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Normothermic Machine Perfusion versus Cold Storage of Liver in Pig Model: A Meta-Analysis

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Background: Normothermic machine perfusion (NMP) is a novel strategy used for organ preservation. We aimed to determine the overall efficacy of NMP for liver preservation versus traditional static cold storage (CS).

Material/Methods: We performed a meta-analysis of the literature to evaluate the efficacy of NMP in experimental pig models of liver preservation. We use the standardized mean difference and 95% confidence intervals (CI) to calculate statistics and used the random effects model for the combined analysis of the results.

Results: A total of 16 studies from 12 published articles were included. The combined results showed that NMP significantly decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST), and hyaluronic acid levels in serum or perfusate, significantly increased bile production, and had a similar 5–7-days survival rate after liver transplantation compared with the CS group.

Conclusions: NMP provides superior graft preservation compared with CS in the pig model.

MeSH Keywords: **Equipment and Supplies • Liver Transplantation • Meta-Analysis • Organ Preservation • Perfusion • Reperfusion Injury**

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Background

Liver transplantation (LT) has become the main treatment for end-stage liver disease. Advances in surgical technology, improvements in perioperative management, and the application of immunosuppressive agents have greatly improved the success rate and long-term survival rate of LT. However, the success of LT has created a new dilemma, as the supply of organs is insufficient to meet the demand. Hence, marginal donor livers, affected by severe fatty degeneration, older donors, prolonged hospitalization, and donation after circulatory death [1], are being considered to compensate for the gap between the supply and demand of livers for transplantation. However, grafts of suboptimal standard increase the risk of ischemic reperfusion injury (IRI) and post-transplant organ failure [2].

The reduction of IRI is currently one of the biggest issues in transplantation. In the past few decades, different measures have been taken to reduce IRI and improve the preservation quality of the donor liver. At present, the standard organ preservation method is static cold storage (CS). The low temperature (0–4°C) environment reduces the metabolism of organs [3], but cannot completely terminate metabolism, and so the lack of metabolic substrates and the accumulation of metabolites will still cause damage to the donor liver [4,5]. Therefore, CS cannot ensure the quality of the donor liver after preservation of marginal donor livers.

Normothermic mechanical perfusion (NMP) does not use low temperature to reduce the rate of organ metabolism, and instead aims to provide an approximately normal physiological environment for organs *ex vivo* [6]. NMP can continuously supply oxygen and metabolic substrates to the organ via a mechanical pump, and the perfusate can dilute the metabolites immediately instead of accumulating in the tissue. Thus, NMP can theoretically avoid cold ischemia injury during preservation and reduce IRI.

Although NMP has been used in clinical trials, the outcomes of NMP preservation remain unclear. One study verified the safety and feasibility of NMP in clinical application [7]. In addition, a retrospective control study showed that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels at postoperative day 3 tended to be lower in the NMP group than the CS group [8], with no significant difference between NMP and CS in postoperative graft function, duration of ICU stay, and duration of postoperative hospitalization [8]. Another clinical study reported no significant difference in 30-day survival rate of NMP versus CS grafts, although the duration of ICU stay and duration of hospitalization were significantly longer in the NMP group than in the CS group [9]. However, the livers preserved by NMP in these clinical trials had been rejected clinically as suboptimal, and were not equal

to livers preserved by CS. In contrast, animal studies investigating liver preservation via NMP versus CS have used equivalent quality livers for each preservation method. A number of animal experiments using NMP have been published, with the pig model being the closest to humans [10]. Hence, we collated these reports and carried out the present meta-analysis to quantitatively analyze the differences in the preservation of the liver using NMP versus CS; this will provide a theoretical basis for the selection of suitable organ preservation methods in clinical practice.

Material and Methods

Literature search

We performed a complete literature search to find articles related to NMP of the liver in the PubMed, EMBASE, and Cochrane Library databases using the following search terms: “normothermic perfusion” or “normothermic preservation” and “liver” and “transplantation”. The retrieval date ended in June 2017 and was updated in October 2017. We excluded non-English literature, unpublished research, and conference summaries. There were no restrictions on the species in the retrieval process, but only the literature on pigs was included in the final literature review.

