curve

Analysis of this curve revealed that the probability of survival (percentage) was inversely proportional to postoperative time.

DISCUSSION

Surgical mortality

According to several investigators[22, 28, 36, 39, 49, 68], the deaths occurring from 24 to 72 hours after surgery for small intestine transplantation in rats should be considered to be related to operative mortality.

In the present study we considered deaths occurring up to the 3rd postoperative day to be caused by surgery. There is reasonable agreement among investigators about the fact that in small intestine allotransplantation in rats, mortality due to rejection starts on the 5th day[22, 36, 50, 68]; when hyperacute rejection occurs, however, death may occur starting on the 3rd postoperative day. On the basis of these literature data, it seemed appropriate to use as a criterion of surgical mortality the deaths that occurred up to the third postoperative day. From this viewpoint, mortality is directly related to the question of the surgical technique of transplantation, especially during the phase of surgeon training in microsurgery.

Complication of vascular anastomoses

In the present study, this type of complication was 40.5% and was mainly caused by thrombosis of the portocaval venous anastomosis. In general, this occurs because of torsion of the anastomosis due to an error in its spatial orientation[67], faulty execution of the vascular anastomosis[5], and faulty application of the microsurgery principles recommended by GUITY et al.[20].

Complications of the intestinal anastomosis

We observed a jejuno-jejunal fistula probably due to rejection since we used an absorbable biological prosthesis (a segment of macaroni) to orient the intestinal anastomosis, a maneuver that, according to ZHONG et al.[68], practically abolishes the risk of an intestinal fistula.

Complications of the intestinal graft

Graft manipulation should be reduced to a minimum and graft ischemia due to hemorrhage during the surgical procedure on the donor animal should be avoided. Care should also be taken not to irrigate the intestinal lumen with an excessive amount of solution[68], although some surgeons consider the use of profuse volumes to be adequate[36]. The quality of the intestinal graft is of fundamental importance for a successful transplant.

Anesthetic complications

Complications of this type are rare. The only case observed in the present series was during anesthetic complementation with inhalatory sulfuric ether. Most centers[22, 65, 68] use intraperitoneal pentobarbital (40 mg/kg) for anesthesia in order to avoid severe respiratory depression.

Hemodynamic alterations

A high incidence of recipient animal death was observed in the present study due to blood sequestration in areas distally located to the clamped vessels of the graft at the time of intestinal reperfusion. According to ZHONG et al.[67, 68], a hemodynamic fall occurs during clamping of the aorta and of the inferior vena cava and at the time of intestinal graft reperfusion due to hemodilution and temporary bleeding through the vascular anastomoses. In an attempt to correct the hemodynamic fall, we infused 5 mL of lactated Ringer solution after the completion of the vascular anastomosis and 5 mL after the end of the surgical procedure, as recommended by ZHONG et al.[67, 68].

Autopsy results

Subgroup T1 - a technical fault was detected in the vascular anastomosis, characterized by hemoperitoneum and palenes of the intestinal graft related to deficient containment of the vascular anastomosis[38, 39, 68]; we also noted ischemia of the graft because of poor graft quality caused by technical faults during its removal from the donor animal, as commented earlier.

Subgroup T2 - macroscopic signs of rejection were observed, characterized by thickening of the mesentery with lymph node enlargement and perforations of the Peyer patches with peritonitis, and a thick intestinal mass of unrecognizable limits. According to MONCHICK and RUSSELL[36], these signs are related to a clinical picture of acute rejection associated with weight loss, decreased food ingestion, diarrhea, and tachypnea.

Subgroup T3 - the animals were submitted to autopsy starting on the 30th postoperative day. Intestinal adhesions, slight thickening of the mesentery and dilatation of the intestinal loops were observed, as well as enlarged lymph nodes and isolated signs of acute rejection, according to MONCHICK and RUSSELL[36], such as diarrhea, piloerection, weight loss, decreased motor activity, hyperemia of paws and ears, and loss of hair. These signs, however, tended to regress after a few days.

Group C - the animals were submitted to autopsy on the 30th postoperative day and loose intestinal adhesions were observed, with no other changes.

Surgical time

The mean time of cold ischemia did not exceed 50 minutes and had no effect on the success of the transplant, probably due to the use of the lactated Ringer solution at 4o C[2, 13, 68] which insures good preservation of the intestinal graft for a period of up to 5 hours in the rat[30, 31, 32, 33, 35, 49].

A tendency to a significant difference between T2 and T3 was observed with respect to time of warm ischemia, and perhaps a significant difference would be observed if the sample were increased. However, according to several investigators[13, 19, 38, 68], this time is considered to be one of the determinant factors of surgical complications.