The literature was included according to the following criteria: 1) studies evaluating *ex vivo* NMP of the liver, 2) CS used as a control, 3) preservation effect was evaluated by LT or simulated transplantation. The perfusion temperature was 35–38°C [11], and there were no restrictions on the NMP perfusate or CS preservation solution. In included studies, the NMP group had the same preservation time as the CS group, and all conditions were basically the same except for the preservation method. Exclusion criteria included: non-randomized or non-controlled experiments from which the data could not be obtained from the literature. We used the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines [12] to evaluate the quality of the included studies. Two authors independently performed the database search and selected the literature for review according to the inclusion and exclusion criteria; any differences in opinion were resolved by discussion.

The following information was collected: name of the first author, publication date, sample size, preservation time, evaluation method, observation time, and outcome. If one outcome was reported by more than 4 articles, the data was extracted and merged for analysis. If the original text did not directly provide the data, the image data was extracted using the image software GetData Graph Digitizer, version 2.20 (<http://getdata-graph-digitizer.com/>).

Statistical analysis

Statistical analysis was carried out in accordance with the Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0 (<http://training.cochrane.org/handbook>). We used the standardized mean difference (SMD, Cohen's method) to compare the results of the different studies using a common measure [13]. We pooled the overall effect using the random effects model and used the Q test and the I^2 value to quantify the heterogeneity between studies. P values less than 0.5 and I^2 values more than 50% were taken to indicate severe heterogeneity. We carried out subgroup analyses based on warm ischemia time (WIT), preservation time, and observation time. Sensitivity analysis was also conducted by excluding these studies from the analysis one-by-one to verify whether the same trend was observed with the remaining trials. STATA software version 12.0 (StataCorp, TX, USA) was used to analyze the data. $P < 0.05$ was considered to indicate statistically significant differences.

Results

Eligible studies

The PRISMA flow diagram was used in the search process (Figure 1). According to the retrieval strategy, 320 articles were preliminarily retrieved (194 from PubMed and 126 from Embase), of which 82 were repeated. We excluded 212 articles based on the titles and abstracts, as they were reviews, letters, other organ studies, studies on mechanisms of IRI, or non-controlled studies. There were 17 studies that met the inclusion criteria, including 3 on rats, 12 on pigs, and 2 in humans. The studies by Boehnert et al. 2013 [14], Brockmann et al. 2009 [15], and Schon et al. 2001 [6] each contained several groups of experiments. After discussion amongst the authors, these groups of experiments within a single study that met the inclusion criteria were selected separately and regarded as different studies. Each group of experiments within a single study were differentiated by "a", "b", and "c". A final total of 16 studies from 12 articles were included, all of which were studies conducted on pigs that were published from 2001–2016, all, with 95 cases in the experimental group and 83 in the control group (Table 1). Quality scoring was carried out according to the ARRIVE guidelines, with an average of 14.9 ± 1.5 (range: 13 to 18) (Table 2).

Hepatocellular injury

The determination of liver enzyme concentrations can reflect the degree of liver damage. Nine studies reported the level of ALT, 8 of which examined the concentration of the perfusate [16–19,21–24], and one study tested the serum concentration

Figure 1. Search strategy for selection of studies.

after orthotopic liver transplantation (OLT) [14]; the SMD and 95% CI were $-3.83 (-4.88, -2.79)$, indicating that the ALT level of the NMP preservation group was significantly lower than that of the CS preservation group (Figure 2). Ten studies reported the level of AST, 6 study examined the concentration of the perfusate [16–19,21,22], and the 4 study tested the serum concentration after OLT [6,14,20]; the SMD and 95% CI were $-3.31 (-4.93, -1.68)$, indicating that the AST level of the NMP preservation group was significantly lower than that of the CS preservation group (Figure 3).