Total surgical time was found to be inversely correlated with survival when subgroup T2 was submitted to intragroup analysis.

Clinical parameters

Body weight - there was progressive weight loss in subgroup T2, suggesting the hypothesis that mortality was caused by acute rejection. Since T3 and C behaved similarly in terms of weight gain, we may

accept the fact that the intestinal transplant was well tolerated, suggesting that immunologic tolerance occurred in T3, as is usually the case for transplants between isogenic animals[36, 39, 67, 68].

Diet ingestion - a fall in ingestion occurred in subgroup T2 compared to subgroup T3 and to group C, which behaved in a similar manner, with no changes in ingestion over a 30 day period. This fact may indicate rejection of the graft in subgroup T2.

Urinary volume - urinary volume was similar in the three groups studied (T2, T3 and C), supporting the notion that the loss of weight observed in T2 effectively represented the more intense catabolism of these animals.

Evaluation of clinical staging according to its worsening showed a significant difference between T3 and C only in terms of motor activity and feces consistency, which were reduced in T3, permitting us to consider these parameter as strongly indicative of rejection[36, 38, 68].

The Kaplan Meier survival curve

The analysis of Kaplan Meier curve is just applied in subgroup T2, the subgroup T1 was excluded because they had early death after surgical complication during the surgical training. The subgroup T3 were not be analysed because they were submitted to sacrifice. The subgroup T2 were homogeneous and submitted to same proceeding of intestinal transplantation, we could study the survival curve during 29 days. This experiment show that risk of rejection was correlated with the worsening of the clinical parameters. The histopathologic evaluation was not done to confirm the rejection phenomenon, but the clinical signs and the necropsy results in subgroup T2 are coincident as same as the medical index[36, 38, 38, 57].

CONCLUSIONS

1. Experimental small intestine transplantation in rats is a procedure accompanied by considerable morbidity and mortality.
2. Intra- and postoperative mortality is mainly due to complications related to vascular anastomoses, especially thrombosis of the portacaval anastomosis.
3. Intra- and postoperative mortality is directly related to total surgical time.

Lee ADW, Gama-Rodrigues J, Galvão FH, Waitzberg DL. Estudo da morbidade em transplante ortotópico de intestino delgado em ratos Wistar. Estudo experimental. Arq Gastroenterol 2002;39(1):39-47.

RESUMO – ***Racional*** – O transplante de intestino delgado é procedimento cirúrgico em estudo visando sua aplicação no tratamento dos pacientes portadores da síndrome do intestino curto, com vistas à reabilitação da dieta oral. ***Objetivo*** - Definir a morbidade e mortalidade do transplante intestinal em animal de pequeno porte com emprego da microcirurgia. Foi estudado a morbidade e a mortalidade intra e pós-operatória no alotransplante intestinal ortotópico em ratos Wistar. ***Material e Método*** - Os animais foram divididos em três grupos: grupo A (37 animais doadores), grupo B (37 animais receptores) e C grupo (10 animais controle), sendo que o grupo B foi subdividido em três subgrupos conforme o tempo de sobrevivência. Os animais que faleceram durante o ato operatório ou de causa relacionada diretamente à intervenção cirúrgica constituíram o subgrupo. Os animais que faleceram entre o quarto e 29º dia de pós-operatório constituíram o subgrupo T2 e os que sobreviveram após 30 dias constituíram o subgrupo T3. Os animais transplantados foram avaliados quanto à técnica cirúrgica empregada (anastomoses vascular e intestinal), qualidade do enxerto, tempo cirúrgico e parâmetros clínicos. Realizou-se autopsia dos animais que tiveram óbito até o 29º dia de pós-operatório, os demais foram sacrificados após 30 dias. ***Resultados*** - Houve alto índice de complicação de natureza cirúrgica; ocorreu 54% de mortalidade precoce, isto é, até o 3º dia de pós-operatório, sendo a anastomose vascular a principal causa de óbito. O tempo cirúrgico foi avaliado num grupo restrito e homogêneo, mostrando forte valor prognóstico quanto ao sucesso do transplante. Os parâmetros clínicos: perda de peso, redução da ingesta, redução da atividade motora e diarréia tiveram correlação direta quanto à rejeição aguda. ***Conclusão*** - O transplante intestinal experimental apresenta expressiva morbimortalidade relacionando-as a complicações cirúrgicas imediatas (anastomose vascular e intestinal, enquanto que no tardio há processo de rejeição).

DESCRITORES – Intestino delgado, transplante. Morbidade. Mortalidade. Síndrome do intestino curto. Ratos Wistar.

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