Subgroup analysis of ALT (Table 3) showed that regarding the WIT and observation time, most of the 95% CI between the subgroups was overlapped, suggesting that there was no significant difference between the subgroups. According to the preservation time, the 95% CI overlap between the 1–4 hour group and the 10 hour group showed no significant difference. However, compared with the 1–4 hour and the 10 hour groups, only a small part of the 95% CI of the 24 hour group overlapped; this difference was statistically significant, which indicates that the effect of NMP versus CS on the ALT level becomes more obvious with the prolongation of the preservation time. However, this trend was not seen in the subgroup analysis of AST (Table 4).

Table 1. Characteristics of the included studies.

References	WIT (min)	n (NMP)	Time (NMP)	n (CS)	Time (CS)	Perfusate	Evaluation Method	Observation time	Outcomes
Banan 2016 [16]	0	3	4	3	4	Whole Blood	Reperfusion	4 h	AST, ALT, Lac, INR, bile production, bile biochemistry, blood gas, β -galactosidase, HA, histological changes, O ₂ consumption, Vascular compliance
Nassar 2016 [17]	60	5	10	5	10	Whole Blood	Reperfusion	24 h	AST, ALT, GGT, LDH, GGT, bile production, bile biochemistry, histological changes, O ₂ consumption, Flow/100 g tissue
Nassar 2015 [18]	60	15	10	5	10	Whole Blood	Reperfusion	24 h	AST, ALT, LDH, histological changes
Liu 2014 [19]	60	5	10	5	10	Whole Blood	Reperfusion	24 h	AST, ALT, LDH, Lac, bile production, histological changes, Oxygen consumption, HA and PV pressures and flows
Boehnert-a 2013 [14]	60	6	8	6	8	Steen solution	Reperfusion	12 h	ALT, bile production, bile biochemistry, HA, histological changes, O ₂ consumption, BUN, CT angiography
Boehnert-b 2013 [14]	60	6	4	6	4	Steen solution	OLT	8 h	AST, bile production
Fondevila 2011[20]	90	6	4	6	4	Blood based	OLT	5 d	AST, LDH, TB, PT, IL-6, TNF, vWF, Glu, weight change of liver, 5d survival
Brockmann-a 2009 [15]	0	5	5	5	5	Whole Blood	OLT	5 d	AST, ALT, HA, histological changes, 5d survival
Brockmann-b 2009 [15]	0	7	20	7	20	Whole Blood	OLT	5 d	AST, ALT, HA, histological changes, Glu consumption, weight change of liver, 5d survival
Brockmann-C 2009 [15]	40	6	20	4	20	Whole Blood	OLT	5 d	AST, ALT, HA, histological changes, 5d survival
Reddy 2005 [21]	60	5	1	6	1	Whole Blood	Reperfusion	23 h	AST, ALT, bile production, β -galactosidase, HA, histological changes, weight change of liver
Reddy 2004 [22]	60	5	4	4	4	Whole Blood	Reperfusion	20 h	AST, ALT, BE, PT, Factor V, Glu, bile production, HA
Imber 2002 [23]	0	5	24	5	24	Whole Blood	Reperfusion	24 h	AST, GGT, free Hb, Urate, PT, Factor V, bile production, histological changes, O ₂ consumption
St Peter 2002 [24]	60	4	24	4	24	Whole Blood	Reperfusion	24 h	ALT, bile production, histological changes
Schon-a 2001 [6]	0	6	4	6	4	Blood based	OLT	7 d	AST, ALT, INR, HA, Glu, histological changes
Schon-b 2001 [6]	60	6	4	6	4	Blood based	OLT	7 d	AST, ALT, INR, HA, Glu, histological changes

WIT – warm ischemia time; NMP – normothermic machine perfusion; CS – cold storage; OLT – orthotopic liver transplantation.

Table 2. Assessment of study quality based on the ARRIVE guidelines.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Quality score
Banan 2016	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	18
Nassar 2016	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	+	+	15
Nassar 2015	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	+	+	-	+	14
Liu 2014	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	-	+	+	+	16
Boehnert 2013	+	+	+	+	+	-	+	+	-	+	+	+	+	-	-	+	-	+	+	+	15
Fondevila 2011	+	+	+	+	+	+	-	-	-	+	+	+	+	-	+	+	-	+	-	+	14
Brockmann 2009	-	+	+	+	+	-	+	+	-	+	+	+	-	-	-	+	-	+	+	+	13
Reddy 2005	+	+	+	+	+	+	-	+	-	+	+	+	+	-	-	+	-	+	-	+	14
Reddy 2004	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	-	+	-	+	15
Imber 2002	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	-	+	-	-	13
St Peter 2002	+	+	+	+	-	+	+	+	-	+	+	+	+	-	-	+	-	+	+	+	15
Schon 2001	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	17

Study quality items are (1) title; (2) abstract; (3) background; (4) objectives; (5) ethical statement; (6) study design; (7) experimental procedures; (8) experimental animals; (9) housing and husbandry; (10) sample size; (11) allocation of animals to experimental groups; (12) experimental outcomes; (13) statistical methods; (14) baseline data; (15) numbers analyzed; (16) outcomes and estimation; (17) adverse events; (18) interpretation/scientific implications; (19) generalizability/translation; and (20) funding.

Figure 2. Effect of NMP vs. CS preservation on ALT level. Results of meta-analysis according to the random effects model.

Sinusoidal endothelial cell injury

Hyaluronic acid (HA) is a matrix polysaccharide enzyme that is known to be associated with inflammation [25] and has been used as a marker of sinusoidal endothelial cell (SEC) damage [21,26]. Five studies reported the level of HA, 3 study examined the concentration of the perfusate [16,21,22], and the 2 study tested the serum concentration after OLT [6]; the SMD and 95% CI were -3.25 (-4.43, -2.06), indicating that the HA

level of the NMP group was significantly lower than that of the CS group (Figure 4).

Liver synthetic function

The amount of bile production is an important index for evaluating the function of liver synthesis. Ten studies reported the level of bile production during the evaluation period [14,16-19,21-24]; the SMD and 95% CI were 3.96 (2.09, 5.84),

Figure 3. Effect of NMP vs. CS preservation on AST level. Results of meta-analysis according to the random effects model.

Table 3. Subgroup analysis of ALT.

Subgroup	No. of studies	Subtotal SMD (95%CI)	I-squared value	p Value
WIT				
0 min	2	-4.75 (-8.22, -1.28)	59.6%	0.116
60 min	7	-3.68 (-4.81, -2.55)	49.8%	0.063
Preservation time				
1-4 h	4	-3.43 (-4.51, -2.34)	0.0%	0.899
10 h	3	-6.42 (-8.98, -3.87)	0.0%	0.813
24 h	2	-3.58 (-5.93, -1.24)	78.4%	0.055
Observation time				
4-8 h	2	-3.20 (-4.70, -1.69)	0.0%	0.971
20-24 h	7	-4.13 (-5.51, -2.75)	59.7%	0.021

Table 4. Subgroup analysis of AST.

Subgroup	No. of studies	Subtotal SMD (95%CI)	I-squared value	p Value
WIT				
0 min	2	-1.70 (-6.82, 3.41)	86.7%	0.006
60-90 min	8	-3.70 (-5.37, -2.04)	83.6%	0.000
Preservation time				
1-4 h	7	-3.51 (-5.79, -1.23)	88.6%	0.000
10 h	3	-3.32 (-4.35, -2.30)	19.8%	0.287
Observation time				
4-8 h	2	-5.72 (-8.06, -3.39)	0.0%	0.422
20-24 h	5	-1.86 (-4.82, 1.09)	89.9%	0.000
5-7 d	3	-3.45 (-4.23, -2.67)	0.0%	0.541

Figure 4. Effect of NMP vs. CS preservation on SEC damage. Results of meta-analysis according to the random effects model.

Figure 5. Effect of NMP vs. CS preservation on liver synthetic function. Results of meta-analysis according to the random effects model.

Table 5. Subgroup analysis of bile production.

Subgroup	No. of studies	Subtotal SMD (95%CI)	I-squared value	p Value
WIT				
0 min	2	3.38 (1.71, 5.05)	0.0%	0.899
60 min	8	4.22 (1.93, 6.52)	89.5%	0.000
Preservation time				
1-4 h	4	2.46 (0.23, 4.69)	80.0%	0.002
8-10 h	4	5.78 (0.83, 10.73)	92.6%	0.000
24 h	2	—	—	—
Observation time				
4-12 h	3	0.84 (-0.43, 2.11)	53.4%	0.117
20-24 h	7	5.80 (2.87, 8.72)	85.9%	0.000

indicating that the amount of bile production in the NMP preservation group was significantly greater than that in the CS preservation group (Figure 5). Subgroup analysis showed that there was no significant difference in the effect of WIT and

preservation time on bile production, but with the prolongation of observation time, the difference between the NMP group and the CS group tended to become more obvious (Table 5).

Figure 6. Effect of NMP vs. CS preservation on survival rate. Results of meta-analysis according to the random effects model.

Survival rate

Seven studies performed orthotopic LT after liver preservation using NMP or CS [6,14,15,20], and 6 of these studies reported the survival rate after transplantation [6,15,20]. The RR and 95% CI were 2.02 (0.79, 5.15), indicating that there was no significant difference in the survival rate between the NMP and CS groups (Figure 6).

Discussion

The purpose of NMP is to restore the normal physiological environment of the liver *ex vivo* through continuous perfusion with oxygen and essential nutrients. NMP is used to preserve donor organs at normal body temperature and create an optimal environment in which to maintain organ activity, metabolism, and even reverse injury [24,27]. In contrast to CS, the fundamental principle of NMP is to maintain cell metabolism in the physiological environment throughout the preservation process. Using a sanguineous perfusate makes it possible to assess the viability of the preserved organ before transplantation [23].

Various transaminases will be released into blood when hepatocytes and mitochondrial membranes are damaged. ALT is a more sensitive indicator of liver damage, as it is found almost only in hepatocytes, while AST also exists in erythrocytes [23]. In hepatocytes, ALT mainly exists outside of the mitochondria, while about 80% of AST exists in mitochondria [28]. Hence, a moderate degree of hepatocyte injury causes a much greater leakage rate of ALT than that of AST. However, when severe

hepatocyte injury occurs, the mitochondrial membrane is also damaged, which leads to the release of the AST contained in mitochondria [29,30]. The results of the present meta-analysis showed that compared with CS, NMP significantly decreased serum ALT and AST levels, and this advantage of NMP became more obvious with the prolongation of preservation time.

HA is the main component of the extracellular matrix that is mainly absorbed and decomposed by the SECs. When the extracellular matrix is destroyed, and the SECs are seriously damaged, the level of serum HA will be substantially increased. Therefore, HA is a sensitive indicator of SEC damage [31]. Five included studies measured the HA level, and the results showed that NMP could better protect the SECs compared with CS.

The bile secretion that corresponds to the liver synthetic function has been used by many research teams to evaluate the activity in the liver *ex vivo* [32]. Some studies have investigated the decrease in the amount of bile after IRI, as ATP consumption reduces the secretion of bile [33]. IRI causes a decrease in the bile acid dependence on microtubules and increases the permeability of closely connected bile ducts, leading to the failure of the osmotic gradient of the microtubules [23]. Three included studies reported no significant difference in bile production between the NMP and the CS groups [14,21], but the remainder of the included studies showed that the amount of bile production in the NMP group was significantly greater than that in the CS group [16–9,22,23][16]. Subgroup analysis showed that when the observation time was 12 hours, there was no significant difference in bile production between the NMP and CS groups; however, when the observation time was 20–24 hours, the NMP group had a significantly greater

amount of bile production than the CS group. Thus, the observation time had a significant effect on whether the amount of bile formation differed between the 2 groups. Three of the included studies reported the total amount of bile generated during the observation period [17–19], while the data from the other studies was presented as the mean value of bile production per hour at the end of the evaluation stage. We excluded these 3 studies and repeated the analysis, but the difference in bile production between the NMP and CS groups still existed. This suggests that bile duct injury may take a long time to result in an observable difference in bile production. Bile duct injury may affect the long-term outcome after LT, but further research is required to determine the most suitable duration of observation.

The survival rate after LT is undoubtedly the most important index for the evaluation of LT. Seven of the included studies performed orthotopic LT after preservation via NMP and CS; 6 of these studies reported the survival rate after transplantation. Our result showed that there was no significant difference in survival rate between the NMP group and the CS group. However, as these 6 studies were from only 3 articles [6,15,20], the conclusion may be one-sided. Therefore, the effect of NMP on postoperative survival rate and long-term efficacy still needs further research.

Pathological diagnosis is clinically recognized as the diagnostic gold standard. Obvious histopathological changes occur in the liver tissues after ischemia-reperfusion. This series of pathological changes varies greatly depending on the duration of ischemia, the method of perfusion, and the duration of reperfusion [23]. The changes in hepatocytes after reperfusion include hepatocyte edema, vacuolization, endothelial cell destruction, neutrophil infiltration, hepatic sinusoid dilation, and hepatic parenchymal hemorrhage [20]. The present results showed that compared with the CS group, there was less necrosis of hepatic cells in the NMP group, and sinusoidal space and endothelial cells were well preserved. However, only 2 included studies used scoring criteria to quantify the pathological changes [17,19], while the other included studies only performed descriptive analyses. Therefore, we could not determine whether this difference was statistically significant. Quantifiable pathological evaluation methods should be recommended in future research to facilitate comparative analysis.

Heterogeneity

In the present meta-analysis, there was a high degree of heterogeneity for the analyses of AST level, bile production, and survival rate. Subgroup analyses were performed according to WIT, preservation time, and observation time because of the different experimental designs. Subgroup analysis showed that heterogeneity could not be eliminated by grouping, so

we speculate that these 3 factors were not the main source of heterogeneity.

Because of the small number of included articles, we did not detect any publication bias. The studies included in the present meta-analysis were all published in English, and so there may have been a certain amount of language bias. In addition, unpublished literature was not included; this unpublished literature may contain negative results, as studies with positive results may be easier to publish. However, not all of the included studies had positive results. Some studies reported that there was no significant difference between the 2 preservation methods regarding the level of AST [6,20] and bile production [14]. Moreover, there was no significant difference between the 2 preservation methods regarding the overall postoperative survival rate.

There was also an inevitable degree of experimental heterogeneity. Although we strictly adhered to the inclusion criteria, the working principle and perfusion parameters of the NMP system used in the various studies were similar, but the equipment and operation methods used were not exactly the same. We consider that this was the main source of heterogeneity. However, the sensitivity analysis in which the included studies were eliminated one-by-one showed that the final results did not change significantly. Hence, we consider that the results of the present meta-analysis are reliable.

Limitations

Our study has some limitations. First, although the NMP system is very expensive, there is no research into the cost of the NMP system. If a comparison of NMP and CS was carried out in combination with health economics, the results may have been different. Second, a relatively small number of studies were included in the present meta-analysis and the sample size was small. Third, animal experiments currently only focus on the short-term effects of the model. Postoperative long-term complications, such as biliary stricture formation, cannot be evaluated after only 5–7 days of observation, and both clinical and animal experiments have shown that biliary stricture formation is related to prolonged preservation time and WIT. Therefore, future studies with large sample sizes are needed to evaluate the long-term benefits of NMP.

Conclusions

Compared with CS, preservation of liver via NMP can protect hepatocytes and SECs, reduce the levels of ALT, AST, and HA, significantly increase bile production, and cause no change in the 5–7 days natural survival rate in the pig model. The NMP system is a novel organ preservation method that requires

further investigation in clinical practice, including cost-effectiveness evaluation.

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Conflict of interest

None.

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